EPSTEIN-BARR VIRUS

1. Virus–Host Interactions

1.1 Structure, taxonomy and viral gene products

1.1.1 Structure

Like other herpesviruses (see Figure 1 of Introduction), Epstein-Barr virus (EBV) has a toroid-shaped protein core that is wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between the nucleocapsid and the envelope and an outer envelope with external glycoprotein spikes. The major EBV capsid proteins are 160, 47 and 28 kDa, similar in size to the major capsid proteins of herpes simplex virus. The most abundant EBV envelope and tegument proteins are 350/220 and 152 kDa, respectively (Kieff, 1996).

1.1.2 Taxonomy

EBV is the most extensively studied gammaherpesvirus and the prototype of the lymphocryptovirus subfamily. The gammaherpesvirus classification was initially established on the basis of similarity in biological properties rather than structural characteristics. Like other gammaherpesviruses, EBV establishes latent infection in lymphocytes and can induce proliferation of the latently infected cells. Taxonomists have renamed EBV human herpesvirus 4 (HHV4).

Two major EBV types have been detected in humans (Gerber *et al.*, 1976a; Adldinger *et al.*, 1985; Zimber *et al.*, 1986; Young *et al.*, 1987; Rowe *et al.*, 1989; Sixbey *et al.*, 1989; Gratama *et al.*, 1992; Walling *et al.*, 1992). Type 1 and type 2 EBV genomes (formerly referred to as types A and B) are nearly identical, except for the genes that encode some of the nuclear proteins (EBV nuclear antigen, EBNA-2, EBNA-3A, -3B, -3C; see section 1.5.2) in latently infected cells (Nonoyama & Pagano, 1973; Bornkamm *et al.*, 1980). These differences are reflected in type-specific and type-common epitopes for antibody (Young *et al.*, 1987) and T-cell responses (Moss *et al.*, 1988). Antibodies to type-2 viruses are somewhat more prevalent in African sera than in sera from people in developed countries, but the recovery of type-2 virus from blood is unusual (Young *et al.*, 1987; Rowe *et al.*, 1989), perhaps because EBV type-2 infected lymphocytes grow less efficiently *in vitro* (Rickinson *et al.*, 1987). A naturally occurring EBV recombinant that encodes both type-1 and type-2 nuclear antigen sequences was isolated from the blood of a healthy adult in Papua–New Guinea (Burrows *et al.*, 1996a).

In addition to type-specific polymorphism, significant DNA sequence heterogeneity has been found by comparison of selected regions of the genome in EBV isolated in certain geographical areas or even from the same area. These polymorphisms define

different viral strains within types 1 and 2 (Aitken *et al.*, 1994). Some of the changes cause amino-acid substitutions in viral proteins and may even affect peptides that are important for the immune control of viral infection (de Campos-Lima *et al.*, 1993a; Lee *et al.*, 1993a; de Campos-Lima *et al.*, 1994; Lee *et al.*, 1995a; Burrows *et al.*, 1996b).

1.1.3 *Host range*

Under normal circumstances, EBV infection is restricted to humans. Antibodies to EBV have been detected in several primate species, probably due to the presence of cross-reactive antibodies against their own species-specific EBV homologues (reviewed by Kieff *et al.*, 1979). Such cross-reactive immunity may explain why these animals are usually refractory to experimental infection, although their B lymphocytes can be efficiently transformed *in vitro* (Miller *et al.*, 1972; Levine *et al.*, 1980a). New World monkeys carry no EBV-like virus, and at least three species appear to be infectable experimentally. The cotton-topped tamarin (*Saguinus oedipus oedipus*) and owl monkey (*Aotus trivirgatus*) are susceptible to EBV-induced B-cell lymphomas (Epstein *et al.*, 1973; Shope & Miller, 1975), while in more limited studies with the common marmoset (*Callithrix jacchus*) induction of a mononucleosis-like syndrome was seen in some animals (Wedderburn *et al.*, 1984). Infection of newborn marmosets with EBV resulted in the establishment of a long-term permissive infection, indicating similarities in the responses of marmosets and humans to EBV (Cox *et al.*, 1996).

1.1.4 *Target cells*

EBV is a B-lymphotropic herpesvirus, and infection of B cells is mediated through interaction of the viral envelope glycoprotein gp350 (gp340)/220 with the receptor for the C3d complement component CR2 (CD21; Fingeroth *et al.*, 1984, 1988). After binding of the viral particle to the surface of the host cell and endocytosis, the viral envelope fuses with the host cell membrane by a mechanism involving three other viral glycoproteins, gp85, gp25 and gp42 (Li *et al.*, 1995a). Interestingly, gp42 can bind to major histocompatibility complex (MHC) class II, and EBV uses this as a cofactor in the infection of B lymphocytes (Li *et al.*, 1997). The notion that EBV is a predominantly if not exclusively B-lymphotropic virus has been challenged, initially on the basis of the detection of EBV in non-B-cell tumours, notably certain T-cell lymphomas and some carcinomas. In addition, it has become apparent that expression of the C3d/EBV receptor is not restricted to B cells. Thus, identical or related molecules have been detected in T cells, follicular dendritic reticulum cells and, possibly, some epithelial cells (Reynes *et al.*, 1985; Fingeroth *et al.*, 1988; Timens *et al.*, 1991; Birkenbach *et al.*, 1992; Hedrick *et al.*, 1992; Sinha *et al.*, 1993). The cellular tropism of EBV is clearly not restricted through CD21, however; thus, the virus has been detected in CD21-negative cells. Moreover, some CD21-positive cells, such as follicular dendritic reticulum cells, have proved difficult to infect *in vitro* and have been shown consistently to contain no EBV *in vivo*.

Current evidence suggests that EBV infection in healthy chronic virus carriers is largely restricted to B cells, although in certain situations the virus can be detected in non-neoplastic epithelial cells and T lymphocytes as well (see section 1.2.1).

1.1.5 Genome, episomal and integrated viral forms and gene products

The EBV genome is a linear, double-stranded, 172-kb DNA molecule (Kieff, 1996). It has been speculated that the observed relative underrepresentation of CpG dinucleotides may correlate with the latent status of the viral genome (Honess et al., 1989). The genome of the prototype laboratory strain B95-8 has been sequenced as a set of BamHI-restriction fragments (Baer et al., 1984). The nomenclature for EBV open reading frames (ORFs) is based on the BamHI-restriction fragment in which they are found. For example, the BARF1 ORF is found in the BamHI A fragment and extends rightwards. The BLLF2 ORF is the second leftwards ORF in the BamHI L fragment (Table 1, Figure 1). The genome has the structural organization of group C herpesviruses with reiterated 0.5-kb terminal direct repeats and a reiterated 3-kb internal direct repeat, which divide the genome into short and long, largely unique sequences. The unique sequences contain several perfect or imperfect tandem repeat elements, many of which encode for repeat domains in proteins. EBV isolates differ in their tandem repeat reiteration frequency. Thus, differences in protein size are often useful markers of specific isolates and strains (Gratama et al., 1990). Furthermore, latent EBV infection is usually characterized by persistence of the genome as an episome with a characteristic number of terminal repeats. The fact that each progeny cell tends to have the same number of terminal repeats can be used to determine whether latently infected cells have arisen from a common progenitor (Raab-Traub & Flynn, 1986).

Circular episomal EBV DNA is detected in the nuclei of blood lymphocytes infected in vitro within 12–16 h of infection (Hurley & Thorley-Lawson, 1988). Probably only DNA repair synthesis is required for circularization, because significant nucleotide incorporation does not occur until 48 h after infection (Sixbey & Pagano, 1985). Stable, latently infected, proliferating so-called 'lymphoblasts' contain multiple EBV episomes (Adams & Lindahl, 1975; Kaschka-Dierich et al., 1976; Lindahl et al., 1976). EBV episomes are replicated early in the S phase by cell DNA polymerase (Hampar et al., 1974; Gussander & Adams, 1984; Shaw, 1985); the initial amplification requires DNA synthesis early in the S phase (Hampar et al., 1974), and the number of episomes increases thereafter over several days (Sugden et al., 1979), probably due to effects of EBNA-1 on S-phase oriP-dependent DNA replication, although the abundance of EBNA-1 does not appear to correlate with the episome copy number (Ernberg et al., 1977; Yates et al., 1984; Sternås et al., 1986). Episomal DNA is likely to be necessary for lytic cycle EBV DNA replication, since viral production has not been observed in cells that contain only integrated EBV DNA, and circular DNA copy numbers increase during lytic infection (Shaw, 1985).

Although episomal forms are commonest, the EBV genome can also persist by integrating into chromosomal DNA (Henderson et al., 1983; Matsuo et al., 1984; Lawrence et al., 1988; Hurley et al., 1991a,b; Delecluse et al., 1993a). Few cell lines with integrated EBV DNA have been studied in detail. In the Burkitt's lymphoma line Namalwa, EBV DNA has persisted by integrating into 1p35, and the entire block of cell DNA with the integrated EBV genome is duplicated as an inverted DNA domain

## Table 1. Identified EBV gene products and their open reading frames

Open reading frame	Common name	Proposed function
Latent genes		
BKRF1	*EBNA-1*	Plasmid maintenance
BYRF1	*EBNA-2*	*trans*-Activation, transformation
BERF1	*EBNA-3A*	*trans*-Activation, transformation
BERF2	*EBNA-3B*	Unknown
BERF3/4	*EBNA-3C*	*trans*-Activation, transformation
BWRF1	*EBNA-LP*	*trans*-Activation, transformation
BNLF1	*LMP-1*	Transformation
BNRF1	*LMP-2A/2B*	Maintenance of latency
BARF0[a]		Unknown
Immediate early genes		
BZLF1	*ZEBRA*	*trans*-Activation, initiation of lytic cycle
BRLF1		*trans*-Activation, initiation of lytic cycle
BI'LF4		*trans*-Activation, initiation of lytic cycle
Early genes		
BMRF1		*trans*-Activation
BALF2		DNA binding
BALF5		DNA polymerase
BORF2		Ribonucleotide reductase subunit
BARF1		Ribonucleotide reductase subunit
BXLF1		Thymidine kinase
BGLF5		Alkaline exonuclease
BSLF1		Primase
BBLF4		Helicase
BKRF3		Uracil DNA glycosylase
Late genes		
BLLF1	*gp350*	Major envelope glycoprotein
BXLF2	*gp85 (gH)*	Virus–host envelope fusion
BKRF2	*gp25 (gL)*	Virus–host envelope fusion
BZLF2	*gp42*	Virus–host envelope fusion, binds MHC class II
BALF4	*gp110 (gB)*	Unknown
BDLF3	*gp100–150*	Unknown
BILF2	*gp55–80*	Unknown
BCRF1		Viral interleukin-10
BHRF1[a]		Viral *bcl-2* analogue

Adapted from Li *et al.* (1995a); Nolan & Morgan (1995)

[a] Expressed in latently infected cells as well

EBNA, EBV nuclear antigen; LP, leader protein; LMP, latent membrane protein; ZEBRA, Z EBV replication activation; gp, glycoprotein; MHC, major histocompatibility complex

Figure 1. *Bam*HI-restriction map of the EBV genome and latent transcripts
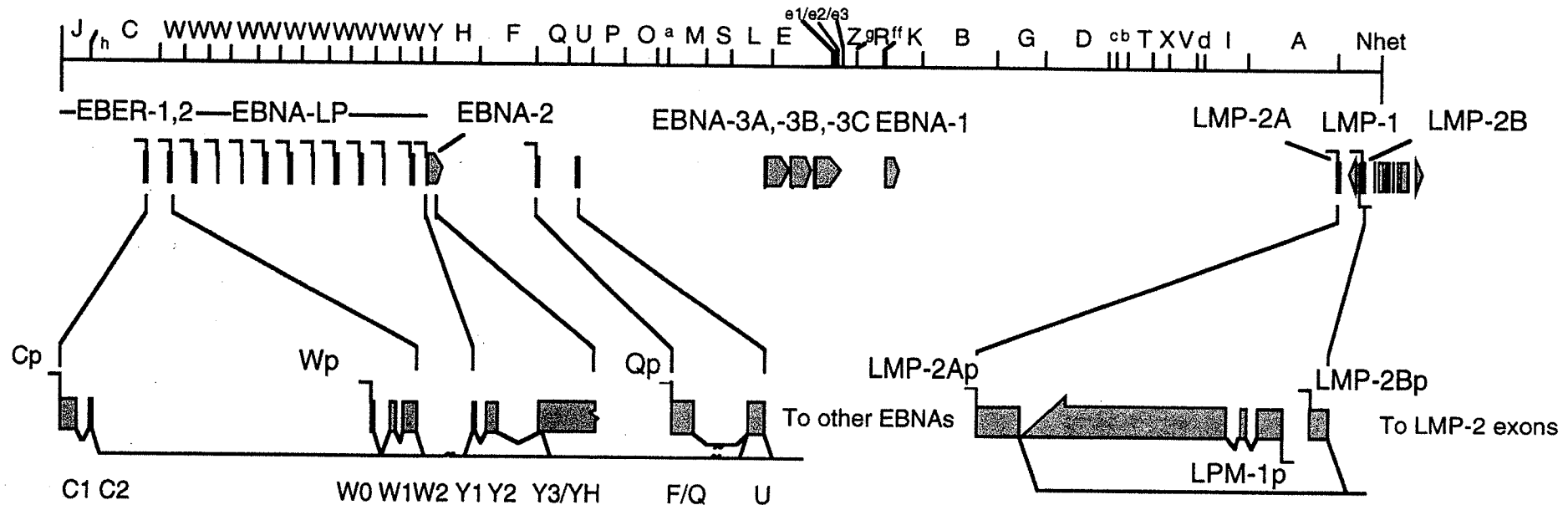
Adapted from Masucci and Ernberg (1996) and Rickinson and Kieff (1996)
EBER, EBV-encoded RNA; EBNA, Epstein-Barr nuclear antigen; LMP, latent membrane protein
Other abbreviations are explained in the text.

(Henderson *et al.*, 1983; Lawrence *et al.*, 1988). In the human fetal lymphoblastoid cell line IB4, multiple EBV genome copies are integrated in tandem into 4q25 (Matsuo *et al.*, 1984; Hurley *et al.*, 1991b). Integration occurs frequently when Burkitt's lymphoma cells are infected with EBV *in vitro* (Hurley *et al.*, 1991a). Clearly, integration is not chromosome site-specific or a regular feature of EBV infection.

In the establishment of latent infection, most of the EBV genome undergoes progressive methylation (Kintner & Sugden, 1981; Larocca & Clough, 1982; Perlmann *et al.*, 1982), whereas regulatory domains involved in maintaining latent infection, such as *oriP*, tend to remain under-methylated (Minarovits *et al.*, 1992). Extensive methylation of parts of the genome not expressed in latent infection may help to maintain latency by inhibiting lytic gene expression (Nonkwelo & Long, 1993). Treatment of latently infected cells with drugs that reduce DNA methylation increases the frequency of cells entering the productive cycle (Ben-Sasson & Klein, 1981).

EBV infection of primate B lymphocytes *in vitro* is usually nonproductive and results in the expression of a restricted number of viral genes and continuous proliferation. EBV-infected proliferating B lymphocytes are similar to lymphocytes that are proliferating in response to antigens, mitogens or interleukin (IL)-4 and anti-CD40 in that they express a similar repertoire of activation-associated markers, secrete similar immunoglobulins and have similar intercellular adherence (Åman *et al.*, 1986; Hurley & Thorley-Lawson, 1988; Alfieri *et al.*, 1991; Banchereau *et al.*, 1991). At least 11 EBV genes are expressed in latently infected B lymphocytes (Table 2, Figure 1). Two of these encode small, non-polyadenylated RNAs (the EBV-encoded RNAs EBER-1 and EBER-2), six encode nuclear proteins (the EBV nuclear antigens EBNA-1, EBNA-2, EBNA-3, -4 and -6, also called EBNA-3A, -3B and -3C, and EBNA-5, also called leader protein, LP), and three encode integral membrane proteins (the latent membrane proteins LMP-1 and LMP-2A and -2B, also called terminal proteins 1 and 2). Other viral mRNAs encoded for example by the *BHRF1* and *BARF0* ORFs have been detected in latently infected cells, but their products are not well characterized (reviewed by Kieff, 1996).

Different patterns of viral gene expression have been identified in latently infected, EBV-carrying cells *in vivo* (Table 3). Expression of all the latent viral genes detected in lymphoblastoid cell lines *in vitro* is a feature of the immunoblastic lymphomas of immunosuppressed patients. This form of latency is commonly defined as latency III. Latency I was first detected in EBV-positive Burkitt's lymphoma cells and is characterized by restricted expression of the EBERs, EBNA-1 and probably the product of the *BARF0* ORF. Latency II, characterized by the expression of EBERs, EBNA-1 and the three LMPs, was first recognized in a proportion of nasopharyngeal carcinomas but also seems to be common in most EBV-carrying non-B-cell tumours and in Hodgkin's disease (reviewed by Rickinson & Kieff, 1996). The latency II type has been reproduced *in vitro* by stable infection of a human T-cell line with a recombinant EBV carrying a selectable marker (Yoshiyama *et al.*, 1995). An additional form of latency characterized by restricted expression of EBERs, EBNA-1 and EBNA-2 has been described in smooth-muscle tumours arising in immunosuppressed children and AIDS patients (Lee *et al.*, 1995b).

Table 2. Nomenclature of latent viral gene products

Adopted terminology	Alternative nomenclature	
EBNA-1	EBNA-1	EBNA-1
EBNA-2	EBNA-2	EBNA-2
EBNA-3A	EBNA-3	EBNA-3A
EBNA-3B	EBNA-4	EBNA-3B
EBNA-3C	EBNA-6	EBNA-3C
EBNA-LP	EBNA-5	EBNA-4
LMP-1		
LMP-2A	TP-1	
LMP-2B	TP-2	
EBER-1		
EBER-2		

EBNA, EBV nuclear antigen; LP, leader protein; LMP, latent membrane protein; EBER, EBV-encoded RNA; TP, terminal protein

Table 3. Patterns of EBV latent gene expression

Type of latency	Gene product	Examples	Reference
I	EBERs, EBNA-1	Burkitt's lymphoma Gastric carcinoma	Rowe et al. (1987a) Imai et al. (1994a)
II	EBERs, EBNA-1, LMP-1, -2A, -2B, BARF0	Hodgkin's disease Nasopharyngeal carcinoma	Deacon et al. (1993) Hitt et al. (1989); Brooks et al. (1992)
III	EBERs, EBNA-1, -2, -3A, -3B, -3C, -LP LMP-1, -2A, -2B	Post-transplant lympho-proliferative disorder Infectious mononucleosis	Young et al. (1989a) Tierney et al. (1994)
Other	EBERs, EBNA-1, -2	Smooth-muscle tumours	Lee et al. (1995b)

EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; LMP, latent membrane protein; LP, leader protein

With a frequency characteristic of each EBV-infected B-lymphocyte line, some progeny spontaneously become permissive for viral replication. In these cells, the viral DNA is amplified several hundred-fold by a lytic origin of DNA replication, oriLyt (Hammerschmidt & Sugden, 1988). Multimeric forms produced from presumably circular templates are generated from oriLyt following a pattern that could result from rolling-circle DNA replication (Pfüller & Hammerschmidt, 1996). The expression of viral genes associated with productive infection follows a temporal and sequential order (reviewed by Kieff, 1996). Some viral genes are expressed early after induction, indepen-

dently of new protein synthesis, and are therefore classified as immediate early genes. Early lytic genes are expressed slightly later, and their expression is not affected by inhibition of viral DNA synthesis. Genes the expression of which is blocked by inhibitors of viral DNA synthesis are categorized as late genes. Because epithelial cells are fully permissive for lytic EBV infection *in vivo* (Lau *et al.*, 1993; Ryon *et al.*, 1993), considerable efforts have been made to adapt EBV to grow in organ cultures of transformed epithelial cell lines. These cultures can be infected, but the infection is often abortive or inefficient and very little virus is produced (Li *et al.*, 1992). Therefore, most of our knowledge of latent or lytic EBV products is derived from the study of EBV-infected B lymphocytes.

1.1.5.1 EBV-encoded RNAs

The two EBV-encoded, small, non-polyadenylated RNAs (EBER-1 and -2) are by far the commonest viral RNAs in latently infected cells, with an estimated abundance of 10^7 copies per cell (Howe & Steitz, 1986; Howe & Shu, 1989). They are usually transcribed by RNA polymerase III, although polymerase II may also be involved (Howe & Shu, 1989). Most EBERs are located in the nucleus and are associated at the 3′ terminus with the cellular La antigen (Howe & Steitz, 1986) and other cellular proteins, including the EBER-associated protein, which shows strong resemblance to an endodermal, developmentally regulated sea-urchin protein called 217 (Toczyski & Steitz, 1991). The primary sequences of EBER-1 and -2 show extensive similarity to those of adenovirus VAI and VAII and cell U6 small RNAs (Glickman *et al.*, 1988). These RNAs form stable secondary structures that allow extensive intermolecular base-pairing and may have, therefore, similar functions. On the basis of the known function of the RNAs of VA and U6, two alternative roles have been proposed for the EBERs. In adenovirus infection, VAI RNA inhibits the activation of an interferon-induced protein kinase which blocks transcription by phosphorylating the protein-synthesis initiator factor eIF-2 α. EBER-1 and -2 can partially complement the replication of an adenovirus with null mutations in VAI and VAII, but their effect on eIF-2 α kinase activity is significantly smaller and they are not found in the cytoplasm (Kieff, 1996). A possible role of the EBERs in the splicing of the primary EBNA and LMP mRNA transcripts is suggested by the partial complementarity to RNA splice sites. Complexes between U6 and U4 RNAs are required for RNA splicing *in vitro*, and the EBER-2 RNA contains a single-stranded loop with six of seven nucleotides identical to those required for U6–U4 interaction (Glickman *et al.*, 1988). Both of the proposed functions of EBERs are somewhat incompatible with the observation that their expression is delayed until after EBNA and LMP gene expression and initiation of DNA synthesis (Alfieri *et al.*, 1991). Nevertheless, the earlier events in primary B-cell infection are sensitive to interferon (IF) (Thorley-Lawson, 1980, 1981), and EBERs may play a role in blocking eIF-2 kinase. EBV recombinants in which the EBERs have been deleted can initiate primary B-cell infection and growth transformation in the same way as wild-type virus, and no differences were seen in the growth of lymphoblastoid cell lines infected with EBER-deleted and control virus or in the permissiveness of these cells for lytic infection (Swaminthatan *et al.*, 1991). The EBER

transcription unit was found to be unmethylated in all types of EBV-carrying cells (Minarovits *et al.*, 1992).

1.1.5.2 Nuclear proteins

Transcription of nuclear proteins is initiated at polymerase II-dependent promoters in the *Bam*HI C (Cp) and *Bam*HI W (Wp) regions (reviewed by Rogers *et al.*, 1992). Approximately at the time of genome circularization, the Wp promoter initiates right-wards transcription that proceeds through almost the entire strand of the genome (Bodescot *et al.*, 1986). The mRNAs contain a 40-base first exon, W0 (Rogers *et al.*, 1992), which is spliced to alternating W1 and W2 exons derived from successive reiteration of the internal *Bam*HI W repeats (Bodescot & Perricaudet, 1986; Sample *et al.*, 1986). The use of an alternative splice acceptor site in W1 results in generation of an ATG codon in about half of the transcript. This initiates translation of an ORF encoded by the repeating W1 and W2 exons and by two short exons in the *Bam*HI Y region (Y1 and Y2). The resulting protein, called EBNA-LP, is encoded in the leader of the EBNA mRNAs and is therefore also called leader protein (LP) (Kieff, 1996).

The Y2 exon is spliced to a long exon, YH, that spans the right end of *Bam*HI W and the left end of *Bam*HI H (Kieff, 1996). The ORF of this exon encodes the nuclear protein EBNA-2. EBNA-LP and EBNA-2 are the first viral proteins expressed in infected B lymphocytes. They are already detected 12 h after infection and reach the levels maintained in transformed lymphoblastoid cell lines within 24–32 h after infection (Alfieri *et al.*, 1991). Probably as a consequence of the action of EBNA-2 on response elements located in the upstream *Bam*HI C fragment, a switch from Wp to Cp usage occurs in most of the infected cells (Woisetschlaeger *et al.*, 1990). Two exons downstream of Cp, C1 and C2, replace the W0 exon in those transcripts. As with the W0 exon, alternative splicing of C2 to W1 or W0 results in mRNAs incapable or capable of translating EBNA-LP, respectively. A potential splice donor site near the beginning of the YH exon, but preceding the EBNA-2 initiation codon, is activated in some of the transcripts and is spliced to a downstream acceptor in *Bam*HI U. The U exon is further spliced to any of four alternate acceptor sites that begin the ORFs encoding EBNA-3A, EBNA-3B, EBNA-3C or EBNA-1. Thus, as a consequence of alternate 5′ splicing of Cp- or Wp-initiated RNAs, some RNAs will encode EBNA-LP or EBNA-LP and EBNA-2, while other RNAs will exclusively code for EBNA-3A, -3B, -3C or EBNA-1. The turning on of EBNA-3A, -3B, -3C and EBNA-1 expression is probably explained by the strength of the Cp, which leads to significantly more transcription. Polyadenylation may determine splicing and thereby regulate the expression of the six EBNA mRNAs from the same promoter. *cis*- or *trans*-Acting factors, such as the expression of certain viral products or the formation of secondary structures in the primary EBNA transcripts, may also play a role in splice choice.

In cells that express a latency I or II phenotype, the Cp and Wp are silenced and extensively methylated (Ernberg *et al.*, 1989; Schaefer *et al.*, 1997). Transcription of the EBNA-1 message is initiated at an alternative promoter located in the FQ transition region and, like the Cp- and Wp-initiated messages, splices to the U and K exons.

Characterization of the transcripts originating from this region has been complicated by the presence of several transcription–initiation start sites and by the overlapping of promoters that are active during the latent and lytic phases of the cycle. It seems now clear that the originally described Fp is a lytic promoter (Nonkwelo et al., 1995, 1996), while transcription of the latency I EBNA-1 mRNAs begins at the adjacent Qp (Nonkwelo et al., 1997).

(a) EBNA-LP

The size of EBNA-LP varies among isolates, owing to varying numbers of internal repeats (IR1) that contain the W1 and W2 exons of LP (Dillner et al., 1986; Finke et al., 1987; Wang et al., 1987a). The W2 exon encodes for a 44-amino acid repeat containing basic Arg-Arg-His-Arg and Arg-Arg-Val-Arg-Arg-Arg domains that could be nuclear localization signals. EBNA-LP is phosphorylated, probably on serine residues located in the W2 exon near a casein kinase II phosphorylation site (Petti et al., 1990; Kieff, 1996). The protein is strongly associated with the nuclear matrix and has an unusual location, as seen by immunofluorescence microscopy (Petti et al., 1990; Jiang et al., 1991). Some EBNA-LP is diffusely spread through the nucleus, while the rest is concentrated in a few granules, frequently distributed in curved linear arrays.

Molecular analysis of EBNA-LP has focused on the last two exons that are deleted in the nontransforming P3HR-1 EBV strain. Deletion of the exons resulted in EBV recombinants with significantly reduced growth transforming ability under standard in-vitro culture conditions (Allan et al., 1992); however, only a modest reduction of transformation efficiency was observed by plating infected B lymphocytes in soft agarose over fibroblast feeder layers (Hammerschmidt & Sugden, 1989; Mannick et al., 1991). The mutant virus-infected lymphoblastoid cell lines also tended to be more differentiated towards immunoglobulin secretion. The unusual fibroblast dependency and enhanced differentiation suggest that EBNA-LP may indirectly or directly up-regulate the expression of autocrine factors required for B-cell growth. Transient transfection of EBNA-LP and EBNA-2 into primary B lymphocytes co-stimulated with gp350 indicated that the two proteins cooperate in the induction of G0 to G1 transition, as marked by induction of cyclin D2 (Sinclair et al., 1994), but the mechanism of this effect remains unknown.

(b) EBNA-2

EBNA-2 differs extensively between the EBV type-1 and type-2 isolates (Aitken et al., 1994) and is the primary determinant of the biological differences that enable the type-1 strains to transform B lymphocytes with greater efficiency (Rickinson et al., 1987). The two proteins consist of 484 and 443 amino acids, respectively; they are over-all acidic, phosphorylated on threonine and serine residues and contain at least seven discrete domains including a polyproline repeat that varies by 10–40 amino acids in different EBV isolates. The carboxy-terminal domain contains two Arg-Gly and Lys-Arg-Pro-Arg repeat sequences that are required for nuclear localization (Cohen et al., 1991). EBNA-2 localizes in large nuclear granules and is associated with nucleoplasmic, chromatin and nuclear matrix fractions (Petti et al., 1990).

EBNA-2 is a specific *trans*-activator of cellular genes, such as the B-cell activation markers CD23 (Wang *et al.*, 1987b, 1990a, 1991) and CD21 (Wang *et al.*, 1990a) and the *c-fge* oncogene (Knutson, 1990), and viral genes including *LMP-1* (Abbot *et al.*, 1990; Ghosh & Kieff, 1990; Wang *et al.*, 1990b; Tsang *et al.*, 1991; Fåhraeus *et al.*, 1993) and *LMP-2* (Tsang *et al.*, 1991) and the *cis*-acting element upstream of the Cp (Sung *et al.*, 1991; Walls & Perricaudet, 1991; Sjöblom *et al.*, 1995a). The EBNA-2-responsive elements have been partially defined by deletion analysis of EBV promoter constructs or by positioning near a heterologous promoter (Laux *et al.*, 1994a). Molecular analysis of EBNA-2 by rescue of transforming virus from P3HR1-infected cells or the *trans*-activation of LMP-1 in transiently transfected Burkitt's lymphoma cells as phenotypic markers reveals a correlation between the sequences of EBNA-2 that are essential for transformation and those important for promoter *trans*-activating activity (Cohen *et al.*, 1991). Two large deletions, between amino acids 112–230 and 463–483, were shown to reduce transformation and *trans*-activating activity but still result in virus capable of transforming cells, albeit with low efficiency. Deletion of the Arg-Gly oligomer domain in residues 337–357 resulted in a 90% reduction of transforming efficiency but increased *trans*-activation of the LMP-1 promoter by at least 10-fold (Tong *et al.*, 1994; Kieff, 1996). This domain can interact with histones, potentially facilitating the interaction of EBNA-2 with DNA. Only three regions, located between residues 95–110, 280–337 and 425–462, appear to be stringently required for transformation and *trans*-activating activity. The 425–462 region is essential for its acidic *trans*-activating characteristics, while the 280–337 region mediates the interactions with DNA sequence-specific binding proteins that bring EBNA-2 to its responsive elements. The function of the 95–110 region is unclear (Kieff, 1996). Detailed analysis of the 425–462 region indicates that this domain is similar in many respects to the prototype VP16 acidic domain (Cohen & Kieff, 1991; Cohen, 1992), part of which can substitute for part of the EBNA-2 domain in the context of reconstituted EBV recombinants (Cohen, 1992). The two domains share affinity for the transcription factors TFIIB, TAF40, TFIIH and RPA70, suggesting a critical role of the 425–462 region in recruiting these factors to EBNA-2-responsive promoters (Tong *et al.*, 1995).

EBNA-2 does not interact directly with its responsive elements. The response elements in the LMP-1, LMP-2 and Cp promoters have been intensively investigated by electrophoretic mobility shift assays with nuclear extracts from EBNA-2-positive cells. Each responsive element contains at least two gel shift activities: the major activity corresponds to an oligonucleotide that includes the GTGGGAA motif (Zimber-Strobl *et al.*, 1991; Jin & Speck, 1992; Ling *et al.*, 1993a). A 28-amino acid polypeptide corresponding to residues 310–336 of EBNA-2 was used to affinity purify a 63-kDa nuclear protein that reproduced the gel shift pattern (Yalamanchili *et al.*, 1994). Sequencing of p63 showed it to be the previously characterized recombination signal sequence binding protein Jκ, a widely expressed and highly conserved protein that probably acts as a key adapter for transcription regulatory factors of cellular genes (Grossman *et al.*, 1994; Henkel *et al.*, 1994). Additional protein binding sites are present in the EBNA-2-responsive elements. Mutation of the PU.1 binding site has a profound effect on the EBNA-2 responsiveness of the LMP-1 promoter (Johannsen *et al.*, 1995; Sjöblom *et al.*,

1995a,b). PU.1 is also likely to be an important factor for many of the cellular genes that are activated by EBNA-2, as it is frequently involved in B lymphocyte-specific gene transcription.

(c)   EBNA-3A, -3B, -3C

EBNA-3A, -3B and -3C are encoded by three genes placed tandemly in the EBV genome. Each protein is encoded by a short and a long exon 3' to the respective mRNA (Hennessy *et al.*, 1985, 1986; Kallin *et al.*, 1986; Joab *et al.*, 1987; Petti & Kieff, 1988; Petti *et al.*, 1988; Ricksten *et al.*, 1988). The mRNAs that encode these proteins are the least abundant EBNA mRNAs, few molecules occurring in each latently infected cell. The proteins encoded by type-1 and type-2 EBV strains are only 84, 80 and 72% identical in the predicted primary amino acid sequence (Sample *et al.*, 1990). Each protein contains different repeating polypeptide domains near their carboxy termini that are responsible for size variation in different viral isolates. These EBNAs are remarkably hydrophobic and contain repeats of hydrophobic leucines, isoleucines or valines that could facilitate homo- and heterodimerization. They are located in large nuclear clumps in the nuclear matrix, chromatin and nucleoplasmic fractions but not in the nucleolus (Petti *et al.*, 1990).

Because of their structure and sequence similarity, these proteins are likely to have similar functions in latent EBV infection and transformation. EBNA-3C was shown to up-regulate the expression of CD21 mRNA in transfected Burkitt's lymphoma cells (Wang *et al.*, 1990a) and the expression of LMP-1 in Raji cells (Allday *et al.*, 1993; Allday & Farrell, 1994), while expression of EBNA-3B was shown to correlate with up-regulation of vimentin and CD40 and down-regulation of CD77 (Silins & Sculley, 1994). EBNA-3A, -3B and -3C can inhibit EBNA-2-activated transcription, probably by their capacity to bind to Jκ (Robertson *et al.*, 1995a; Krauer *et al.*, 1996; Robertson *et al.*, 1996). The complexes do not bind to DNA *in vivo*, suggesting a possible mechanism by which these proteins could modulate B-cell activation.

(d)   EBNA-1

EBNA-1 is the only EBNA that is associated with chromosomes during mitosis (Reedman *et al.*, 1974; Ohno *et al.*, 1977; Grogan *et al.*, 1983). The predicted sequence of the prototype EBV type 1 strain consists of 641 amino acids that form four obvious domains: an amino terminus of 89 amino acids rich in basic residues, a 239-amino acid Gly-Ala copolymer that could form β sheets and may participate in intermolecular interactions, a short basic domain and a long hydrophobic domain from residues 459–607 which have sequence-specific DNA binding and dimerization activities (Ambinder *et al.*, 1991; Shah *et al.*, 1992; Kieff, 1996). *EBNA-1* is phosphorylated on serine residues in the carboxy terminus of the molecule (Hearing & Levine, 1985; Polvino-Bodnar *et al.*, 1988).

EBNA-1 binds to DNA by recognizing the partial palindrome TAGGATAGCATA-TGCTACCCAGATCCAG that is found at three sites in the EBV genome (Rawlins *et al.*, 1985; Ambinder *et al.*, 1990; Kieff, 1996). The site with the greatest affinity consists of 20 tandem direct repeats of the cognate sequence about 7 kb from the left end

of the genome. The second highest affinity site is 1 kb to the right and consists of two cognate sequences in dyad symmetry and two in tandem. The third site is in the BamHI Q fragment about 10 kb downstream of the EBNA-2 coding exon (Rawlins et al., 1985). This site is composed of two divergent tandem repeats and appears to be important for negative regulation of the alternative promoter for latent EBNA-1 transcription in BamHI FQ (Lear et al., 1992; Sample et al., 1992). Binding of EBNA-1 to the tandem repeat and dyad symmetry sites enables covalently closed, circular DNA molecules to replicate and persist as episomes (Reisman et al., 1985; Yates et al., 1985; Yates & Guan, 1991). The EBV DNA segment containing both sites is therefore designated oriP, for origin of plasmid DNA replication. The tandem repeat component acts as an EBNA-1-dependent enhancer on heterologous or neighbouring EBV promoters in transient transfection assays (Reisman & Sugden, 1986). The dyad symmetry component is stringently required for episome replication. The structure is partially denatured as a consequence of EBNA-1 binding, forming bubble and cruciform structures that are characteristic of other efficient origins (Williams & Kowalski, 1993). The interaction of EBNA-1 with the tandem repeat and dyad symmetry sites is cooperative and results in higher-order structures that lead to bending of the DNA, distortion of the duplex and looping out of the intervening sequences (Frappier & O'Donnell, 1991; Orlowski & Miller, 1991; Frappier & O'Donnell, 1992). The amino terminal of the dimerization domain is important for the formation of macromolecular complexes of EBNA-1 homopolymers after association with the DNA templates (Goldsmith et al., 1993). The carboxy terminus of EBNA-1 determines its nuclear location by interacting with a specific protein that is homogeneously distributed on chromosomes (Ohno et al., 1977; Harris et al., 1985; Petti et al., 1990). This property is likely to be important for segregation of episomes into progeny nuclei during mitosis. Part of EBNA-1 is also associated with the nuclear matrix. EBNA-1 is the only EBNA that continues to be made during lytic infection. The lytic mRNA begins at a BamHI F promoter near 62.2 kb (Lear et al., 1992; Kieff, 1996). The two EBNA-1 binding sites that are not part of ori-P are downstream of the adjacent latency I promoter in BamHI Q (kb 62.3). Thus, EBNA-1 is likely to play a role in regulation of these promoters (Sample et al., 1992; Kieff, 1996).

1.1.5.3 Latent membrane proteins

(a) LMP-1

LMP-1 mRNA is the second most abundant viral transcript in latently infected cells (Fennewald et al., 1984; Sample & Kieff, 1990). The product, encoded by three exons, is an integral membrane protein with at least three domains: a 20-amino acid hydrophilic amino terminus; six hydrophobic, 20-amino acid, alpha helical transmembrane segments separated by five reverse turns, each five to 10 amino acids in length and a 200-amino acid carboxy terminus, rich in acidic residues. The results of studies of protein cleavage in live cells are consistent with a model of plasma membrane insertion in which both the N and C termini are positioned on the cytoplasmic side and there are only three short reverse turns on the extracellular side of the membrane (Liebowitz et al., 1986). At least half of LMP-1 is associated with the cytoskeleton, as defined by resistance to extraction

with nonionic detergents and co-localization with the intermediate filament protein vimentin (Mann *et al.*, 1985; Liebowitz *et al.*, 1986; Moorthy & Thorley-Lawson, 1990, 1993a). Nascent, nonionic detergent-soluble LMP-1 has a half-life of less than 2 h and is converted to an insoluble, closely cytoskeleton-associated form that is phosphorylated on serine and threonine residues in the carboxy-terminal domain (Moorthy & Thorley-Lawson, 1990, 1993a). The cytoskeletal form has a half-life in the order of 3–15 h. After phosphorylation, *LMP-1* is cleaved near the beginning of the carboxy-terminal domain, resulting in a soluble product of about 25 kDa (Moorthy & Thorley-Lawson, 1990, 1993b). LMP-1 forms patches in the cell membrane that are often organized into a single cap-like structure (Hennessy *et al.*, 1984; Liebowitz *et al.*, 1986). Although vimentin co-localizes to the patches, patch formation does not require vimentin or other EBV proteins (Wang *et al.*, 1988a; Liebowitz *et al.*, 1992) and is likely to be due to the capacity of LMP-1 to interact with itself through the transmembrane domains. Like *EBNA-1*, *LMP-1* is transcribed during lytic infection and can be induced by treatment with activators of protein kinase C (Rowe *et al.*, 1987a; Laux *et al.*, 1988a). In late lytic infection, a promoter in the third *LMP-1* exon transcribes the part of the ORF that encodes for the last two transmembrane domains and the cytoplasmic domain (Hudson *et al.*, 1985). This truncated *LMP-1* does not associate with vimentin or other cytoskeletal elements and does not have transforming or cell-activating properties (Mann *et al.*, 1985; Wang *et al.*, 1985, 1988a,b; Liebowitz *et al.*, 1992). The full-size LMP-1 is incorporated into virions (Mann *et al.*, 1985), indicating that the virion-associated products may affect the growth of newly infected cells.

Transfection of *LMP-1* with heterologous promoters has transforming effects in rodent fibroblast cell lines (Wang *et al.*, 1985; Baichwal & Sugden, 1988; Wang *et al.*, 1988a; Moorthy & Thorley-Lawson, 1993a). LMP-1 expression in Rat-1 or NIH 3T3 correlates with altered cell morphology and growth in low concentrations of serum. The cells lose anchorage dependence, acquire growth capacity in soft agar and become tumo-rigenic in nude mice. Expression at levels above those detected in ordinary lympho-blastoid cell lines results in toxicity (Wang *et al.*, 1988a). LMP-1 also dramatically alters the growth of EBV-negative B lymphoblasts and induces many of the changes that are usually associated with EBV infection of primary B cells, including cell clumping, increased numbers of villous projections and vimentin expression, increased surface expression of CD23, CD39, CD40, CD44, class II MHC and the cell adhesion molecules LFA-1, ICAM-1 and LFA-3 and decreased expression of CD10 (Wang *et al.*, 1988b; Birkenbach *et al.*, 1989; Wang *et al.*, 1990a; Liebowitz *et al.*, 1992; Peng & Lundgren, 1992; Zhang *et al.*, 1994a; Kieff, 1996). LMP-1 has been shown to protect B lymphocytes from apoptosis by inducing *bcl-2* (Rowe *et al.*, 1994) and probably also *A20* (Henderson *et al.*, 1991; Martin *et al.*, 1993; Fries *et al.*, 1996). In addition, LMP-1 was shown to up-regulate certain cytokines with B-cell promoting activity, such as IL-10 (Nakagomi *et al.*, 1994). *LMP-1* expression also alters the growth of multipotent haema-topoietic stem cells and epithelial cells (Dawson *et al.*, 1990; Fåhraeus *et al.*, 1990a; Hu *et al.*, 1993). In monolayer cultures of immortalized human keratinocytes, LMP-1 induces morphological changes, alters cytokeratin expression and inhibits cell differen-

tiation (Dawson *et al.*, 1990; Fåhraeus *et al.*, 1990a). Similar effects were observed in the hyperplastic skin of *LMP-1* transgenic mice.

Studies with *LMP-1*-deletion mutants in rodent and human cells and the isolation of EBV recombinants specifically mutated in *LMP-1* have yielded information on the role of different protein domains in cell growth and transformation. Transfection studies with *LMP-1*-deletion mutants indicate that the cytoplasmic amino terminus is not responsible for the activating effects, the transmembrane domains are critical, probably due to their importance for LMP-1 aggregation in the plasma membrane, and the carboxy-terminal domain is essential (Wang *et al.*, 1988a,b; Baichwal & Sugden, 1989; Martin & Sugden, 1991; Liebowitz *et al.*, 1992; Moorthy & Thorley-Lawson, 1993a,b). As expected, EBV recombinants lacking *LMP*-1 were unable to induce growth transformation of primary B cells (Kaye *et al.*, 1993). Growth transformation was also abolished by deletion of progressively large parts of the amino terminal and transmembrane domains, and the same was true for deletion of all of the carboxy-terminal cytoplasmic domain. Primary B lymphocytes infected with EBV recombinants that express the entire amino terminus and transmembrane domain and the first 44 amino acids of the carboxy-terminal domain grow well on diploid fibroblast feeder layers, suggesting that at least two functional domains exist in the carboxy terminus (Kaye *et al.*, 1995). A cellular protein interaction with the first 44 amino acids of the C-terminal domain was identified in a two-hybrid screen. The LMP-1-associated protein has a ring finger and extended coiled-coil structure and is homologous to factors associated with the murine tumour necrosis factor receptor. A second LMP-1-associated protein, EBI6, is the human homologue of the murine factors. The LMP-1-associated protein binds directly not only to the LMP-1 C-terminal domain but also to the p80 tumour necrosis factor receptor, CD40 and lymphotoxin-β receptor cytoplasmic domains (Mosialos *et al.*, 1995) and to lesser extent to the cytoplasmic domains of Fas/apo-1 protein and p60 tumour necrosis factor receptor. Thus, LMP-1 seems to interact with cellular proteins that are mediators of cytoplasmic signalling from the family of tumour necrosis factor receptors and thereby induce constitutive activation of the growth, death and necrosis factor-κB signalling pathways of those receptors (Hammarskjöld & Simurda, 1992). An additional domain in the distal C-terminal end of LMP-1 seems to be involved in necrosis factor-κB activation (Huen *et al.*, 1995).

Although the standard type-1 and type-2 EBV strains are closely similar (Sample *et al.*, 1994), numerous sequence variations were identified in *LMP-1* genes from different EBV isolates. A major (*Xho*)I restriction enzyme polymorphism was detected in type-1 strains from southeast China (Hu *et al.*, 1991a,b) and in type-2 strains from Alaska. In addition, consistent nucleotide variations in the amino terminus of *LMP-1* were identified in strains marked by the *Xho*I polymorphism. Amino acids 343–352 are deleted in the Chinese strains but not in those from Alaska, and numerous other changes were detected in the amino terminus that did not co-segregate with either disease phenotype, EBV type or specific geographic region (Miller *et al.*, 1994a; see also section 1.3.2).

(b)   LMP-2A and -2B

LMP-2A and -2B are encoded by spliced mRNAs transcribed from the circularized EBV genome across the terminal repeats (Laux *et al.*, 1988a). The *LMP-2A* transcription starts 3 kb downstream of the *LMP-1* polyadenylation site, whereas that of *LMP-2B* starts 0.2 kb upstream of the *LMP-1* transcription start site (Laux *et al.*, 1988a; Sample *et al.*, 1989). The *LMP-2B* and *LMP-1* promoters form a bi-directional transcription unit containing a common *EBNA-2* response element, while a separate *EBNA-2* response element regulates *LMP-2A* transcription (Zimber-Strobl *et al.*, 1993). The two proteins have unique first exons but share the remaining exons derived from the terminal repeats. These encode for 12 hydrophobic transmembrane sequences separated by short reverse turns and a 27-amino acid hydrophilic C-terminal domain. LMP-2 co-localizes with LMP-1 in the plasma membrane of latently EBV-infected B lymphocytes (Longnecker & Kieff, 1990; Longnecker *et al.*, 1991).

1.1.5.4   Other latent viral genes

BHRF1 (Austin *et al.*, 1988) and *Bam*HI A (Brooks *et al.*, 1993a) mRNAs have been identified in strictly latently infected cells. *BHRF1* RNA and protein are abundantly expressed early in lytic infection, but the protein is not found in latently infected cells or newly infected primary B lymphocytes (Alfieri *et al.*, 1991). An ORF, *BARF0*, is contained in a highly spliced RNA originally identified from cDNA cloning of naso-pharyngeal carcinoma RNA (Sadler & Raab-Traub, 1995a). Antibodies specific for the in-vitro translated polypeptide can be detected in sera from healthy people and from nasopharyngeal carcinoma patients (Gilligan *et al.*, 1991). The antisera produced to a synthetic peptide were used to identify a doublet of 30–35 kDa in immunoblots of EBV-carrying but not EBV-negative lines and in biopsy samples of nasopharyngeal carci-noma. Cellular fractionation indicates that the protein is associated with the membrane. Although its function in EBV infection is unknown, a recombinant virus carrying a deletion encompassing the *BARF0* coding region can transform lymphocytes *in vitro* (Robertson *et al.*, 1994).

1.1.5.5   Genes of the productive viral cycle

Only a small fraction of latently infected B lymphocytes spontaneously enters the productive cycle; thus, lytic infection is usually induced by chemicals (Luka *et al.*, 1979; Saemundsen *et al.*, 1980; Laux *et al.*, 1988b). Of the latently infected cell lines, marmoset cells tend to be more inducible than adult human lymphoblasts, and neonatal human lymphoblasts are the least inducible (Miller & Coope, 1974). A few lympho-blastoid cell lines can be induced to permit viral replication in approximately 10% of the cells. The Akata cell line, which carries an *LMP-2A*-deleted virus, can be induced by cross-linking of surface immunoglobulin (Ig) to the extent that more than 50% of the cells enter the lytic cycle (Takada, 1984; Takada & Ono, 1989). A second approach to studying viral replication is to induce the lytic cycle by superinfection of Raji cells with defective EBV from the P3HR-1 cell line (Mueller-Lantzsch *et al.*, 1980). Raji is an EBV-positive Burkitt's lymphoma line with an unusually high EBV episome copy

number. The Raji EBV genome has at least two deletions that make it defective for DNA replication and late gene expression; the cell line is, therefore, tightly latent (Polack *et al.*, 1984a). Defective virions from P3HR-1 contain rearranged DNA molecules in which the immediate early *trans*-activators of the lytic cycle are expressed after super-infection (Cho *et al.*, 1984; Miller *et al.*, 1984).

Cells that have become permissive for viral replication undergo cytoplasmic changes that are typical of herpesviruses, including margination of nuclear chromatin, synthesis of viral DNA, assembly of nucleocapsids, envelopment of the virus by budding through the inner nuclear membrane and inhibition of host macromolecular synthesis (Gergely *et al.*, 1971). Expression of the CD21 EBV receptor is down-regulated in 12-*O*-tetra-decanoyl phorbol-13-acetate (TPA)-treated EBV-positive but not EBV-negative cells, probably as a consequence of binding of viral particles (Yamamoto *et al.*, 1982).

(a) Immediate early genes

Three leftward mRNAs are transcribed after P3HR-1 superinfection of Raji or surface Ig cross-linking of Akata cells in the presence of protein synthesis inhibitors. The *BZLF1*, *BRLF1* and *BI'LF4*-encoded proteins are potent *trans*-activators of early EBV gene expression (Takada & Ono, 1989; Marschall *et al.*, 1991; Kieff, 1996). Two key early promoter regulatory elements with left and right duplications that include the origins of lytic viral DNA replication are co-ordinately up-regulated by *BZLF1* and *BRLF1* (Hudewentz *et al.*, 1982; Hummel & Kieff, 1982a; Freese *et al.*, 1983; Nuebling & Mueller-Lantzsch, 1991). The two proteins acted synergistically on the bi-directional BHRF1 and BHLF1 promoter of left duplication in assays for transient transfection (Cox *et al.*, 1990). The *BSMLF1* and *BMRF1* promoters are also coordinately regulated, probably by binding to specific response elements (Holley-Guthrie *et al.*, 1990).

The R protein is a DNA sequence-specific acidic *trans*-activator that has distant homology to *c-myb* (Gruffat & Sergeant, 1994; Kieff, 1996). The Z protein varies slightly in size among EBV strains, but the sequence differences have not been investigated (Packham *et al.*, 1993). The *BZLF1* mRNA is spliced and consists of three exons (Lieberman & Berk, 1990): the first exon encodes amino acids 1–167 which include the *trans*-activating domain (Taylor *et al.*, 1991; Chi & Carey, 1993); the second encodes amino acids 168–202 which include a strong basic domain that has homology to a conserved region of the *c-jun/c-fos* family of transcription modulators (Chang *et al.*, 1990; Lieberman & Berk, 1990) and confers the ability to interact with AP1-related sites in DNA and also targets Z to the nucleus (Mikaélian *et al.*, 1993); the third exon encodes amino acids 203–245 which include a perfect leucine or isoleucine heptad repeat capable of coiled-coil dimer formation (Chang *et al.*, 1990; Kouzarides *et al.*, 1991). This domain is required for interaction of Z with *p53* (Zhang *et al.*, 1994b). Over-expression of wild-type *p53* inhibits the ability of Z to disrupt viral latency. Thus, direct interaction between Z and *p53* may play a role in regulating the switch from latency to lytic infection. Dimerization may facilitate the interaction with templates that have multiple Z responsive elements. The similarity between TPA response elements, AP1 sites and Z recognition sites may partially explain the capacity of TPA to activate the lytic cycle (Borras *et al.*, 1996).

(b) Early genes

The early genes are expressed when the lytic cycle is induced in the presence of inhibitors of DNA synthesis. By this criterion, at least 30 EBV mRNAs are early gene products (Hummel & Kieff, 1982a,b; Baer *et al.*, 1984). Because of the difficulty in studying EBV replicative functions, proteins encoded by early genes have been identified or assigned functions by analysis of their predicted sequences or by conducting functional assays of proteins translated *in vitro* or expressed *in vivo* (Wong & Levine, 1986; Nuebling & Mueller-Lantzsch, 1991). In many cases, functions have been suggested from comparison with the primary amino-acid sequence of the herpesvirus proteins of known function. Two very abundant early proteins have been mapped to specific DNA sequences. The BALF2 protein is homologous to the HSV DNA binding protein ICP8 and is important in DNA replication (Hummel & Kieff, 1982a; Kieff, 1996). The BHRF1 protein, which is expressed in moderate abundance, has extensive collinear homology with bcl-2 (Pearson *et al.*, 1983a; Austin *et al.*, 1988). BHRF1 can protect EBV-negative Burkitt's lymphoma cells from apoptosis (McCarthy *et al.*, 1996); however, EBV recombinants lacking the *BHRF1* ORF are fully able to initiate and maintain cell growth transformation and they can also enter the lytic cycle and produce virus (Lee & Yates, 1992; Marchini *et al.*, 1991).

Several of the early genes are linked to DNA replication. These include the genes for DNA polymerase (*BALF5*), the major DNA binding protein (*BALF2*), ribonucleotide reductase (*BORF2* and *BARF1*), thymidine kinase (*BXLF1*) and alkaline exonuclease (*BGLF5*), which are distributed through the unique long domain of EBV DNA (Kieff, 1996). The ribonucleotide reductase is confined to multiple discrete regions in the cytoplasm of productively infected cells (Pearson *et al.*, 1983b, 1987). Acetone fixation destroys its immunological reactivity, suggesting that it may be a major component of the restricted early antigen complex originally identified in EBV-immune sera from African Burkitt's lymphoma patients. Transfection experiments demonstrate that some of these genes are activated in the process of cell differentiation in the absence of other viral gene products, suggesting a possible role of cellular factors in regulating the productive cycle, at least in certain cell types (Marschall *et al.*, 1991).

(c) Late genes

The late genes with partially known products or which can be identified by homology with other herpesviruses code for structural glycoproteins or proteins that modify the infected cells in order to permit viral envelopment or egress. Among the non-glycoproteins, the major nucleocapsid protein is probably encoded by *cLF1*, *NRF1* encodes the major external non-glycoprotein of the virion, and *BXRF1* is likely to encode a basic core protein (Kieff, 1996). The *BFRF3* ORF encodes a highly basic viral structural capsid protein or tegument protein, VCA p18, which is strongly immunogenic in humans (van Grunsven *et al.*, 1994). The known EBV glycoprotein genes are *BLLF1* (gp350/220), *BALF4* (gp110), *BXLF2* (gp85), *BILF2* (gp55/80), *BDLF3* (gp100–150) and *BZLF2* (gp42) (see Table 1) (Hummel *et al.*, 1984; Beisel *et al.*, 1985; Gong *et al.*, 1987; Heineman *et al.*, 1988; Oba & Hutt-Fletcher, 1988; Li *et al.*, 1995a; Nolan & Morgan, 1995; Kieff, 1996). Gp110 is one of the most abundant late EBV proteins (Emini *et al.*,

1987; Gong *et al.*, 1987; Gong & Kieff, 1990). Immune light microscopy and electron microscopy have been used to localize gp110 to the inner and outer nuclear membrane and to the cytoplasmic membranes frequently surrounding enveloped virus but not to the Golgi or plasma membrane, suggesting that the protein is not processed in the Golgi apparatus. In contrast, gp85 and gp350/220 are processed efficiently through the Golgi apparatus and are found on the virus and in the plasma membrane of lytically infected cells (Gong & Kieff, 1990; Kieff, 1996). Gp85 is a relatively minor viral component that appears to be important in fusion between the virus and cell membranes (Miller & Hutt-Fletcher, 1988). Viral penetration involves two other EBV glycoproteins, gp25 and gp42, which form a tripartite complex with gp85. Gp42 also binds to HLA DR; thus, MHC class II molecules may serve as cofactors in human B-cell infection (Li *et al.*, 1997). Gp350/220 is the dominant external viral protein involved in mediating viral binding to the B-lymphocyte receptor CD21 and is the major target of the human EBV neutralizing antibody response (Thorley-Lawson & Geilinger, 1980; Thorley-Lawson & Poodry, 1982; Tanner *et al.*, 1988; Zhang *et al.*, 1991). Injection of purified gp350/220 or infection with vaccinia recombinants that express this protein protects cotton-topped tamarins against a lethal, lymphomagenic EBV challenge (Morgan *et al.*, 1988a,b).

The late *BCRF1* gene, which is located in the middle of the EBNA regulatory domain between *ori-P* and the Cp, is a close homologue of the human *IL-10* gene, with nearly 90% collinear identity in amino-acid sequence (Moore *et al.*, 1990; Vieira *et al.*, 1991; Touitou *et al.*, 1996). *BCRF1* has most of the activities of human *IL-10*, including negative regulation of macrophage and NK cell functions and inhibition of IF γ production. Thus, virally expressed *IL-10* may have a local effect on these responses to reactivated infection. Nonsense or deletion mutations involving *BCRF1* have no effect on the ability of EBV recombinants to initiate growth transformation or to maintain latent infection or on the ability of B lymphocytes to enter the lytic cycle *in vitro* (Swaminathan *et al.*, 1993).

1.2 Methods of detection

1.2.1 Assays to detect antibodies to EBV

The measurement of antibodies to EBV in biological fluids remains the major means of diagnosis of EBV infection. As discussed in section 1.2.3, distinct patterns of antibody response have been identified during primary infection, latent infection of immuno-competent carriers and viral reactivation and in various EBV-associated diseases (Evans, 1972).

1.2.1.1 Immunofluorescence

Immunofluorescence assays are, in general, labour-intensive and time-consuming, and experience is required to interpret the resulting patterns. Preparation of the antigen slides is also subject to batch variation. Although some commercial kits are available, their use has not been accepted universally.

Antibodies to viral capsid antigen, early antigen and membrane antigen: Detection of anti-viral capsid antigen (VCA) and anti-early antigen (EA) in serum by immuno-fluorescence was one of the earliest tests developed and is currently the 'gold standard' with which other EBV antibody assays are compared (Henle & Henle, 1967, 1985). Burkitt's lymphoma cell lines (e.g. Raji) superinfected with P3HR1 virus or induced with TPA and/or sodium *n*-butyrate are used as the source of VCA and EA antigens. After reaction with the test serum and its dilutions, the antibody is revealed by fluorescein iso-thiocyanate-conjugated, class-specific anti-human Ig. Detection of specific antibodies of different isotypes is used for the diagnosis and investigation of various EBV-associated diseases such as infectious mononucleosis (IgM), nasopharyngeal carcinoma (IgA) and Burkitt's lymphoma and Hodgkin's disease (IgG). EAs can be divided into restricted (R) and diffuse (D) forms on the basis of their pattern of distribution and sensitivity to methanol (Henle *et al.*, 1971a,b). As discussed in section 1.3.3.1, antibodies against EA(D) and EA(R) may show different prevalence patterns in different EBV-associated diseases.

In order to detect anti-membrane antigen by immunofluorescence, live cells must be used.

EBNA antibodies: Anti-EBNA antibodies are detected by the anti-human complement immunofluorescence assay first developed by Reedman and Klein (1973; Reedman *et al.*, 1974). While this test is generally used for the detection of EBNA complex antibodies, more detailed analysis is now possible using cell lines transfected with individual EBNA genes.

1.2.1.2   *Enzyme-linked immunosorbent assay*

Some of the technical difficulties associated with immunofluorescence tests have been overcome by the development of specific enzyme-linked immunosorbent assays (ELISAs). The specificity of most ELISAs used in EBV serology suffers, however, in comparison with that of immunofluorescence assays. A variety of antibodies to EBV antigens can be detected in ELISA with affinity-purified native or recombinant proteins. Serological reactions to antibody-specific epitopes have been mapped with the use of synthetic peptides. ELISAs have been developed for EBNAs, EA(D), EA(R), VCA, membrane antigen and ribonucleotide reductase (Luka *et al.*, 1984; Halprin *et al.*, 1986; Uen *et al.*, 1988; Weber *et al.*, 1996). Recombinant gp350 is used for the detection of antibodies to membrane antigen in ELISAs.

1.2.1.3   *Immunoblotting*

Lysates of EBV-positive cell lines fractionated by sodium dodecyl sulfate–poly-acrylamide gel electrophoresis are used to detect anti-EBV antibodies in western blot methods. Individual antibody polypeptides can be distinguished by their characteristic size and migration patterns.

1.2.1.4 *Complement fixation*

Soluble complement-fixing antibodies were characterized when tested against soluble, non-sedimentable, nonstructural antigens extracted from non-virus-producing cells such as Raji, and were shown to appear only months after primary EBV infection. The results of this test parallel the presence of EBNA in cases of asymptomatic infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma (Gerber & Birch, 1967; Sohier & de Thé, 1972; Vonka *et al.*, 1972).

1.2.1.5 *Functional assays*

Antibodies to EBV-encoded proteins have been detected by their capacity to neutralize viral function or to mediate lysis of virus-infected target cells. For the detection of EBV DNase neutralizing antibodies, the EBV-encoded enzyme is purified from productively infected cells or expressed as a recombinant protein (Cheng *et al.*, 1980; Stolzenberg *et al.*, 1996). The last serum dilution capable of neutralizing a given amount of the enzyme (e.g. six units) gives the anti-DNase titre. In the antibody-dependent cellular cytotoxicity assay, antibodies to EBV membrane antigen are detected by their capacity to mediate killing of ^{51}Cr-labelled, productively EBV-infected cells by Fc-receptor-positive killer cells (Chan *et al.*, 1979a; Mathew *et al.*, 1981). The significance of the functional activity of these antibodies for the control of EBV infection *in vivo* is uncertain.

1.2.2 *Detection of EBV in tissues*

While serological studies can provide indirect evidence of an association of EBV with human tumours, definite identification of EBV-associated tumours rests ultimately on the detection of viral genomes and/or viral gene products in tumour tissues. Over the years, a number of methods have become available, which can be divided broadly into those based on the extraction of nucleic acids from tissues (Southern blot hybridization and the polymerase chain reaction (PCR)) and those which detect viral genomes or gene products *in situ* in single cells (in-situ hybridization and immunohistology). Some methods are used to detect and characterize viral genomes (Southern blot hybridization, PCR, DNA in-situ hybridization), and others to detect viral gene products (reverse transcriptase (RT)-PCR, RNA in-situ hybridization, immunohistology). The latter require that the viral genome be actively expressed; it is important to realize that this is also the case for EBER in-situ hybridization (see below), which has largely replaced EBV DNA in-situ hybridization for the identification of EBV. Thus, ultimate proof that the virus is absent from a given tissue can come only through the demonstration that the viral genome is absent. The suitability of these methods for the detection of EBV in tissues will be discussed using Hodgkin's disease as an example. Hodgkin's disease is particularly suited to illustrate the advantages and disadvantages of the various techniques because of its unique pathological features (see section 2.3.1), including the scarcity of neoplastic cells, the Hodgkin and Reed-Sternberg (HRS) cells, which rarely amount to more than 2% of the total cell population in an affected lymph node and which are embedded in a

background of abundant reactive cells (Herbst & Niedobitek, 1993). This scarcity made the association between EBV and Hodgkin's disease particularly difficult to establish.

1.2.2.1 *Southern blot hybridization and the polymerase chain reaction*

Prompted by seroepidemiological studies, several groups have attempted to detect viral genomes in extracts of DNA from Hodgkin's disease tissues. Studies with nucleic acid reassociation kinetics failed to do so, probably because of the limited sensitivity of this method (zur Hausen, 1976). With the development of Southern blot hybridization, a more sensitive and specific technique became available and, when probes directed, e.g. against the *Bam*HI W internal repetitive fragment of the viral genome, were used, EBV DNA was indeed detected in approximately 20% of cases of Hodgkin's disease (Weiss *et al.*, 1987; Herbst *et al.*, 1989). Southern blot hybridization involving probes for characterization of the terminal repeat region of the viral genome was used to demonstrate that in most cases the viral genomes are present as monoclonal episomes (Raab-Traub & Flynn, 1986; Weiss *et al.*, 1987; Anagnostopoulos *et al.*, 1989; Masih *et al.*, 1991). This suggested that the virus-carrying cell population is monoclonal and implicated the HRS cells as the likely cellular source. Several groups have subsequently used PCR to detect viral genomes in Hodgkin's disease tissues. These experiments were often designed to amplify sequences from the *Bam*HI W internal repetitive fragment of the viral genome, since this promised maximal sensitivity. Using this approach, viral genomes were detected in 50–80% of biopsy samples from cases of Hodgkin's disease in western countries (Herbst *et al.*, 1990; Knecht *et al.*, 1991; Shibata *et al.*, 1991a).

Interpretation of the results obtained with Southern blot hybridization and PCR has, however, been difficult. The sensitivity of Southern blot hybridization is limited, and target DNA must be present in at least 1% of the total cell population. Furthermore, because DNA extracts from heterogeneous cell populations are analysed, the cellular source of any viral DNA detected cannot be determined unequivocally. Examination of the configuration of the terminal repeat region of the EBV genome by Southern blot hybridization can provide evidence for the clonal composition of the viral genomes. Detection of monoclonal viral genomes in DNA from clonal neoplasms indicates that the tumour cells are the cellular source of the viral DNA and that EBV infection took place before clonal expansion of the neoplastic cell population. The nature and clonal origin of HRS cells are, however, uncertain; the results of a few recent studies suggest the possibility that HRS cells may be polyclonal at least in some cases (Hummel *et al.*, 1995a; Delabie *et al.*, 1996).

Analysis of DNA extracts by PCR faces the same difficulties, which are compounded by the high sensitivity of this method. It is now clear that circulating, EBV-carrying, non-neoplastic lymphocytes are found in a variety of tissues, including Hodgkin's disease lymph nodes (Herbst *et al.*, 1992; Khan *et al.*, 1992; Niedobitek *et al.*, 1992a). Thus, the demonstration of EBV DNA in any tumour by PCR cannot be taken as evidence of viral infection in the tumour-cell population. A number of RT-PCR strategies have been devised for the analysis of EBV gene expression in peripheral blood or tissues (Raab-Traub *et al.*, 1991; Brooks *et al.*, 1992; Busson *et al.*, 1992a; Qu & Rowe, 1992; Tierney

et al., 1994). With the possible exception of the demonstration of EBER expression, these methods are not suitable for diagnostic purposes but rather represent a tool for the characterization of EBV infection under conditions in which the association with EBV is known.

1.2.2.2   In-situ hybridization and immunohistochemistry

The study of the relationship between EBV infection and Hodgkin's disease illustrates the necessity of identifying the cellular location of the virus. The first unequivocal demonstration of EBV within the HRS cells of Hodgkin's disease came from two independent studies in which DNA in-situ hybridization was used with ^{35}S-labelled probes. Again in these studies, the *Bam*HI W internal repetitive fragment of the viral genome was used as a target, because it promises greater sensitivity than other unique genome fragments. In both studies, EBV DNA was detected exclusively in the HRS cells of a few selected cases (Anagnostopoulos *et al.*, 1989; Weiss *et al.*, 1989a). These results were subsequently confirmed, with the use of both radioactive and non-radioactive DNA probes (Herbst *et al.*, 1990; Uccini *et al.*, 1990; Coates *et al.*, 1991a,b; Delsol *et al.*, 1992). The detection rate of DNA in-situ hybridization was always lower than that of PCR, indicating comparatively low sensitivity. Moreover, when biotin-labelled probes are used, the presence of endogenous biotin may give rise to artefactual cytoplasmic labelling (Niedobitek *et al.*, 1989a; Hawkins *et al.*, 1990; Santucci *et al.*, 1993).

A major breakthrough in the analysis of EBV infection by in-situ hybridization came with the introduction of probes specific for the small nuclear EBV-encoded RNAs, *EBER-1* and *-2* (Wu *et al.*, 1990). The EBERs are abundantly expressed in all forms of EBV latency and are therefore uniquely suitable as targets for in-situ hybridization. Moreover, because of their high copy number, EBERs can be detected with non-radioactive RNA and oligonucleotide probes, making this method suitable for many laboratories (Wu *et al.*, 1991). Because of its high sensitivity, this method allows precise determination of the incidence of EBV infection in HRS cells. Thus, EBER in-situ hybridization has revealed that EBV infection in Hodgkin's disease is not restricted to HRS cells but may occur in non-neoplastic, so-called 'bystander' lymphocytes (Herbst *et al.*, 1992; Khan *et al.*, 1992; Jiwa *et al.*, 1993a). This method has been applied in many laboratories to the study of not only Hodgkin's disease but also other malignancies, leading to the identification of a variety of new EBV-associated tumours.

In parallel with these developments, several monoclonal antibodies have become available which are suitable for the detection of EBV-encoded latent and replicative proteins in sections of routinely formalin-fixed, paraffin-embedded tissues (Rowe *et al.*, 1987b; Young *et al.*, 1989b, 1991; Grässer *et al.*, 1994). This technique has been vastly improved by the development of antigen retrieval techniques with microwave irradiation or pressure cooking (Shi *et al.*, 1997), allowing the detection of epitopes in paraffin sections masked by formalin fixation and which have been undetectable hitherto. Thus, monoclonal antibodies specific for EBNA-1, EBNA-2, LMP-1 and LMP-2A can now be used for the immunohistochemical analysis of latent EBV gene expression (Grässer *et al.*, 1994; Delecluse *et al.*, 1995a; Murray *et al.*, 1996; Niedobitek *et al.*, 1997a).

Similarly, monoclonal antibodies and probes that recognize several lytic cycle gene products, e.g. BZLF1 and EA(D), are available (Young *et al.*, 1991; Brousset *et al.*, 1992, 1993; Rowlands *et al.*, 1993; Ryon *et al.*, 1993; Niedobitek *et al.*, 1997a). Unlike transcriptional studies involving PCR or western blot analysis, immunohistochemistry allows the analysis of EBV gene expression in single cells. This is particularly relevant for the study of Hodgkin's disease because of the frequent presence of EBV-carrying, non-neoplastic lymphocytes in such tissues. With the exception of EBNA-1, however, these viral proteins are not invariably expressed in virus-associated tumours. *EBNA-1* is expressed only weakly in many cases of Hodgkin's disease and is therefore often not detectable by immunohistochemistry (Grässer *et al.*, 1994). For these reasons, immuno-histology cannot substitute for in-situ hybridization for the detection of latent EBV infection. Immunohistology for the detection of LMP-1 in Hodgkin's disease represents a possible exception to this rule, since this protein has been shown to be expressed in virtually all cases (Herbst *et al.*, 1991a; Pallesen *et al.*, 1991a).

1.3   Biology of EBV infection

1.3.1   *Target tissues*

1.3.1.1   *Infection* in vitro

A characteristic feature of EBV is its ability to infect human B cells *in vitro* and to convert them into permanently growing lymphoblastoid cell lines. Several studies have shown that the virus not only immortalizes mature B cells but can also infect precursor B cells, including those with immunoglobulin heavy and light chain genes in germ-line configuration (Hansson *et al.*, 1983; Ernberg *et al.*, 1987; Gregory *et al.*, 1987a). More-over, EBV infection of immature B cells induces the expression of 'late' lymphocyte activation antigens (CD30, CD70), as also seen in Ig-expressing lymphoblastoid cell lines (Gregory *et al.*, 1987a). This finding is consistent with the detection of EBV in CD30$^+$ HRS cells with pro-B- and pre-B-cell phenotypes and genotypes (Herbst *et al.*, 1993).

The efforts to generate in-vitro models for EBV-associated non-B-cell neoplasia have been particularly successful for T lymphocytes, in which EBV infection through CD21 or a related molecule has been achieved. Several groups have recently reported the infection of immature thymocytes with EBV. Interestingly, the viral genome in these cells appears to be maintained in linear form, without episome formation. Qp-derived EBNA-1 mRNA expression has been demonstrated in such cells, and there is a high degree of lytic cycle antigen expression (Watry *et al.*, 1991; Kaufman Paterson *et al.*, 1995; Kelleher *et al.*, 1995). This suggests that the virus–cell interaction in these cells is fundamentally different from that in B-cell lines. A more conventional result was reported by Yoshiyama *et al.* (1995), who infected a human T-cell lymphotropic virus (HTLV)-I-carrying T-cell line with EBV. This resulted in a stable latent infection with viral epi-somes and expression of EBNA-1 and LMP-1, reminiscent of the type-II latency seen in many EBV-associated T-cell lymphomas. In contrast to EBV-infected B cells, up-regu-lation of CD23, LFA-1, LFA-3 and ICAM-1 was not observed in these cells; and in

contrast to the results obtained with immature thymocytes, lytic cycle antigens were not inducible.

In view of the frequent association of EBV with carcinomas, efforts have been made to infect epithelial cells with the virus *in vitro*; however, this has proved difficult. Some reports suggest that direct infection of nasopharyngeal and cervical epithelial cells with EBV may be possible (Sixbey *et al.*, 1986; Furukawa *et al.*, 1990; Yoshizaki *et al.*, 1994); however, in most recent studies mechanisms to facilitate this process had to be devised. One such mechanism is transfection of CR2-negative keratinocytes with the gene that encodes the B-cell EBV receptor: thus, Li *et al.* (1992) achieved transient infection of CR2-transfected human keratinocytes. Subsequently, Knox *et al.* (1996) generated stable EBV-infected subclones from such cells. In these cells, EBNA-1 and low levels of LMP-1 were expressed, reminiscent of the situation in nasopharyngeal carcinomas. Moreover, the ability of these cells to undergo terminal squamous differentiation was impaired, suggesting that stable EBV infection of epithelial cells requires an undifferentiated cellular environment.

A different approach was chosen by Sixbey and co-workers, who demonstrated that coating EBV with gp350-specific polymeric IgA could facilitate binding to and infection of a human colon carcinoma cell line which was otherwise refractory to EBV infection. The EBNA complex, EBNA-2, and lytic cycle antigens were expressed in a fraction of these cells (Sixbey & Yao, 1992). The relevance of this model for EBV infection *in vivo* is as yet uncertain.

Infection of a hepatoma cell line with EBV has been reported (Lisi *et al.*, 1995), but this model has yet to be fully characterized. Of uncertain significance also is the reported generation of precursor monocyte cell lines (Revoltella *et al.*, 1989) which are EBV DNA-positive, as demonstrated by Southern blot hybridization and expressed EBNA complex.

Lindhout *et al.* (1994) achieved infection of follicular dendritic cells with EBV, presumably mediated through CR2, generating permanently growing cell lines that express LMP-1 and EBNA-2. A report of EBV infection of mouse fibroblasts after CR2 transfection demonstrates that, at least in certain cell types, the absence of CR2 is the main obstacle for EBV infection (Ahearn *et al.*, 1988).

Yet another mechanism that may be important for EBV infection of CR2-negative cells of various lineages was suggested by the results of Bayliss and Wolf (1980, 1981), who demonstrated that EBV induces cell fusion between virus-carrying B cells and other cell types, including T cells and fibroblasts. The importance of this mechanism is suggested by the reported EBV infection of endothelial cells by co-cultivation with irradiated EBV-carrying B-cell lines (Jones *et al.*, 1995). While the mechanism of entry of the virus into endothelial cells was not elucidated in this study, it suggests that it is due to cell–cell contact. This result, together with the detection of the virus in CD21-negative tumour cells, raises the possibility that cell fusion may facilitate the entry of EBV in the absence of the C3d/EBV receptor (Bayliss & Wolf, 1980; Ahearn *et al.*, 1988).

1.3.1.2   *Infection of non-neoplastic cells* in vivo

As anticipated, it has been shown in several studies that EBV-carrying B cells are readily identifiable in lymphoid tissues during infectious mononucleosis and in persistent infection. When EBER in-situ hybridization is used, numerous EBV-infected cells are found in lymphoid tissues from patients with infectious mononucleosis, mainly in the expanded paracortical areas (Niedobitek *et al.*, 1992a; Hamilton-Dutoit & Pallesen, 1994; Anagnostopoulos *et al.*, 1995; Niedobitek *et al.*, 1997b). These cells are mostly of B-cell phenotype, and a proportion has been shown to undergo plasma-cell differentiation (Robinson *et al.*, 1981; Niedobitek *et al.*, 1992a; Anagnostopoulos *et al.*, 1995; Niedobitek *et al.*, 1997b). Significantly, the expression of lytic-cycle gene products has been reported in plasma cells during infectious mononucleosis (Anagnostopoulos *et al.*, 1995; Niedobitek *et al.*, 1997b). Small numbers of EBV-positive lymphocytes are detectable in lymphoid and other tissues as well as in peripheral blood from chronic virus carriers, again, usually in the paracortex of lymphoreticular tissues; many display the phenotype of small, resting B cells (Niedobitek *et al.*, 1992a; Deamant *et al.*, 1993; Miyashita *et al.*, 1995). The number of EBV-positive cells can increase dramatically under conditions of impaired T-cell immunity, e.g. in individuals infected with the human immunodeficiency virus (HIV). On rare occasions, expansion of EBV-infected cells in germinal-centre reactions has been observed, suggesting that at least some EBV-positive circulating B cells show physiological response to antigenic stimulation (Niedobitek *et al.*, 1992a).

The possibility of infection of other cell types by EBV is more controversial. The ability of EBV to infect T cells is demonstrated by the frequent detection of the virus in T-cell non-Hodgkin lymphomas (Pallesen *et al.*, 1991b). Moreover, EBV infection has been demonstrated in certain atypical non-neoplastic T-cell proliferations, often in association with primary infection. Kikuta *et al.* (1988) demonstrated EBV infection in CD4$^+$ T cells from a boy with chronic active EBV infection and Kawasaki disease. Yoneda *et al.* (1990) reported the detection of EBV in a transient polyclonal T-cell proliferation in a young man. Moreover, several groups have demonstrated the presence of EBV in non-neoplastic T cells and natural killer (NK) cells in patients with virus-associated haemophagocytic syndrome (Kawaguchi *et al.*, 1993; Su *et al.*, 1994; Dolezal *et al.*, 1995). In agreement with these observations, EBV infection of T cells has been reported in patients with infectious mononucleosis as well as in persistent infection (Deamant *et al.*, 1993; Anagnostopoulos *et al.*, 1995, 1996); however, Niedobitek *et al.* (1992a, 1997b) could not confirm this finding, and EBV infection of T cells in healthy individuals appears to be a rare event.

Similarly, the question of the infection of normal epithelial cells with EBV is controversial. There is unequivocal evidence that in oral hairy leukoplakia, an AIDS-associated lesion of the tongue, viral replication occurs in the differentiated upper epithelial cell layers (Greenspan *et al.*, 1985; Niedobitek *et al.*, 1991a; Thomas *et al.*, 1991a; Young *et al.*, 1991; see also section 1.2.2); however, several authors have indicated that this is not accompanied by latent EBV infection in the basal epithelial cells of the lesion, suggesting that hairy leukoplakia represents a focus of isolated EBV replication without

a detectable latent phase (Niedobitek *et al.*, 1991a; Thomas *et al.*, 1991a; Sandvej *et al.*, 1992; Murray *et al.*, 1996). Lemon *et al.* (1977) and Sixbey *et al.* (1984) reported the detection by in-situ hybridization of EBV DNA in oropharyngeal epithelial cells from patients with infectious mononucleosis and in one chronic virus carrier. More recent studies of tonsils from patients with infectious mononucleosis, however, have shown no evidence of EBV infection in the tonsillar epithelium (Niedobitek *et al.*, 1989b; Weiss & Movahed, 1989; Niedobitek *et al.*, 1997b), and Karajannis *et al.* (1997) demonstrated the presence of isolated EBV-positive B cells in throat washings from infectious mono-nucleosis patients in the absence of detectable virus in desquamated epithelial cells. Moreover, there is no evidence of EBV infection in epithelial cells of normal naso-pharyngeal mucosa, in tissues from either healthy individuals or from the vicinity of EBV-positive nasopharyngeal carcinomas (Niedobitek *et al.*, 1991b; Sam *et al.*, 1993; Tao *et al.*, 1995). The recent description of EBV infection in nasopharyngeal carcinoma *in situ* indicates that viral infection of the nasopharyngeal mucosa takes place before the infiltrative growth of the carcinoma commences (Pathmanathan *et al.*, 1995a); however, when exactly in the pathogenic process of nasopharyngeal carcinoma this occurs is as yet unclear, and present evidence would suggest that EBV infection of normal oro- and naso-pharyngeal epithelial cells is at best a rare event. Similarly, EBV infection of normal gastric mucosa has not yet been demonstrated convincingly (Rowlands *et al.*, 1993; Fukayama *et al.*, 1994; Osato & Imai, 1996).

A few studies have suggested that EBV may persist in the epithelial cells of the parotid and other salivary glands, first on the basis of reassociation kinetics and DNA in-situ hybridization studies of parotid glands from healthy individuals (Wolf *et al.*, 1984). Subsequent studies using EBV DNA in-situ hybridization and immunohistochemistry showed the presence of EBV in ductal epithelial cells of salivary and lachrymal glands from patients with Sjögren's syndrome (Pflugfelder *et al.*, 1993; Wen *et al.*, 1996). Inte-restingly, a difference between the results of EBV DNA and EBER in-situ hybridization was noted in one of these studies. Thus, EBV DNA was localized to lymphocytes and epithelial cells, whereas latent EBV infection was detected by EBER in-situ hybri-dization only in lymphocytes and not in epithelial cells. This result is difficult to explain, particularly in view of the reported absence of antigens associated with lytic EBV infection (Wen *et al.*, 1996). Other studies of salivary glands from patients with Sjögren's disease and of normal tissue adjacent to salivary gland carcinomas by EBER and EBV DNA in-situ hybridization also showed no detectable EBV infection in salivary gland epithelial cells. This issue therefore remains controversial (Hamilton-Dutoit *et al.*, 1991a; DiGuiseppe *et al.*, 1994; Hamilton-Dutoit & Pallesen, 1994; Leung *et al.*, 1995a). Interestingly, EBV-infected hepatocytes and adrenocortical epithelial cells have been demonstrated occasionally in transplant recipients and in one patient with virus-asso-ciated haemophagocytic syndrome (Randhawa *et al.*, 1992; Kikuta *et al.*, 1993). These reports suggest that the cellular tropism of EBV may be broader in patients with impaired EBV-specific immunity.

Convincing evidence of infection of normal follicular dentritic cells, endothelial cells, smooth muscle cells and other cell types has not yet been reported.

1.3.1.3 *Infection in neoplasms*

The detection of EBV in certain B-cell non-Hodgkin's lymphomas is in keeping with the known ability of the virus to infect and immortalize human B cells *in vitro*. The association of EBV with endemic Burkitt's lymphoma is well documented (Epstein *et al.*, 1964; Lenoir & Bornkamm, 1987; Magrath *et al.*, 1992). Moreover, the virus is present in the majority of B-cell lymphoproliferations that develop in immunosuppressed transplant patients and in a large proportion of AIDS-related B-cell lymphomas (Hamilton-Dutoit *et al.*, 1991b; Craig *et al.*, 1993). The presence and role of EBV in tumour cells is discussed in Section 4. EBV has been detected in T-cell non-Hodgkin's lymphomas of the CD4$^+$, CD8$^+$ and NK cell phenotypes (Harabuchi *et al.*, 1990; Su *et al.*, 1990; Pallesen *et al.*, 1993). The ability of EBV to infect a broad range of target cells within the lymphoid system has also been demonstrated by the frequent detection of the virus in HRS (Herbst *et al.*, 1991a, 1993). While the precise nature and clonal origin of HRS cells are uncertain, these cells may express B- or T-cell antigens (Herbst *et al.*, 1993; Haluska *et al.*, 1994).

The ability of EBV to infect cells of non-lymphoid lineages was first illustrated by its detection in undifferentiated nasopharyngeal carcinoma (Klein, 1979). The presence of EBV in squamous-cell nasopharyngeal carcinomas and in gastric and other adenocarcinomas further demonstrates the ability of the virus to infect variously differentiated epithelial cells (Raab-Traub *et al.*, 1987; Shibata & Weiss, 1992; Pathmanathan *et al.*, 1995b; Osato & Imai, 1996).

The virus has also been detected in tumours derived from smooth-muscle cells, i.e. in leiomyomas and leiomyosarcomas, in immunocompromised patients (Prévot *et al.*, 1994; Lee *et al.*, 1995b; McClain *et al.*, 1995). Intriguingly, in some of these cases, EBNA-2 appears to be expressed in the absence of detectable LMP-1 expression (Lee *et al.*, 1995b). Moreover, the presence of clonal EBV genomes has been reported in so-called inflammatory pseudotumours. In some of these cases, the virus-carrying cells were of smooth-muscle phenotype, while in other cases the immunophenotype was consistent with derivation from follicular dendritic cells (Arber *et al.*, 1995; Selves *et al.*, 1996a). In one case with a follicular dendritic cell phenotype, LMP-1 expression was detected by immunostaining (Selves *et al.*, 1996a).

The expanding list of virus-associated tumours serves to illustrate that the target cell tropism of EBV *in vivo* is much broader than was originally anticipated. The conditions and mechanisms that allow EBV infection of these diverse cell types are, however, in most cases unknown.

1.3.2 *Persistence and latency*

Figure 2 illustrates the cellular events thought to be involved in EBV infection and persistence. Primary infection, occurring via transmission of cell-free virus and/or of productively infected cells in saliva, is associated with entry of the virus into the circulating B-cell pool. The detection of productively infected epithelial cells in the throat washings of patients in the acute phase of infectious mononucleosis has been reported (Sixbey *et al.*, 1984), and EBV can also replicate in the stratified squamous epi-

thelium lining the oral cavity, as clearly demonstrated in hairy leukoplakia lesions in immunosuppressed individuals (Greenspan *et al.*, 1985; Niedobitek *et al.*, 1991a; Lau *et al.*, 1993). It is not clear, however, whether epithelial cells are the primary targets of orally transmitted virus or whether the virus must be amplified in locally infiltrating B lymphocytes, e.g. in the tonsillary crypts. Uncertainty about the role of epithelial cells is further increased by the failure to demonstrate a putative EBV receptor on these cells. In a recent study of throat washings from infectious mononucleosis patients, EBV was not detected in desquamated epithelial cells (Karajannis *et al.*, 1997). A 200-kDa glyco-protein expressed on differentiating squamous epithelium has only some antigenic simi-larity to the CD21 molecule on B cells, and its presence does not correlate with EBV-binding activity (Young *et al.*, 1986; Birkenbach *et al.*, 1992). Very low levels of CD21 have been detected in some epithelial tumour lines *in vitro* (Birkenbach *et al.*, 1992) and in transplantable nasopharyngeal carcinomas, suggesting that low levels of the receptor could be expressed *in vivo*. Other routes of viral entry, such as fusion with EBV-infected, infiltrating B lymphocytes (Bayliss & Wolf, 1981) or transport as an immune complex (Sixbey & Yao, 1992), may be more relevant to epithelial-cell infection.

Figure 2. Model of EBV–host interaction during primary infection and persistence
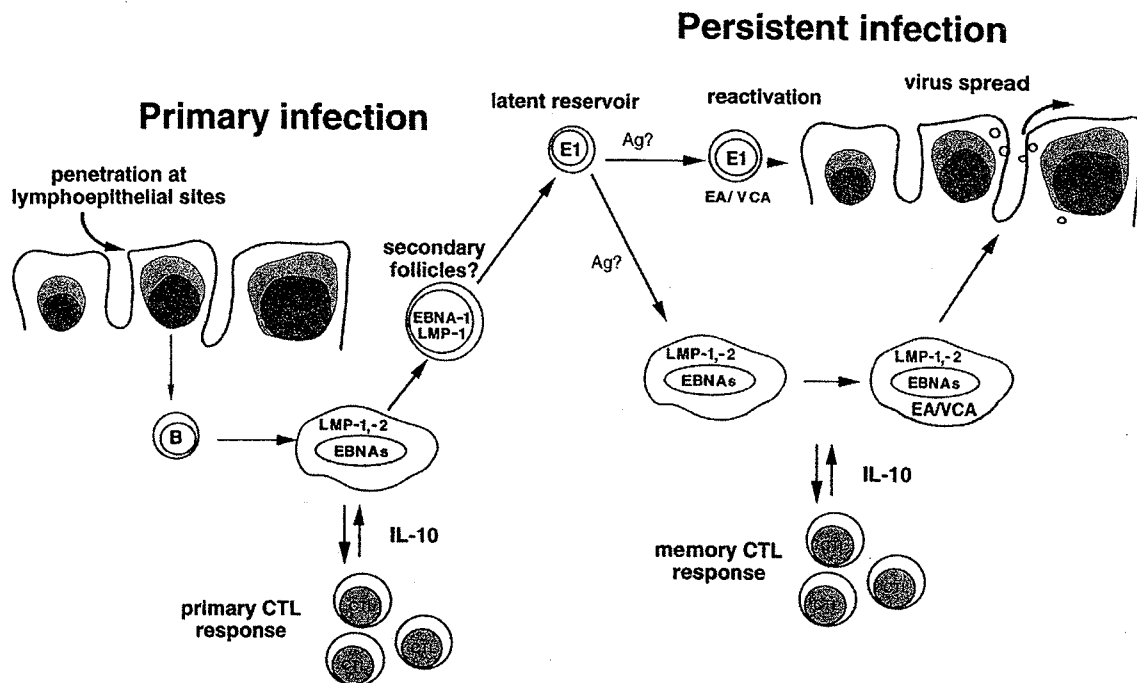
Ag, antigen; EA, early antigen; VCA, viral capsid antigen; EBNA, EBV nuclear antigen; E1, EBV nuclear antigen 1; LMP, latent membrane protein; IL, interleukin; CTL, cytotoxic T lymphocyte

The initial events of B-cell infection *in vivo* appear to be similar to those seen on experimental infection of resting B cells *in vitro*, i.e. transcription from Wp, Cp and LMP promoters and expression of the full spectrum of latent proteins (Falk *et al.*, 1990; Tierney *et al.*, 1994). This pattern of latent viral gene expression has two important effects, both serving to increase the chances that viral persistence will be established in the lymphoid system. First, it can drive rapid polyclonal expansion of the infected B-cell

pool in the very early phases of the infection, before an effective cellular immune response has developed. Second, LMP-1 can up-regulate at least two cellular proteins, bcl-2 and A20, that are capable of extending cell survival *in vivo*. Virus-induced entry of infected cells into the long-lived B-cell pool could be important for viral persistence, since the majority of B lymphocytes are short-lived, and only a very small fraction of the newly generated cells is selected in the long-lived compartment by physiological means.

The early phase of virus-induced B-cell proliferation is curtailed by the developing T-cell response, and large numbers of infected cells appear to be eliminated by virus-specific cytotoxic T lymphocytes (CTLs) at this stage (Klein *et al.*, 1981; Svedmyr *et al.*, 1984; Callan *et al.*, 1996; Steven *et al.*, 1996), which correlates with the decrease in virus shedding in the oropharynx. Despite the strength of this primary immune response, viral latency within the B-cell pool and viral replication in the oropharynx are never completely eliminated. The B-cell compartment appears to be the true reservoir of latent virus, since pharmacological elimination of the autologous lymphoid system in patients receiving allogeneic bone-marrow transplants may result in eradication of the virus (Gratama *et al.*, 1988). Furthermore, treatment with the nucleoside analogue, acyclovir, abolished virus production in the oropharynx but had no effect on the number of latently infected cells in the blood (Yao *et al.*, 1989a). B Lymphocytes cultured from peripheral blood or lymphoid tissues show spontaneous outgrowth of lymphoblastoid cell lines, due predominantly to reactivation of viral replication and infection of co-resident B cells (Rickinson & Epstein, 1978; Rickinson *et al.*, 1978). In a very few cases, direct outgrowth of B lymphocytes latently infected *in vivo* has been suggested by the establishment of spontaneous lymphoblastoid cell lines in cultures supplemented with inhibitors of viral replication and virus-neutralizing antibodies (Lewin *et al.*, 1987, 1988). When assays for spontaneous outgrowth are performed in the presence of cyclosporin A, to prevent T-cell activation, the minimum number of cells required to be seeded for spontaneous outgrowth varies between 10^4 and 10^6 B cells per culture (von Knebel Doeberitz *et al.*, 1983; Rickinson *et al.*, 1984). Similar figures were recently obtained by semi-quantitative PCR analysis of purified B lymphocytes (Chen *et al.*, 1995a; Miyashita *et al.*, 1995). In healthy virus carriers, the same EBV strain appears to predominate in the blood and in throat washings (Gratama *et al.*, 1990, 1992). In the few cases in which detailed studies have been conducted, it was noted that individuals with large numbers of EBV-infected cells in the blood also tended to shed relatively high levels of virus in the throat (Yao *et al.*, 1985a,b). Altogether, these findings are consistent with the idea that latently infected B cells are necessary and sufficient for persistence and that epithelial infection may depend upon seeding from the lymphoid reservoir .

The persistence of EBV-infected B cells in healthy, immunocompetent virus carriers appears to involve a form of latency that is different from that seen in B cells during primary infection. PCR analysis of viral transcripts in circulating B cells from healthy donors showed expression of *EBNA-1* and *LMP-2A* mRNAs only (Qu & Rowe, 1992; Tierney *et al.*, 1994; Chen *et al.*, 1995a). Such a restricted pattern of latent viral gene expression is consistent with the conclusion that virus-carrying cells have a resting phenotype (Miyashita *et al.*, 1995) and could explain how these cells can persist in the face of efficient CTL surveillance (reviewed by Masucci & Ernberg, 1994). A switch

from the activated to the resting cell pattern of viral gene expression has never been seen to occur spontaneously in lymphoblastoid cell lines *in vitro* but can be induced experimentally by enforcing a change in cellular phenotype in cell hybrids (Contreras-Salazar *et al.*, 1989; Contreras-Brodin *et al.*, 1991; Kerr *et al.*, 1992). A similar switch might occur naturally *in vivo* if some of the infected cells could progress down a normal B-cell differentiation pathway.

Cells that express the full repertoire of growth transformation-associated antigens are likely be generated continually in asymptomatic virus carriers, since memory CTLs that are reactive against most EBNAs are maintained at high levels for life. Furthermore, some B cells must be continually triggered into the lytic cycle in order to maintain oropharyngeal shedding of infectious virus. Both events are likely to occur by reactivation from a latently infected memory B-cell pool. An interesting possibility is that both events may be governed by physiological signals that control normal B-cell behaviour. The switching of EBV-infected B cells to a blast-like phenotype and pattern of viral gene expression may therefore be triggered by conventional antigen-driven activation of the latently infected cells; similarly, physiological stimuli may be delivered to circulating B cells at mucosal surfaces which can trigger initiation of the lytic cycle.

1.3.3 *Immune responses*

1.3.3.1 *Antibody responses*

As outlined in Section 1.2, the seroepidemiology of EBV infection still relies on a set of immunofluorescence assays developed within the first decade of EBV research. These are used to measure antibody responses to the nuclear antigens (EBNA; Reedman & Klein, 1973); EA(D) and EA(R); the VCA, expressed in the cells late during the productive virus cycle (de Schryver *et al.*, 1972) and the membrane antigen, expressed on the surface of lytically infected cells (Klein *et al.*, 1969). Each of these antigens is a composite of several viral products, and detection of individual reactivity requires complex assays. Several partially successful attempts have been made to replace the fluorescence tests with ELISA-based and other assays in which recombinant proteins or synthetic peptides are used as substrates (Sternås *et al.*, 1986; Zhang *et al.*, 1991).

Studies of infectious mononucleosis patients have provided the bulk of the information available on primary antibody responses. By the time of onset of clinical symptoms, most such patients have substantial titres of IgM antibodies to VCA and rising IgG titres to both EA and VCA (Henle & Henle, 1973a; Horwitz *et al.*, 1975; Svedmyr *et al.*, 1984; Horwitz *et al.*, 1985; Marklund *et al.*, 1986; van Grunsven *et al.*, 1994). IgA antibodies to these antigens may also appear at this stage. Anti-VCA IgM disappears over the next few months, whereas anti-VCA IgG titres rise to a peak that may fall slightly and anti-EA IgG becomes either undetectable or stabilizes at a very low level. Neutralizing antibodies are detectable during the acute phase of infectious mononucleosis but only at very low titres; these increase to stable levels thereafter (Horwitz *et al.*, 1975; Lennette *et al.*, 1982). An interesting feature of the serology of infectious mononucleosis is the pattern of anti-EBNA responses. Patients in the acute phase of the disease show an IgG response to EBNA-2 (and probably also to EBNA-3A, -3B and

-3C), whereas an IgG response to EBNA-1 is not usually detectable until convalescence (Henle *et al.*, 1987). IgM antibodies to EBNA-1 are usually detected during the acute phase, but these are thought to be part of an autoantibody response with cross-reactivity to the Gly-Ala repeat domain, which EBNA-1 probably shares with several cellular proteins (Rhodes *et al.*, 1987). Anti-EBNA persists for life, whereas the persistence of other EBV antibodies, including anti-EBNA-2, varies (Henle *et al.*, 1987). The delayed antibody response to EBNA-1 is difficult to understand. The original hypothesis that it is due to delayed destruction of latently infected cells *in vivo* is difficult to reconcile with the detection of good responses to EBNA-2. One possible explanation is that EBNA-1 is somewhat protected from certain pathways of antigen processing. Alternatively, the changes in antibody reactivity between primary and persistent infection could reflect the predominance of different types of latently infected cells. In addition to EBV-specific antibodies, the early phase of infectious mononucleosis is characterized by a general increase in total IgM, IgG and IgA, which is consistent with virus-driven polyclonal activation of the B-cell system. Heterophile antibodies with the capacity to agglutinate sheep and horse erythrocytes are the basis of the Paul-Bunnel-Davidson test for the diagnosis of classic EBV-associated infectious mononucleosis (Fleisher *et al.*, 1979; Halbert *et al.*, 1982).

Healthy virus carriers consistently have antibodies to VCA, neutralizing anti-gp350 antibodies and antibodies to EBNA-1 (Henle & Henle, 1976a; Henle *et al.*, 1987). The titres can differ markedly among individuals and are usually stable over time. Only a proportion of healthy carriers have antibodies to EA or to one or more of the other EBNA proteins. Usually, there is no direct correlation between the titres of anti-VCA or anti-EA antibodies and the levels of virus replication, at least as measured by the titres of transforming virus in throat washings (Yao *et al.*, 1985a,b,c), although some individuals with extremely high titres of anti-VCA or anti-EA and low anti-EBNA-1 antibodies show signs of chronically active EBV infection (Johansson *et al.*, 1971; DuBois *et al.*, 1984; Straus *et al.*, 1985; Henle *et al.*, 1987; Miller *et al.*, 1987). The role of antibodies in maintaining persistent infection is not clear. Anti-gp350 antibodies may sensitize lytically infected cells to lysis by CD16^{+} effectors (Pearson *et al.*, 1978a, 1979), but cannot recognize latently infected cells. Gp350 antibodies can neutralize a broad spectrum of EBV strains (Thorley-Lawson & Geilinger, 1980), however, and this may be one of the mechanisms that helps to prevent superinfection of the immune host with orally transmitted virus.

1.3.3.2 *Cell-mediated responses*

Clinically manifested primary EBV infection is associated with an unusually strong cell-mediated immune response. 'Atypical' mononuclear cells, the large numbers of which are a key feature of infectious mononucleosis, are predominantly T lymphocytes of the CD8^{+} subset, with a small contribution of CD4^{+} cells (Sheldon *et al.*, 1973; Svedmyr *et al.*, 1984). These activated T cells express the CD45RO marker and, when expanded *in vitro*, are programmed to die by apoptosis unless rescued by exogenously added cytokines (Moss *et al.*, 1985). The main stimulus for this response appears to be

latently infected B lymphocytes. In this context, it is possible that viral IL-10 produced at foci of lytic replication in the oropharynx serves to dampen local cellular immune responses. IL-10 also acts as a B-cell growth factor (Rousset *et al.*, 1992). Both these effects could promote spread of the virus from the initial focus of infection into the circulating B-cell pool which later elicits the generalized T-cell response.

Functional characterization of infectious mononucleosis T cells has been the subject of numerous investigations. Early observations demonstrated that freshly isolated infectious mononucleosis cells can kill a wide range of EBV-positive cells of different HLA type while not recognizing EBV-negative targets (Svedmyr & Jondal, 1975; Seeley *et al.*, 1981). This apparent lack of MHC restriction has now been resolved by the demonstration that, although CD8$^+$ CTLs with a classical pattern of MHC class I-restricted virus-specific reactivity are activated during primary infection (Strang & Rickinson, 1987a,b), these cells often exhibit cross-reactive recognition of certain alloantigens (Schendel *et al.*, 1992; Burrows *et al.*, 1994). This appears unlikely to be a complete explanation, however, and other CD8$^+$ T-cell clones coincidentally expanded *in vivo* alongside the EBV-specific response may well contribute to the alloreactivity (Klein *et al.*, 1981). The activated CD4$^+$ population may also contain an EBV-specific component (Misko *et al.*, 1991). The magnitude of the response in acute infectious mononucleosis has led to the speculation that EBV may encode, or induce the expression of, a superantigen with the capacity to activate certain T-cell subsets in a polyclonal manner. This possibility has recently received some experimental support (Sutkowski *et al.*, 1996a,b). It has become clear that an important component of the primary EBV-specific response in infectious mononucleosis is indeed the expansion of specific CTL clones that show the same pattern of EBV antigen recognition and epitope choice as the memory response (Steven *et al.*, 1996).

A range of cell-mediated immune mechanisms is probably involved in the control of persistent EBV infection. Early work on experimentally infected cultures allowed identification of a number of activities that can delay the initial B-cell proliferation. Many of these are independent of immune status and have been ascribed to CD16$^+$ NK cells (Masucci *et al.*, 1983), to IF α release (Thorley-Lawson, 1981) or to IF γ release by CD4$^+$ T cells (Farrell *et al.*, 1997); however, these reactivities can only rarely prevent the transformation process. Complete regression of lymphoblastoid cell growth occurs specifically in cultures from virus-immune donors and is mediated *in vitro* by CTL reactivated from the pool of circulating memory T cells (Moss *et al.*, 1978, 1979; Rickinson *et al.*, 1979). The frequency of CTL precursors, estimated from regression assays, is between 1 in 10^3 and 1 in 10^4 circulating T cells (Rickinson *et al.*, 1981a). A prospective study of infectious mononucleosis patients showed that CTL memory increases during convalescence and soon reaches a stable steady state (Rickinson *et al.*, 1980a).

Techniques for expansion of reactivated CTLs, as either bulk T-cell lines or clones in IL-2-conditioned medium, have greatly facilitated analysis of their function (Wallace *et al.*, 1981, 1982a,b,c). Most of the CTL precursors are CD8$^+$ cells and recognize autologous and MHC class I-matched lymphoblastoid cells but not autologous mitogen-activated B blasts, while a much smaller fraction of the EBV-specific CTLs are CD4$^+$- and MHC class II-restricted (Rickinson *et al.*, 1980b, 1981b; Misko *et al.*, 1984). The

importance of this T-cell subpopulation in the control of latent EBV infection is supported by the finding that CD4$^+$ CTLs are associated with protective immunity in the cotton-topped tamarin model (Wilson *et al.*, 1996a). CD4$^+$ cells could also express their function by triggering Fas/apo-1 protein/Fas–ligand interaction and inducing apoptosis (Durandy *et al.*, 1997). The CD8$^+$ effectors recognize small peptide fragments of endogenously synthesized viral proteins presented on the cell surface in the peptide binding groove of HLA class I molecules. This implies that all EBV antigens constitutively expressed on lymphoblastoid cells are potential sources of immunogenic peptides. Further, since antigen presentation selects for peptides with high affinity for a particular binding groove, the identity of the dominant peptide epitopes, and hence the proteins from which they are derived, differs between individuals, depending on their HLA class I type.

Studies on a large panel of virus-immune donors, encompassing a wide range of HLA types, have shown that reactivity against epitopes from EBNA-3A, -3B and -3C forms a substantial part of the EBV-specific CTL response in a high proportion of individuals (Gavioli *et al.*, 1992; Khanna *et al.*, 1992; Murray *et al.*, 1992a; Gavioli *et al.*, 1993). To some extent, this reflects the fact that class I alleles that are common in Caucasian populations, such as A3, B7, B8 and B44, all tend to select epitopes from these proteins. In addition, certain alleles such as A11 and B44, when present, tend to be the dominant restricting determinant for EBV-specific responses and focus the reactivity to epitopes in EBNA-3B and EBNA-3C, respectively. CTL responses to EBNA-2, EBNA-LP, LMP-1 and LMP-2 have also been identified in some individuals, while class I-restricted CTLs that recognize EBNA-1-expressing cells have not been demonstrated to date. The failure to trigger EBNA-1-specific class I-restricted CTLs seems to be due to a *cis*-acting inhibitory effect of the internal Gly-Ala repeat on MHC class I-restricted presentation (Levitskaya *et al.*, 1995). While this may not necessarily prevent the triggering of CTL responses, EBNA-1-specific MHC class II-restricted CTLs were in fact demonstrated (Khanna *et al.*, 1995). The inability of the immune system to recognize EBNA-1 expressed in latently infected targets would be a major advantage to the virus in its strategy for persistence in immunocompetent hosts.

An interesting feature of the EBV system is the extent to which an individual HLA allele focuses the CTL response, not only against a single viral protein but against a single immunodominant peptide epitope within that protein. Well-documented examples of immunodominant EBV peptides and their restricting class I alleles are listed in Table 4. Rare components of the memory response can be selectively activated *in vitro* by stimulation with autologous cells that express individual EBV antigens carried by recombinant adenovirus vectors (Morgan *et al.*, 1996). The precise constraint on both peptide–HLA interaction and recognition of the complex by the T-cell receptor imply that quite small changes in peptide sequence may abrogate CTL recognition. Some CTL responses are type-specific, in that they recognize proteins encoded by type-1 but not type-2 EBV strains, while other responses recognize epitopes that are shared between EBV types (Burrows *et al.*, 1990a,b; Brooks *et al.*, 1993b; Lee *et al.*, 1993a; Hill *et al.*, 1995; Lee *et al.*, 1995a). Sequence polymorphism affecting CTL epitope regions can also occur between strains of the same type. For example, the major A11-restricted epitope in

EBNA-3B residues 416–424 is conserved in most EBV type-1 strains worldwide but is specifically mutated in all the type-1 strains from Southeast Asian populations, in whom the A11 allele itself is unusually prevalent (de Campos-Lima et al., 1993a; Burrows et al., 1996b). The mutated peptide sequences either do not bind to HLA A11 or, in some cases, do bind but are no longer immunogenic for T-cell responses (de Campos-Lima et al., 1994). This raises the possibility that viral strains with mutations in an otherwise immunodominant epitope may have enjoyed selective advantage in a population in which the presenting allele is expressed in a high proportion of the individuals.

Table 4. Cytotoxic T lymphocyte target epitopes in latent EBV antigens

Antigen	EBV type	Amino acid residues	Cognate peptide	HLA class I restriction
EBNA-2	1	67–76	DTPLIPLTIF	B51/A2
EBNA-3A	1	158–166	QAKWRLQTL	B8
	1	325–333	FLRGRAYGL	B8
	1 and 2	596–604	SVRDRLARL	A2
	1	609–617	RLRAEAQVK	A3
	1	379–387	RPPIFIRRL	B7
	1	458–466	YPLHEQHGM	B35
EBNA-3B	1	101–115	NPTQAPVIQLVHAVY[a]	A11
	1	399–408	AVFDRKSDAK	A11
	1	416–424	IVTDFSVIK	A11
	1	481–495	LPGPQVTAVLLHEES[a]	A11
	1	551–564	DEPASTEPVHDQLL[a]	A11
	1	243–253	RRARSLSAERY	B27
EBNA-3C	1 and 2	881–889	QPRAPIRPI	B7
	1	258–266	RRIYDLIEL	B27
	1 and 2	290–299	EENLLDFVRF	B44
	1	335–343	KEHVIQNAF	B44
LMP-2A/2B	1 and 2	426–434	CLGGLLTMV	A0201
	1 and 2	329–337	LLWTLVVLL	A0201
	1 and 2	453–461	LTAGFLIFL	A0206
	1 and 2	340–349	SSCSSCPLSK	A1101
	1 and 2	419–427	TYGPVFMCL	A2404
	1 and 2	200–208	IEDPPFNSL	B40011
	1 and 2	236–244	RRRWRRLTV	B27

Adapted from de Campos-Lima et al. (1996)
EBNA, EBV nuclear antigen; LMP, latent membrane protein
[a] The minimal epitopes have not been identified.

Given the unique features of the in-vitro model available for the EBV system, it is not surprising that much more is known about cell-mediated immune responses to latent than to lytic infection; however, recent evidence suggests that several of the immediate and early antigens of the EBV lytic cycle can serve as targets for specific CD8^{+} CTLs

(Bogedain *et al.*, 1995; Steven *et al.*, 1997). Cells moving through the lytic cycle may therefore be subject to a number of lytic antigen-specific responses operating either through direct cytotoxicity and/or lymphokine release. Studies with in-vitro models also show that lytically infected cells become more susceptible to lysis by NK cells (Blazar *et al.*, 1980; Patarroyo *et al.*, 1980) and, in the presence of IgG antibodies to gp350, are sensitized to antibody-dependent cellular cytotoxicity effectors (Pearson *et al.*, 1978a, 1979).

1.4 Epidemiology of EBV infection

Extensive seroepidemiology studies have shown that EBV is ubiquitous throughout the world (Evans & Niederman, 1989), including the most remote populations, such as the Aleutian Islanders in Alaska (Tischendorf *et al.*, 1970) and Amazonian tribes, in whom other viral infections, such as measles, are absent (Black *et al.*, 1970).

By the third decade of life, 80–100% of individuals have become carriers of the infection. The age-specific seroprevalence of younger persons generally varies inversely with level of socioeconomic development, family size and hygienic standards. The oral route is the primary route of transmission of the virus; however, transmission by transfusion has been documented. In developing countries, infection is acquired in the first few years of life (Figure 3). Crowding and/or the practice of pre-chewing food for infants may be contributing factors. In the developed world, infection is often delayed to adolescence, when transmission is more likely because of intimate oral exposure (Evans & Niederman, 1989) (Figure 4). About 50% of primary infections during young adulthood result in clinical infectious mononucleosis (see section 1.4.1; Evans *et al.*, 1968; University Health Physicians & PHLS Laboratories, 1971; Hallee *et al.*, 1974; Gratama & Ernberg, 1995). Psychological and behavioural factors may influence the severity of clinical manifestations of EBV infections occurring after childhood (Kasl *et al.*, 1979).

The antibody response in healthy carriers is to a symptomless primary infection, as described in section 1.3.3 and reviewed recently by Evans and Kaslow (1997).

Use of molecular methods to characterize EBV genomes in human tumours led to the identification of several non-random polymorphisms, which are variably distributed between the two major EBV types and among several strains. As a result, there has been some controversy about the relationships between these polymorphisms. The major polymorphic markers identified to date and their geographic distribution are listed in Table 5. Little information is available about the incidence of these polymorphisms in the general population. On the basis of the results of a comparative study, Khanim *et al.* (1996) suggested that detection of EBV gene polymorphisms in human tumours simply reflects their overall prevalence in the respective normal population. Thus, although there is evidence from studies *in vitro* to suggest that some of these polymorphisms may be of functional relevance (see sections 4.3.1.2 and 4.5.1.6), their implication in the pathogenesis of EBV-associated tumours is not firmly established.

Figure 3. EBV antibody prevalence at age 4–6 years in various populations
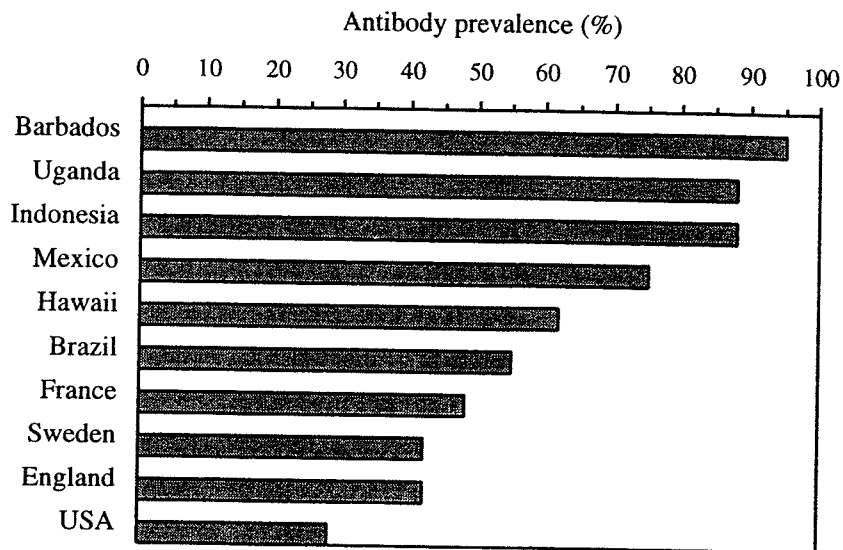
From Evans & Niederman (1989)

Figure 4. Acquisition of EBV antibody by age in three areas
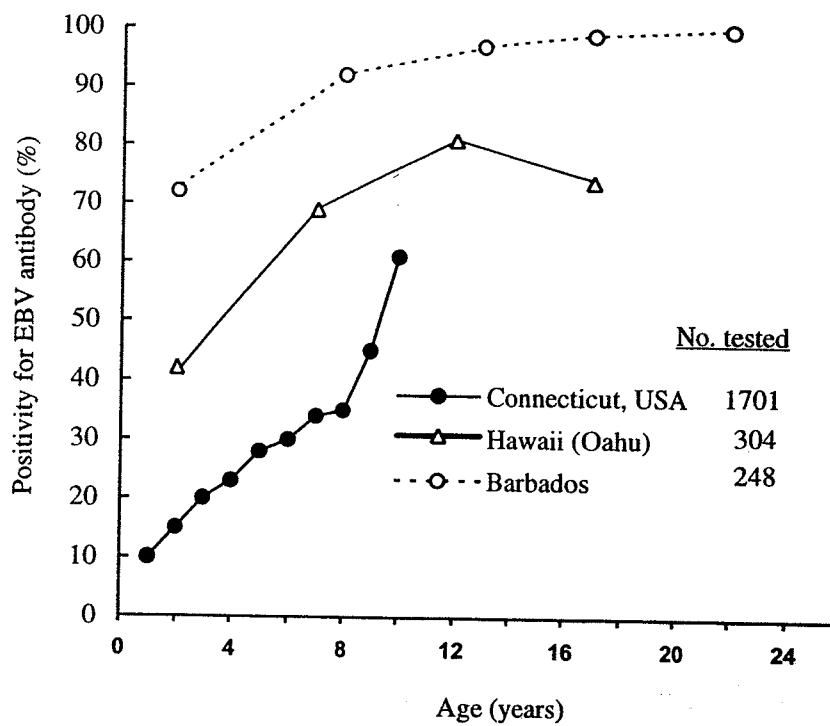
From Evans & Niederman (1989)

Table 5. EBV strain polymorphisms and their geographical distribution

Gene	Polymorphic marker	Goegraphical distribution	Reference
EBNA-2	Type 1 and type 2	Type 2 prevalent in central Africa and Alaska	de Campos-Lima et al. (1993a); Gratama & Ernberg (1995)
EBNA-3A, -3B, -3C	Type 1 and type 2	Type 2 prevalent in central Africa and Alaska	Gratama & Ernberg (1995)
EBNA-3B	Amino acid 424 K to R, T, N	South-east Asia, Papua–New Guinea	de Campos-Lima et al. (1993b, 1994)
EBNA-1	Amino acid 487: A, T, P, L, V	Worldwide (generated in individual virus carriers)	Bhatia et al. (1996); Gutiérrez et al. (1997)
LMP-1	C variant (loss of BamHI site between W1 and I1 regions)	Southern China, Japan	Lung et al. (1990); Hu et al. (1991a); Chen et al. (1992a); Miller et al. (1994a); Gratama & Ernberg (1995)
	f variant (additional BamHI site in BamHI F)	Southern China	
	XhoI polymorphism	Southern China, Alaska	
	30-bp deletion (site loss)	China, Brazil, Africa, Europe (20–100% of the isolates)	
	33-bp repeat variation	Worldwide (generated in individual virus carriers)	
ZEBRA	Akata type (102 416 T to C mutation)	Far East	Packham et al. (1993)
	B95-8 type (102 743 G to A mutation)	United States, Europe, North Africa	
	Jijoye type (102 591 C to T mutation)	Central Africa	

EBNA, EBV nuclear antigen; LMP, latent membrane protein; ZEBRA, Z EBV replication activator; bp, base pair

1.5 Clinical conditions other than malignancy

1.5.1 Infectious mononucleosis

Infectious mononucleosis (glandular fever) is characterized clinically by fever, sore throat, tonsillitis, lymphadenopathy and splenomegaly. Tonsillitis may give rise to ulcers, leading to an erroneous diagnosis of bacterial infection. The diagnosis of infectious mononucleosis relies on the detection of atypical lymphoid cells in the peripheral blood, the occurrence of so-called heterophile antibodies and EBV seroconversion. In most cases, the disease runs its course within a few weeks, but more protracted cases are occasionally observed (Evans, 1972).

As primary EBV infection is usually asymptomatic, most of what is known about it has come from the study of patients with infectious mononucleosis and is based on the implicit assumption that asymptomatic primary EBV infection is milder than but not fundamentally different from infectious mononucleosis. This disease represents a benign lymphoproliferative disorder with prominent expansion of the paracortex of lymphoid tissues by numerous activated B blasts. Morphological, molecular and tissue culture studies have demonstrated the proliferation of EBV-infected polyclonal B blasts, accompanied by the growth of activated T cells (Svedmyr & Jondal, 1975; Tosato *et al.*, 1979; Svedmyr *et al.*, 1984; Brown *et al.*, 1986; Niedobitek *et al.*, 1992a; Anagnostopoulos *et al.*, 1995; Callan *et al.*, 1996; Niedobitek *et al.*, 1997b).

In-situ hybridization for the detection of EBERs has shown numerous latently EBV-infected cells in the paracortical areas of the tonsils of patients with infectious mono-nucleosis (Niedobitek *et al.*, 1992a; Anagnostopoulos *et al.*, 1995; Reynolds *et al.*, 1995; Niedobitek *et al.*, 1997b). Morphologically, these cells are a mixture, ranging from large immunoblasts, including HRS-like cells, to small lymphoid cells. Phenotypic studies in conjunction with in-situ hybridization have shown that the cells are largely B cells which often undergo plasma-cell differentiation (Anagnostopoulos *et al.*, 1995; Niedobitek *et al.*, 1997b), in keeping with the results of Robinson *et al.* (1981), who showed that during acute infectious mononucleosis up to 80% of EBNA-positive cells in peripheral blood may express cytoplasmic immunoglobulins. The detection of EBV infection in cells with evidence of plasmacytic differentiation could be due to infection of cells already committed to plasma-cell differentiation; alternatively, and more probably, this process may be secondary to EBV infection, as indicated by the demonstration that EBV infection can induce polyclonal immunoglobulin synthesis in B cells *in vitro* (Rosén *et al.*, 1977; Kirchner *et al.*, 1979). It has been suggested that plasma-cell differentiation of EBV-infected cells may in part explain the occurrence of heterophile antibodies in infectious mononucleosis patients (Robinson *et al.*, 1981).

In addition to latently EBV-infected cells, a small number of cells expressing lytic cycle antigens, such as BZLF1 and EA, are detectable in tissues from patients with infectious mononucleosis. These tend to be small lymphoid cells that are often found adjacent to crypt epithelium. Phenotypic characterization by double-labelling immunohisto-chemistry has shown that BZLF1-positive cells with features of plasmacellular differentiation are frequent (Niedobitek *et al.*, 1997b), confirming the results of a study showing expression of VCA in B cells with plasmacytoid differentiation *in vitro* (Crawford & Ando, 1986). Although the expression of structural viral antigens has not been analysed, it would appear that plasma cells may sustain full replication of EBV and may represent a cellular source of infectious virus in the saliva of patients with infectious mono-nucleosis.

Transcriptional analysis suggests that a type-III EBV latency prevails in infectious mononucleosis, with expression of the full set of EBV latent genes, including Cp/Wp-driven *EBNA-1* (Falk *et al.*, 1990; Tierney *et al.*, 1994). A more detailed analysis of EBV gene expression at the level of the single cell, reveals, however, a more heterogeneous picture. Only a subset of cells co-expresses *EBNA-2* and *LMP-1*, characteristic of type-III latency. Most cells appear to be EBER-positive but negative for EBNA-2 and LMP-1,

suggesting a type-I latency, and some large immunoblasts are seen which appear to express LMP-1 in the absence of EBNA-2 (type-II latency). Moreover, there are many small lymphocytes that express EBNA-2 but no detectable LMP-1 (Niedobitek *et al.*, 1997b). It is uncertain if this represents a new type of EBV latency or merely a transitory phenomenon. The latter possibility is supported by the observation that *EBNA-2* expression precedes that of *LMP-1* in EBV-induced B-cell immortalization *in vitro* (Alfieri *et al.*, 1991).

While infectious mononucleosis is usually a benign, self-limiting disease, complications may ensue, including rupture of the spleen when there is pronounced spleno-megaly. Neurological complications, interstitial nephritis with renal failure, hepatitis, interstitial pneumonia and anaemia have also been reported (Lloyd-Still *et al.*, 1986; Imoto *et al.*, 1995; Mayer *et al.*, 1996; López-Navidad *et al.*, 1996; Morgenlander, 1996; Schuster *et al.*, 1996; Sriskandan *et al.*, 1996). The underlying mechanisms of these diseases remain uncertain. It has been suggested that such complications of acute infectious mononucleosis are due mainly to tissue infiltration by EBV-carrying B cells and reactive activated T cells or are mediated by autoantibodies (Purtilo & Sakamoto, 1981). Indeed, Sriskandan *et al.* (1996) reported recently the detection of EBV-positive lymphoid cells in the interstitium of the lungs of a patient with interstitial pneumonia after acute infectious mononucleosis. Similarly, EBV-carrying lymphocytes have been demonstrated in the portal tracts and sinusoids of the liver in patients with EBV-asso-ciated hepatitis (Markin *et al.*, 1990; Randhawa *et al.*, 1990; Hubscher *et al.*, 1994; Imoto *et al.*, 1995); however, EBV infection of occasional hepatocytes has also been reported in acute cases (Imoto *et al.*, 1995). In a renal biopsy sample from a patient with renal failure in conjunction with acute infectious mononucleosis, no EBER-positive cells were found. It has been suggested that at least some of the cases with renal impairment can be explained by a concurrent streptococcal infection causing glomerulonephritis (Mayer *et al.*, 1996).

Control of primary EBV infection and transition into a state of asymptomatic viral persistence is mediated by T-cell immunity (Rickinson & Kieff, 1996). A clonal antigen-driven expansion of CD8$^+$ T cells was recently demonstrated in acute infectious mono-nucleosis (Callan *et al.*, 1996). The clonal response has been shown to include virus-specific components against both latent and lytic-cycle viral antigens and to disappear during convalescence (Callan *et al.*, 1996; White *et al.*, 1996). Cases in which T-cell control of primary EBV infection fails and fatal infectious mononucleosis ensues may be due to defined genetic defects such as in X-linked lymphoproliferative syndrome. Patients with this disorder suffer from an immune defect which makes them sensitive to EBV-induced diseases; most affected men die by the age of 40 (Purtilo, 1991). Sporadic cases of fatal infectious mononucleosis without defined defects in T-cell immunity also occur. In the majority of such cases, the host appears to be overrun by excessive, uninhibited proliferation of polyclonal B cells (Falk *et al.*, 1990; Purtilo, 1991), consi-dered to be EBV-driven on the basis of the observation of a type-III latency. In some cases, however, the proliferating B cells appear to be monoclonal, and development of morphologically malignant lymphoma has also been reported (Falk *et al.*, 1990; Purtilo, 1991; Schuster *et al.*, 1996). Thus, patients with X-linked lymphoproliferative syndrome

may develop Burkitt's lymphoma carrying *c-myc* translocations (Purtilo, 1991). Moreover, cases with transition of an infectious mononucleosis-like illness into malignant lymphoma have been reported. These include high-grade B-cell non-Hodgkin's lymphomas, acute lymphoblastic leukaemia and even cases of Hodgkin's disease (Abo *et al.*, 1982; Veltri *et al.*, 1983; Finlay *et al.*, 1986). In some of these cases, detection of EBV in the tumour cell populations was not attempted or was unsuccessful; thus, the relationship with the virus remains tenuous.

These findings suggest a scenario in which patients with impaired T-cell immunity develop massive polyclonal EBV-driven B-cell lymphoproliferation, which may show transition into classical malignant lymphoma. The observation that Burkitt's lymphomas in patients with X-linked lymphoproliferative syndrome harbour *c-myc* translocations (Purtilo, 1991) indicates that the transition from polyclonal lymphoproliferative disorder to frankly malignant lymphoma requires additional genetic changes.

1.5.2 Oral hairy leukoplakia

Oral hairy leukoplakia is an epithelial lesion of the tongue which was originally described in HIV-infected individuals but was subsequently also found in immunosuppressed transplant recipients. Oral hairy leukoplakia manifests itself as a raised white lesion, typically located at the lateral border of the tongue, but which may extend to other parts of the oral mucosa. Histologically, this lesion is characterized by intracellular oedema of the more superficial squamous cells, some nuclear alterations and pronounced hyperparakeratosis. While some of these changes suggested that oral hairy leukoplakia represents a human papillomavirus-induced lesion, this has not been substantiated. In 1985, EBV DNA was detected in this lesion, and the virus has been localized to the superficial epithelial cells. Linear virion DNA and the expression of viral lytic cycle antigens, e.g., BZLF1 and VCA, have been shown, indicating that epithelial cells may support EBV replication (Greenspan *et al.*, 1985; Gilligan *et al.*, 1990a; Young *et al.*, 1991). Walling *et al.* (1992) reported the presence of multiple viral strains in patients with oral hairy leukoplakia and suggested that the intense viral reproduction in this lesion contributes to the generation of EBV variants.

Morphological studies have demonstrated that the expression of lytic cycle antigens such as BZLF1 and VCA is restricted to the more differentiated upper epithelial cell layers (Greenspan *et al.*, 1985; Young *et al.*, 1991). Similarly, the expression of some latent proteins, such as EBNA-1 and LMP-1, has been reported in these cells in the context of lytic EBV infection (Thomas *et al.*, 1991a; Niedobitek *et al.*, 1991a; Sandvej *et al.*, 1992; Murray *et al.*, 1996). Current evidence suggests that EBERs are expressed at low levels, if at all (Gilligan *et al.*, 1990a; Niedobitek *et al.*, 1991a; Lau *et al.*, 1993).

In contrast to the abundance of the virus in upper epithelial cells, viral genomes and EBV gene products associated with latent infection are consistently absent from the basal or parabasal epithelial cells of oral hairy leukoplakia (Niedobitek *et al.*, 1991a; Thomas *et al.*, 1991a; Sandvej *et al.*, 1992). Together with the absence of a detectable episomal population of EBV genomes (Gilligan *et al.*, 1990a), this indicates that oral hairy leukoplakia is an isolated focus of lytic EBV infection, with no detectable latent phase.

Furthermore, oral hairy leukoplakia can be induced to regress by treatment with acyclovir, indicating that this lesion is indeed caused by EBV infection (Resnick et al., 1988).

1.5.3 The X-linked lymphoproliferative syndrome

The X-linked lymphoproliferative syndrome is a hereditary immunodeficiency disorder characterized by a self-destructive immune response to primary EBV infection (Provisor et al., 1975; Purtilo, 1976; Turner et al., 1992). Patients are usually asymptomatic until they encounter EBV, but may present symptoms of immunodeficiency prior to EBV infection. After primary EBV infection, the majority of patients develop a fulminant infectious mononucleosis with a virus-associated haemophagocytic syndrome, which results in liver and bone-marrow destruction, with a fatal outcome. Patients who survive the primary infection are at high risk for developing malignant lymphoma, hypogammaglobulinaemia or aplastic anaemia. Because the immune response to EBV is ineffective, a serological response to the EBV infection may be weak or missing (Seemayer et al., 1995). The XLP gene has been localized to Xq25 (Skare et al., 1987; Sylla et al., 1989), and the region spanning the smallest deletion in patients has been completely cloned (Lamartine et al., 1996; Lanyi et al., 1997) and sequenced; nevertheless, a good candidate gene has still to be identified. Identification of the function of the XLP gene is of prime importance for better understanding the complex interaction between EBV and its host.

Antiviral agents such as IF γ and α and acyclovir are ineffective, and the only curative treatment for X-linked proliferative disorder is allogeneic bone-marrow transplantation (Williams et al., 1993).

1.6 Control and prevention

1.6.1 Drugs

Few drugs prevent replication of EBV, and even fewer are clinically useful; in fact, there are no clinically effective anti-EBV drugs with acceptable side-effects. Most of the drugs that prevent EBV replication were originally tested in studies with herpes simplex virus (HSV). The principal reagent effective against EBV is 9-(2-hydroxyethoxymethyl)-guanine, known as acyclovir when phosphorylated (Colby et al., 1980; King, 1988), which is a substrate for EBV deoxynucleoside kinase and inhibits EBV DNA polymerase (King, 1988). Related compounds that are being evaluated include gancyclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine] (Cheng et al., 1983), famcyclovir, phosphono-formic acid (Foscarnet) and valacyclovir (L-valyl ester of acyclovir); these are being used particularly in the management of viral infections in patients who have received bone-marrow transplants, with varying degrees of success (Dix & Wingard, 1996). Other drugs with activity against EBV include acyclic nucleoside phosphonate analogues (Lin et al., 1991), a thymidine L-nucleoside (Yao et al., 1996), (E)-5-(2-bromovinyl)- and 5-vinyl-1-β-D-arabinofuranosyluracil (Lin et al., 1992), phosphorothioate oligodeoxy-nucleotides without sequence specification (Yao et al., 1993), cucurbitane triterpenoids

(Konoshima *et al.*, 1994, 1995) and triterpenoids from ferns (Konoshima *et al.*, 1996) and quinones (Konoshima *et al.*, 1989).

A distinction should be made between those compounds that directly inhibit viral functions and those that indirectly inhibit EBV replication by interfering with interactions between host-cell components and viral components. For example, retinoic acid down-regulates *BZLF1* expression, thus indirectly reducing lytic cycle induction (Sista *et al.*, 1995), and ciclosporin A (see IARC, 1990) affects lytic cycle EBV gene expression induced by anti-immunoglobulin (Goldfeld *et al.*, 1995; Liu *et al.*, 1997). Retinoids irreversibly inhibit the growth of EBV lymphoblastoid cell lines *in vitro* by unknown mechanisms (Pomponi *et al.*, 1996). Other forms of second-messenger regulation of *BZLF1* expression undoubtedly exist (Daibata *et al.*, 1994). Induction of the EBV lytic cycle by *n*-butyrate and phorbol esters (Ito *et al.*, 1981) may also be blocked by a number of compounds; however, the effect may occur not by direct interference with EBV DNA replication but by blocking of the induction pathway activated by phorbol esters and *n*-butyrate (Daniel *et al.*, 1984; Lin & Smith, 1984). Cucurbitane and fern triterpenoids and quinones fall into this category (Konoshima *et al.*, 1989, 1994, 1995, 1996). The anti-HIV agent zidovudine (AZT) also appears to reduce EBV lytic cycle replication, but again probably by an indirect action on the intracellular environment and not directly on EBV replicative functions (Lin & Machida, 1988; Mar *et al.*, 1995).

Antiviral drugs are ineffective in the treatment of infectious mononucleosis. The clinical symptoms were not reduced by acyclovir in a number of double-blind trials. Viral excretion in throat washings was reduced in acyclovir-treated patients but resumed on cessation of treatment (Andersson & Ernberg, 1988; van der Horst *et al.*, 1991). Because EBV infection is primarily latent and only very few infected cells actually enter the lytic replicative cycle, drugs that interfere with the replication cycle are clearly of limited use in clearing EBV infection. Thus, administration of acyclovir to EBV-seropositive patients, while reducing the shedding of infectious virus in the saliva, has no effect on the level of EBV-infected circulating B lymphocytes (Yao *et al.*, 1989a,b). Treatments with acyclovir in combination with prednisolone had beneficial effects in patients with fulminant mononucleosis (Andersson & Ernberg, 1988) but had no effect on the course of less severe disease in a multicentre, placebo-controlled, double-blind trial, although oropharyngeal shedding of EBV was reduced (Tynell *et al.*, 1996). Oral hairy leukoplakia regresses on treatment with acyclovir (Resnick *et al.*, 1988), but treatment of X-linked proliferative syndrome with acyclovir has been unsuccessful (Sullivan & Woda, 1989).

The search continues for drugs that block EBV replication efficiently but have low toxicity. Their toxicity appears to be due to their incorporation into chromosomal or mitochondrial DNA. Nucleoside analogues continue to be a rich source of potentially useful compounds, the most recent being a thymidine L-nucleoside, 2'-fluoro-5-methyl-β-L-arabinofuranosyluracil, which appears to act by a different mechanism, as it is not incorporated into DNA and is of comparatively low toxicity *in vitro* (Yao *et al.*, 1996).

The EBV latency C promoter is the origin of transcripts for six EBV latent viral proteins; it is active in lymphoblastoid cell lines but silent in many EBV-associated tumours and tumour cell lines. As the C promoter is hypermethylated in these tumour lines, only *EBNA-1* is expressed. 5-Azacytidine inhibits DNA methyltransferase, resulting in demethylation of EBV genomes, activates Cp transcription and induces the expression of *EBNA-2* (Masucci *et al.*, 1989; Robertson *et al.*, 1995b). 5-Azacytidine and similar reagents may therefore render EBV tumour cells susceptible to T-cell control by inducing expression of EBV genes other than *EBNA-1* (Robertson *et al.*, 1995c). Numerous other pharmaceutical agents have been investigated that are cytotoxic to EBV tumour cells. These reagents and the effects of interferons, interleukins and RNA anti-sense oligomers are not considered here. Clearly, much progress will be required before drugs that are effective against EBV replication and/or transcription and are of low toxicity become available in the clinical setting.

1.6.2 Prospects for vaccines

The rational design of an EBV vaccine depends on an understanding of the EBV life cycle and the natural immune responses generated by the virus in humans *in vivo*. How the virus sustains a persistent latent infection in the face of a healthy immune response is not fully understood. Consequently, the development of EBV vaccines has so far been speculative. Since immunosuppressed patients are predisposed to EBV B-cell lymphomas and increased shedding of virus in the saliva (Yao *et al.*, 1985a; Thomas *et al.*, 1991b), immune control must normally exist *in vivo*. Immune responses to EBV include the generation of virus-neutralizing antibodies against envelope glycoproteins (Pearson *et al.*, 1970; de Schryver *et al.*, 1974), recognition of latent antigens by MHC class I-restricted CTLs (Rickinson *et al.*, 1980b), antibody-dependent cellular cytotoxicity against cells carrying surface gp350 (Khyatti *et al.*, 1991) and MHC class II-restricted T-cell responses (Ulaeto *et al.*, 1988; Wallace *et al.*, 1991; Lee *et al.*, 1993b; White *et al.*, 1996). Primary MHC class II-restricted CTLs have been found in seronegative people (Misko *et al.*, 1991). More recently, an EBV superantigen activity was discovered that stimulates Vβ 13 CD4$^+$ T cells from cord blood (Sutkowski *et al.*, 1996a). The various strategies adopted for EBV vaccination have changed as the understanding of the biology of the virus has evolved.

The development of a vaccine to control diseases associated with EBV must be based on the premise that most primary infections occur during the first few years of life and that sooner or later almost the entire population is exposed. The simplest view is that prevention of EBV infection, if attainable, will prevent the diseases associated with EBV, but prophylactic, post-infection and therapeutic EBV vaccination strategies should be distinguished:

- The aim of prophylactic vaccination is to prevent or modify primary infection.
- The aim of post-infection vaccination is to modify the existing immune status of an infected individual.
- Therapeutic vaccination is selectively targeted against viral antigens expressed in tumour cells.

One rationale for developing post-infection vaccines is that the rapid rise in serum IgA antibodies against lytic cycle antigens could serve as a diagnostic indicator of the onset of nasopharyngeal carcinoma and could be used to identify high-risk populations (Zeng, 1985). This marker presumably represents an increase in viral replication at some site that is linked to the emergence of the tumour. Intervention with a gp350 vaccine before this event takes place might well alter the immune balance at a crucial time and prevent development of nasopharyngeal carcinoma. Similarly, high anti-VCA titres have been used as indicators of the development of endemic Burkitt's lymphoma in Ugandan children (de Thé *et al.*, 1978a).

Therapeutic vaccination is intended to enhance the immune response against EBV-associated tumours that have already developed. In this approach, strategies involving the few EBV latent genes that are expressed in the tumours themselves, such as *EBNA-1*, *LMP-1* and *LMP-2*, would be most appropriate (Rickinson, 1995; Ambinder *et al.*, 1996; Moss *et al.*, 1996). The use of EBV latent antigen peptides representing MHC class I-restricted CTL epitopes in EBNA-3A to induce CTL memory by vaccination has recently been reviewed (Moss *et al.*, 1996). No studies on therapeutic or post-infection vaccines have yet been reported.

Current approaches to the development of prophylactic vaccines may also have applications for post-infection and therapeutic vaccines. The latter strategies have obvious target populations; the main purpose of prophylactic EBV vaccination in western countries would be to prevent infectious mononucleosis, which occurs in 50% of persons in whom EBV infection is delayed until adolescence. The incidence of infectious mono-nucleosis in the United States is approximately 65/100 000 (Evans, 1993), which is greater than the incidence of all other reportable diseases except gonorrhoea. Prophy-lactic vaccination to prevent or modify EBV infection and disease in the less developed world would require vaccination of children during the first year of life.

1.6.2.1 Selection of an EBV vaccine antigen molecule

Immunization can prevent tumour induction in non-human primates infected with *Herpesvirus saimiri* (Laufs & Steinke, 1975). Attenuated and killed EBV variants cannot be used as vaccines because of their oncogenic potential. The first prototype EBV vaccine was based on a purified viral envelope glycoprotein, since a correlation had been observed in serum samples between their viral neutralization activity and the reactivity of antibodies against viral membrane antigens on productively infected lymphocytes *in vitro* (Pearson *et al.*, 1970; de Schryver *et al.*, 1974). When these virus-neutralizing antibodies were used to immunoprecipitate viral glycoproteins from lysates of infected and radiolabelled cells, the viral membrane antigens were found to consist of three principal glycoprotein components (North *et al.*, 1980; Qualtière & Pearson, 1980; Thorley-Lawson & Geilinger, 1980). The larger and most abundant of the three are gp350 (gp340) and gp220, the latter being a spliced variant of the former which is encoded by the same ORF, *BLLF1* (Beisel *et al.*, 1985). Gp350 contains up to 50% carbohydrate, much of which is *O*-linked (Morgan *et al.*, 1984; Serafini-Cessi *et al.*, 1989). At least 20 ORFs in the EBV genome could potentially code for glycoproteins as

they have potential sites for N-linked sugars (Baer et al., 1984). With the exceptions of the 110-kDa EBV counterpart of HSV gB coded for by BALF4 (Gong & Kieff, 1990; Gong et al., 1987), the product of BILF2 (Mackett et al., 1990), gp85, which is the HSV gH analogue (Heineman et al., 1988; Miller & Hutt-Fletcher, 1988), and the product of BDLF3 (Nolan & Morgan, 1995), none have been identified or characterized and certainly not evaluated for incorporation into a vaccine. Several EBV glycoprotein products will perhaps have to be incorporated into any vaccine formulation.

A number of interesting properties of gp350 have emerged since the first studies showed it to be a protective immunogen in an animal model of EBV-induced lymphoma (Epstein et al., 1985). Gp350 is the virus ligand which binds to the complement receptor CD21 (Tanner et al., 1987, 1988; Moore et al., 1989; Birkenbach et al., 1992). Cross-linking of CD21 by gp350 induces synthesis of IL-6 (Tanner et al., 1996) and can modulate the synthesis of IL-1 and tumour necrosis factor (Gosselin et al., 1992). CD21 is part of a membrane-signalling complex involved in the activation of B-cell immune responses, and cross-linking by gp350 may be an important early event in driving infected B cells into the cell cycle before immortalization (Sinclair & Farrell, 1995). Indeed, it might be expected that gp350 could affect other cells that express CD21, including follicular dendritic cells, T cells, monocytes and epithelial cells. These possible effects of gp350 could be both advantageous and disadvantageous in vaccination; however, the potential effects of gp350 vaccines in these respects have not been evaluated.

As serious problems have arisen in designing vaccines against HIV and influenza virus because of their capacity to generate antigenic variants, the question has arisen as to whether wild-type variation in the gp350 antigen is significant and would render a conventional vaccine based on a single laboratory strain ineffective. The sequences of gp350 genes from several type-1 and type-2 virus isolates have been compared and no significant differences found. Furthermore, a panel of monoclonal antibodies recognizes the gp350s from all sources equally well (Lees et al., 1993).

As mentioned above, the selection of a viral molecule for use as a subunit vaccine was based originally on the assumption that protective immunity would be provided by virus-neutralizing antibodies directed at glycoproteins on the surface of the virus. This is clearly not the case either in the tamarin model or the common marmoset model (see below) and is unlikely to be the only immunological criterion for protection against EBV-associated diseases in humans.

1.6.2.2 Animal models of EBV infection, disease and vaccination

Two primate model systems have been used in the development of a vaccine against EBV: the cotton-topped tamarin (Saguinus oedipus oedipus) (Miller et al., 1977) and the common marmoset (Callithrix jacchus) (Wedderburn et al., 1984; Emini et al., 1986). Both have shortcomings which must be taken into account in interpreting results. Early studies showed that inoculation of EBV into the common marmoset gives rise to a poorly defined, mononucleosis-like syndrome. These observations have been extended to show that infection of the common marmoset with the M81 strain of EBV gives rise to the

long-term maintenance of antibodies to viral antigens. Furthermore, the presence of EBV DNA has now been reliably demonstrated in tissues and oral fluids of infected animals by PCR analysis (Farrell *et al.*, 1997). A crucial finding was that when infected common marmosets were paired in the same cages with uninfected animals, the uninfected animals seroconverted within four to six weeks, indicating that this animal model of EBV infection will be much more useful than previously thought (Cox *et al.*, 1996).

Injection of large doses of EBV into cotton-topped tamarins gives rise to multicentric oligoclonal B-cell lymphomas, which closely resemble those found in immuno-suppressed human patients, all of which express a type-III EBV latency pattern (Cleary *et al.*, 1985; Young *et al.*, 1989b). There appears to be a threshold dose below which lymphomas do not develop. Cotton-topped tamarins have never been shown to be infected by the oral route and do not support persistent latent infection at the same level as humans, although small numbers of EBV-positive B cells have been detected in animals that have been immunized and protected from a lymphomagenic dose of EBV (Niedobitek *et al.*, 1994). This finding indicates that sterilizing immunity is not induced. The model is useful for testing the efficacy of an EBV vaccine, since protection can be achieved against a massive tumorigenic dose of virus injected intraperitoneally. This severe challenge contrasts with the normal mode of infection of humans, who receive a very small quantity of virus orally.

Other animal models have been developed for EBV-related herpesviruses. Malignant lymphomas can be induced in rabbits by an EBV-related herpesvirus from *Macaca arctoides* (Wutzler *et al.*, 1995); and a murine herpesvirus, MHV-68, although a gamma-2 herpesvirus (see Section 5 and Table 2 in the introduction) may be useful in vaccine development since it can cause lymphomas in mice and maintains a persistent infection in B cells, appearing to be a reservoir for infection *in vivo* (Nash *et al.*, 1996). Inocu-lation of mice with severe combined immunodeficiency with human EBV-infected lym-phocytes gives rise to human B-cell lymphomas (Mosier, 1996), but this model has not yet been exploited in the development of a vaccine. [In the absence of a satisfactory animal system, it seems reasonable to progress directly to human trials, after immuno-genicity and toxicity have been evaluated in animals, because of the difficulty of relating data obtained in the available animal models to human EBV infection and disease.]

1.6.2.3 Natural gp350 subunit vaccines

The first demonstration that gp350 is an effective subunit vaccine in the tamarin lymphoma model was obtained using material isolated from large bulk cultures of cells infected with the B95-8 laboratory isolate of EBV and induced to productive infection with sodium butyrate and phorbol esters (Morgan *et al.*, 1983). Only very small quan-tities of protein were isolated, which were purified by sodium dodecyl sulfate–polyacry-lamide gel electrophoresis, eluted from the gel and renatured after removal of sodium dodecyl sulfate. These small quantities of protein were incorporated into artificial lipo-somes made from phosphatidylcholine. Despite the small quantities of protein and the crude adjuvant system, complete protective immunity was induced in tamarins against a tumorigenic dose of EBV (Epstein *et al.*, 1985).

Gp350 prepared by mono-Q anion exchange (David & Morgan, 1988) and incorporated into immunostimulating complexes (iscoms) (Morein *et al.*, 1984; Morgan *et al.*, 1988a; Morein *et al.*, 1995) or into Syntex adjuvant formulation (Allison & Byars, 1986; Morgan *et al.*, 1989) induces protective immunity in tamarins against the standard lymphomagenic dose of virus and results in high titres of virus-neutralizing antibodies. Protective immune responses were obtained with a dose of 5 μg or less of antigen when Syntex adjuvant formulation or iscoms were used.

1.6.2.4 Recombinant gp350 subunit vaccines

In order to overcome the problems of yield, purity and the presence of EBV DNA, efforts have been made to express the *gp350* gene in bacteria, yeast and eukaryotic cells. Parts of the gene have been expressed in bacteria (Beisel *et al.*, 1985; Zhang *et al.*, 1991; Pither *et al.*, 1992a,b), and the complete gene has been expressed in yeast (Schultz *et al.*, 1987); however, glycosylation does not occur in bacteria, and carbohydrates different from those found on mammalian cells are added when the gene is expressed in yeast (Emini *et al.*, 1988). Emphasis was also placed on producing gp350 that could induce virus-neutralizing antibodies, since this was believed to be the key immune response required to obtain protective immunity against the virus. Although the bacterial product was recognized by antibodies from normal seropositive individuals, none of the antibodies neutralized the virus (Pither *et al.*, 1992a,b). The yeast gp350 product was not sufficiently antigenically similar, presumably because of glycosylation, and this approach was discontinued (Emini *et al.*, 1988). Cell-mediated immune responses to gp350 are known to be protective against EBV-induced lymphoma in the tamarin, however, and are likely to be important in protective immunity in humans. Since tertiary structure and glycosylation are unlikely to impede generation of the appropriate T-cell responses, both the bacterial and yeast products should be re-examined as candidate vaccines. A truncated version of *gp350* has been expressed in a baculovirus system, but the product has not been characterized in terms of its ability to induce virus-neutralizing antibodies or to induce protective immunity in the tamarin lymphoma model (Nuebling *et al.*, 1992).

The *gp350* gene has now been expressed in a number of mammalian cell systems in which glycosylation and post-translational modifications occur, which are closely similar to those found on the natural product (Motz *et al.*, 1986; Whang *et al.*, 1987; Conway *et al.*, 1989; Hessing *et al.*, 1992; Madej *et al.*, 1992). No difference has been found between these products and the natural product gp350 in terms of their ability to induce virus-neutralizing antibodies, bind a range of monoclonal antibodies and, in some studies, to stimulate the proliferation of gp350-specific T cells (Ulaeto *et al.*, 1988; Wallace *et al.*, 1991). In some studies, the membrane anchor sequence has been removed from the gene, allowing secretion of the expressed eukaryotic product into the culture medium (Whang *et al.*, 1987; Hessing *et al.*, 1992; Madej *et al.*, 1992). This approach offers major advantages in the large-scale preparation of a defined product that is relatively easy to purify. A bovine papillomavirus expression system and a Chinese hamster ovary cell system have been adapted to secrete a gp350 product in large quantities

(Hessing *et al.*, 1992; Madej *et al.*, 1992). This product is also indistinguishable from authentic gp350 in immunological terms. Another major benefit of use of this system is the guaranteed absence of potentially oncogenic EBV DNA.

1.6.2.5 *T- and B-cell epitopes on the gp350 molecule*

The generation of a panel of monoclonal antibodies (Qualtière *et al.*, 1987) allowed categorization of gp350 B-cell epitopes into at least seven groups on the basis of antibody competition. The mapping of particular immune functions to specific regions of the molecule could ultimately allow the design of a synthetic peptide or a recombinant-derived vaccine of predetermined immunological specificity. The immunological profile of gp350 is certainly complex. Most, if not all, of those epitopes associated with virus-neutralizing antibodies are discontinuous and are dependent on the conformation of the molecule, including the contribution of the large mass of carbohydrate. A possible exception is a linear epitope in the amino-terminal region between residues 317 and 327, which is recognized by antibodies from rabbits immunized either with gp350 bacterial fusion proteins containing the sequence, with intact virus or with gp350 iscoms. One approach for the identification of B- and T-cell epitopes is to express overlapping fragments of the gene in bacteria as β-galactosidase fusion proteins (Pither *et al.*, 1992a,b). The ability of antibodies made against gp350 in vaccinated animals and present in normal human sera to bind to the various fragments of gp350 expressed as bacterial fusion proteins was determined by western blotting. This procedure allows the detection only of linear epitopes, and a number of immunodominant epitopes were recognized with antisera from normal seropositive individuals. None of these epitopes was able to bind virus-neutralizing antibodies. Clear differences in the recognition patterns of sera from normal seropositive people and from immunized animals were seen, which may simply reflect species differences in immune responses but which may also reflect different immune responses after natural infection and after vaccination with a purified molecule. This difference might explain why neutralizing antibodies against linear epitopes were found in sera from vaccinated rabbits but not in sera from naturally infected humans. No method is yet available for identifying or reconstructing discontinuous B-cell epitopes, although the use of random-sequence synthetic peptides or random peptide sequences in phage libraries should be pursued (Geysen *et al.*, 1986; Cwirla *et al.*, 1990; Scott & Smith, 1990).

Gp350 bacterial fusion proteins have also been used to detect T-cell epitopes and, in some cases, to map them. It would be expected that, although the bacterial products do not retain the native conformation of gp350 and do not carry their carbohydrate complement, they should still have the capacity to be recognized by T cells after MHC class I or class II presentation. Bacterial fusion proteins were screened for their ability to induce proliferation in previously isolated gp350-specific T-cell clones from normal seropositive individuals. A large number of potential T-cell epitopes have been tentatively identified using an algorithm for this purpose, but only a few epitopes have been located in the amino-terminal region (Wallace *et al.*, 1991; Pither *et al.*, 1992a,b). Given the difficulty of identifying appropriate epitopes and the inevitable variation in

epitopes among individuals, the development of synthetic gp350 peptide vaccines seems to be remote.

1.6.2.6   Choice of adjuvant

Most proteins and glycoproteins are weakly immunogenic when inoculated alone into animals, and gp350 is no different in this respect. An adjuvant is invariably required to stimulate the immune response to the antigen, except when it is presented as part of a live virus or other vehicle, in which case a range of natural immune responses is triggered. A variety of effective immunological adjuvants is now available, and new ones appear from time to time (Gupta & Siber, 1995). They probably do not all work in the same way and are certainly not equally effective. Antigen presentation, targeting and delayed decomposition may all be important functions of the delivery vehicle. The mode of action of the small immuno-activator molecule may include lymphokine production, antigen processing, mitogenicity and upregulation of HLA expression. A key element in the action of adjuvants is probably their effect on the differentiation of $CD4^+$ T cells into T-helper 1 or 2 subsets after vaccination, which can influence the ratio of the two types (Dotsika et al., 1997). T-helper 1 cells produce IL-2 and IF γ and enhance cell-mediated immune responses, while T-helper 2 cells produce IL-4, -5 and -10 and augment humoral immune responses (Mosmann & Coffman, 1989). It has been shown that IL-12 can substitute for certain bacterial adjuvants in enhancing cell-mediated immune responses, at least against the protozoan parasite Leishmania major (Afonso et al., 1994). Studies of protection in the tamarin lymphoma model with natural product gp350 subunit vaccines have so far been confined to the use of artificial liposomes (North et al., 1982), a threonyl derivative of muramyl dipeptide in pluronic triblock copolymers (Syntex adjuvant formulation) (Allison & Byars, 1986, 1987), iscoms (Morgan et al., 1988a; Dotsika et al., 1997) and aluminium salts (Finerty et al., 1994).

Because some of the more powerful adjuvants have not yet been licensed for human use, studies have focused on evaluating aluminium salts, the only adjuvants licensed for use in humans, in the tamarin lymphoma model. In experiments with recombinant gp350 (Finerty et al., 1994), the formulations raised protective immunity against EBV-induced lymphoma. It is worth remembering that challenge with a lymphomagenic dose of virus represents an extreme, completely unphysiological event and, in some respects, a very stringent test of the efficacy of any vaccine. Given that three out of five animals immunized with gp350 in alum were protected against lymphoma, this formulation might be sufficiently effective in humans. It also induced protective immunity in the Callithrix jacchus model of an EBV-induced mononucleosis-like syndrome (Emini et al., 1986, 1989).

1.6.2.7   Live virus–vector recombinants

The disadvantage of subunit vaccines most often cited is that they generate poor and sometimes inappropriate responses, although the new generation of adjuvants mentioned above should overcome this objection. Any failure to induce a broad, powerful immune response should be set against the advantages of using biologically dead material of

absolutely defined composition (Murphy, 1989; Moss, 1996). The choice will depend on a variety of factors, including the immune responses that are required, assuming that the induction of both T- and B-cell responses is necessary together with the establishment of immunological memory. Both iscoms (Takahashi et al., 1990) and Syntex adjuvant formulation induce proliferative, cytotoxic T-cell responses and memory (Byars et al., 1991). Aluminium salts also allow the induction of limited cell-mediated immune responses (Dillon et al., 1992).

Many recombinant viruses have been made that express one or more important vaccine molecules; so far, these have been used only rarely in humans. Gp350 has been expressed in vaccinia virus (Mackett & Arrand, 1985), adenovirus (Ragot et el., 1993) and varicella virus (Lowe et al., 1987). Recombinant vaccinia viruses expressing gp350 have been derived from both the relatively virulent WR laboratory strain and the atte-nuated Wyeth vaccine strain and both have been tested in the tamarin lymphoma model (Morgan et al., 1988b). Protective immunity was induced in three of four animals by the recombinant WR strain but in none of the animals immunized with the Wyeth strain derivative. Both groups of animals responded to vaccination, with high levels of antibody against vaccinia proteins, but no antibodies against gp350 could be detected in any animal. The levels of antibodies to vaccinia proteins were substantially lower when the Wyeth derivative was used, and this is presumably a reflection of the degree to which the strain has been attenuated. The key observation in these experiments is that the WR strain derivative gives protective immunity in the absence of antibodies to gp350, so that the immunity in this case is provided by some form of cell-mediated immune response. When protective immunity was induced in the tamarin lymphoma model by injection of a replication-defective adenovirus expressing gp350, antibodies against gp350 were induced but had no capacity to neutralize EBV in vitro (Ragot et al., 1993).

More work is needed to develop effective vaccinia recombinants which strike the correct balance between attenuation and immunogenicity. The gp350 gene may be expressed in a canarypox vector (Taylor et al., 1992) or in a vaccinia recombinant derived from the Copenhagen strain which has been specifically attenuated by the removal or inactivation of individual genes (Tartaglia et al., 1992). The vaccinia gp350 recombinant previously tested in the tamarin model (Morgan et al., 1988b) was also tested in the common marmoset model but with the M81 EBV strain, which resembles more closely the wild-type strains of EBV in the general population than the standard B95-8 strain. Replication of the challenge virus in the vaccinated group was reduced in comparison with control groups (Mackett et al., 1996). The Oka varicella–zoster viral strain has been used to make recombinants expressing gp350 (Lowe et al., 1987), but protection was not induced in animals.

A number of recombinant adenoviruses that express foreign antigens have been constructed, and recombinant adenovirus-expressing gp350 has been tested in the tamarin model (Ragot et al., 1993). Replication-defective recombinants were made in which the E1 region is deleted and the virus can be propagated only in a helper cell line which provides the deleted E1 function (Graham et al., 1977). Adenoviruses have the capacity to down-regulate HLA expression in the cells they infect, but the E3 region responsible for this function has also been deleted in these replication-defective recom-

binants. Several features of adenoviruses have made them attractive for vaccine delivery. Firstly, adenoviruses type-4 and type-7 vaccines have already been used on a large scale in the United States Armed Forces to prevent respiratory disease, with a good safety record (Top *et al.*, 1971a,b). Secondly, the adenovirus can be encapsulated and given orally; although primary immune contact is in the gut lymphoid tissue, mucosal immunity is induced in the respiratory tract. Mucosal immune responses may be advantageous, but they could allow IgA-enhanced entry into epithelial cells (Sixbey & Yao, 1992).

The most significant results obtained with a recombinant virus expressing gp350 are those of a study in which the Chinese vaccinia strain (*Tien Tan*) was used to vaccinate a small group of seronegative and seropositive children in southern China (Gu *et al.*, 1995). Antibody levels to gp350 were reported to have been raised in subjects who were already seropositive and to have been induced in those who were seronegative at the beginning of the trial. Six of nine vaccinated children who were seronegative for EBV at the time of vaccination showed no evidence of natural EBV infection 16 months after vaccination.

1.6.2.8 Cell-mediated immune responses to gp350

Tamarins mount cell-mediated immune responses after inoculation with a lymphomagenic dose of EBV, as measured in a regression assay in which the outgrowth of autologous EBV-infected B cells was inhibited in the presence of blood lymphocytes from tamarins with spontaneously regressed EBV-induced tumours. This capacity to inhibit growth could be boosted by reinoculation of the tamarins with a sub-lymphomagenic dose of virus 18 months after the first challenge (Finerty *et al.*, 1988). More recently, EBV-specific CTLs which are $CD4^+$ MHC class II-restricted have been identified in the re-stimulated animals (Wilson *et al.*, 1996a).

Demonstration of the presence of gp350-specific T cells in vaccinated animals which can proliferate in response to gp350 *in vitro* is an immediate goal. Efforts are also being made to detect gp350-specific CTLs, but the creation of an effective target cell is a problem. Recombinant vaccinia virus expressing gp350 could be used to infect autologous fibroblasts or lymphocytes, as was done for the latent antigens (Khanna *et al.*, 1992; Murray *et al.*, 1992a). T-Cell clones specific for gp350 have been detected in normal seropositive individuals (Ulaeto *et al.*, 1988; Wallace *et al.*, 1991; Lee *et al.*, 1993b), and gp350 can stimulate the production of T cells that prevent EBV-induced transformation *in vitro* (Bejarano *et al.*, 1990). In certain circumstances, cells that express gp350 can be good targets for antibody-dependent cellular cytotoxicity (Khyatti et al., 1991). This activity is readily detected in sera from rabbits immunized with whole EBV, but its detection in sera from tamarins or rabbits vaccinated with purified gp350 has not been reported. The mechanism of cell-mediated immune protection in the tamarin lymphoma model is difficult to explain if a protective function for antibody is excluded, since protection is gp350-specific. The tumour cells themselves seem unlikely targets for gp350-specific responses as they do not express gp350 in readily detectable amounts. Cell-mediated responses induced by vaccinia–gp350 vaccination may in some way reduce the effective viral challenge dose to below the threshold required for tumour

induction. Immune responses in tamarins that had spontaneously recovered from a lymphomagenic viral challenge were possibly directed through MHC class II-restricted T cells positive for both CD4 and CD8 (Wilson *et al.*, 1996a). These cells may inhibit proliferation of lymphoblastoid cell lines by other mechanisms than perforin-mediated lysis.

1.6.2.9 Vaccines against EBV latent antigens

A completely different approach to EBV vaccination has emerged during the past few years, based on the knowledge that CD8^{+} T cells are responsible, at least in part, for limiting the number of EBV-infected B lymphocytes that express most or all of the viral latency genes in the circulation of normal seropositive individuals. Could induction of these immune cells before primary infection be effective in preventing or modifying primary infection? The central problem with this approach is the same as that with gp350. How does the virus persist despite the presence of presumably effective cellular and humoral immune responses? Normal seropositive individuals have CTLs that are specific for EBNA-3A and EBNA-3C and some with activity against LMP-2 and EBNA-2. An important observation is that a large proportion of the total CTL activity in normal seropositive individuals cannot be accounted for by the EBV latent genes (Khanna *et al.*, 1992; Murray *et al.*, 1992a). The target antigens for this proportion of the CTL population have yet to be identified.

A number of epitopes that are recognized by CTLs have been located in EBNA-3A. Moss *et al.* (1996) consider that these epitopes could be used in a vaccine to elicit T-cell memory, which could then be activated to produce EBNA-3A-specific CTLs. Since the target epitope varies between HLA types, several synthetic peptides corresponding to different epitopes would have to be incorporated in such a vaccine. A relatively small number of CTL epitopes would be able to elicit an immune response in the vast majority of the population. Phase 1 trials are in progress of an EBNA-3A peptide, FLRGRAYGL, which is restricted through the HLA-B8 allele. Some circulating, latently infected B cells in bone marrow and/or the circulation, however, express only EBNA-1, which is refractory to MHC class I-restricted antigen processing (Levitskaya *et al.*, 1995; Frisan *et al.*, 1996); these B cells thus cannot be targets for MHC class I-restricted CTLs induced by either vaccination or natural infection.

1.6.2.10 Conclusions

The potential usefulness of gp350-based EBV vaccines depends on the roles of gp350 and lytic replication in infection and disease. Primary infection in the oropharynx, an essential stage in the infectious cycle, is mediated by gp350 in the viral envelope. Vaccine-induced mucosal IgA against gp350 could well act at this level, controlling to some extent but probably not preventing infection (Yao *et al.*, 1989a), but there is also the theoretical possibility that mucosal IgA could enhance EBV infection (Sixbey & Yao, 1992). It is impossible to know at this stage what effects gp350 vaccine-induced immune responses will have on primary EBV infection. Will it be possible to induce sterilizing immunity, or is latent infection inevitable, and, once latency has been established, will

the immune responses of the vaccinee be more effective in preventing EBV disease than those of a normal, unvaccinated, seropositive person?

Patients with infectious mononucleosis have some circulating B cells that express lytic cycle antigens (Anagnostopoulos *et al.*, 1995; Niedobitek *et al.*, 1997b), but acyclovir treatment has no effect on the course or symptoms of the disease, although virus shedding is reduced (Tynell *et al.*, 1996). It is possible that CD4$^+$ T cells primed by gp350 vaccination would be reactivated on viral challenge. Such cells could influence the course of infectious mononucleosis by inducing apoptosis of EBV-infected B cells and by down-regulating the large monoclonal or oligoclonal populations of CD8$^+$ T cells which account for much of the lymphocytosis symptomatic of infectious mononucleosis (Callan *et al.*, 1996).

The observations that the onset of nasopharyngeal carcinoma is accompanied by a rise in the titre of serum IgA antibodies against lytic cycle antigens (Zeng, 1985) and that high anti-VCA titres in Ugandan children are prognostic for the development of endemic Burkitt's lymphoma (de Thé *et al.*, 1978a) also indicate a potential role for intervention with a gp350 vaccine. Answers to the above questions will not be obtained until candidate vaccines have been evaluated in human trials. The major EBV envelope glycoprotein gp350, when expressed as a genetically engineered product in mammalian cells, could now be evaluated in trials in which the target population is first-year university students — a population at high risk of contracting infectious mononucleosis. The endpoints of a trial in this population would be EBV seroconversion, possible development of infectious mononucleosis and changes in a range of immunological parameters, such as virus-neutralizing antibodies. Any further progress in EBV vaccine development will depend heavily on the outcome of such trials.

1.6.3 Passive immunotherapy

As discussed in section 1.3.3, although CTLs cannot eliminate EBV from the body, they appear to be essential for maintaining control of latently infected cells. Thus, infusion of such cells should be a safe, effective form of treatment for immunodeficient patients with EBV-related lymphoproliferation. This therapeutic approach has been explored in a number of studies and clinical trials of bone-marrow transplant recipients. Papadopoulos *et al.* (1994) and Heslop *et al.* (1994) used unseparated leukocytes from EBV-seropositive donors to treat patients with EBV-associated immunoblastic lymphomas that arose after transplantation of T cell-depleted bone-marrow allografts. All of the patients responded, as judged by clinical and laboratory criteria, but some developed fatal pulmonary complications. Severe graft-versus-host disease was observed in the survivors. Thus, although adoptive transfer of unseparated leukocytes seems to be effective, this therapeutic strategy may not be generally applicable owing to the high risk of secondary complications. The problem of graft-versus-host disease has been overcome in more recent studies in which EBV-specific CTLs of donor origin reactivated *in vitro* were infused into allograft recipients (Rooney *et al.*, 1995; Heslop *et al.*, 1996). The patients did not develop complications that could be attributed to the infusion, and in three patients with signs of EBV reactivation (> 100-fold increase in EBV DNA concen-

tration measured by semiquantitative PCR), infusion of CTLs reduced the EBV load to control levels within three to four weeks. In one case, clinical resolution of the immuno-blastic lymphoma was observed. Genetic marking of the infused CTLs was used to demonstrate that specific T cells that responded to challenge with virus-infected cells *in vivo* or *ex vivo* persisted for as long as 18 months. These findings support more wide-spread use of CTLs in the treatment of infections and cancer. Their applications may extend to other EBV-associated malignancies, such as Hodgkin's disease and naso-pharyngeal carcinoma, which express a relatively restricted range of EBV-encoded anti-gens that could serve as target cells for CTL therapy (see Tables 3 and 6).

2. Studies of Cancer in Humans

2.1 Burkitt's lymphoma

2.1.1 *Clinical features and pathology*

2.1.1.1 *Clinical features*

The jaw is the most frequently involved site and the commonest presenting feature in patients with Burkitt's lymphoma in equatorial Africa (Burkitt, 1958, 1970a) and Papua–New Guinea (ten Seldam *et al.*, 1966; Burkitt, 1967; Magrath *et al.*, 1992). The tumour frequently affects multiple jaw quadrants (Figure 5). Jaw involvement is age-dependent, occurring much more frequently in young children, since it arises in close proximity to the developing molar tooth buds. In series of cases of Burkitt's lymphoma in Uganda, 70% of children under five years of age and 25% of patients over 14 had jaw involve-ment (Burkitt, 1970a). Very young children who do not have overt jaw tumours often have orbital involvement (Olurin & Williams, 1972; Figure 6); some of these orbital tumours may arise in the maxilla. In general, jaw involvement appears to be more frequent in regions of higher incidence, even within equatorial Africa; however, patients from, for example, highland regions in Africa, in which the annual incidence rate of Burkitt's lymphoma is much lower, are also of higher median age, and this probably accounts for the lower frequency of jaw tumours (Burkitt & Wright, 1966; Kitinya & Lauren, 1982). It has been suggested that the frequency of jaw tumours is decreasing in some regions of equatorial Africa, with a corresponding increase in the fraction of abdominal tumours but with no clear change in the age-related incidence (Nkrumah, 1984).

Abdominal involvement is found in a little more than half of equatorial African patients at presentation (Burkitt & Wright, 1963; Burkitt, 1970b; Williams, 1975) and as many as 80% of patients in other countries (Magrath, 1991, 1997). There appear to be differences in the intra-abdominal sites of involvement in endemic countries (e.g. equa-torial Africa and Papua–New Guinea) and in those where the disease is sporadic (Europe, Australia and North America). Presentation with a resectable mass in the right iliac fossa or with intussusception (arising from intraluminal tumours), for example, both of which are common in sporadic tumours, is uncommon in African patients. Thus, Wright (1970)

Table 6. Cellular origin and patterns of viral gene expression in EBV-associated malignancies

| Cell type	Tumour	EBV asso-ciation (%)	EBV gene expression			Latency type	Reference
			EBNA-1	EBNA-2, -3A, -3B, -3C, -LP	LMP-1/2		
B Lymphocyte	Immunoblastic B lymphoma	100	+	+	+	III	Thomas *et al.* (1990)
B Lymphocyte	Endemic Burkitt's lymphoma	> 95	+	–	–	I	Rowe *et al.* (1987a)
T Lymphocyte	Immunoblastic T lymphoma	50–90	+	–	+	II	Jones *et al.* (1988); Kikuta *et al.* (1988)
T lymphocyte	Midline granuloma	100	+	–	+	II	Minarovits *et al.* (1994a)
HRS cells	Hodgkin's disease	40–50	+	–	+	II	Herbst *et al.* (1991a); Pallesen *et al.* (1991c); Deacon *et al.* (1993); Grasser *et al.* (1994)
Nasal epithelium	Nasopharyngeal carcinoma	100	+	–	+ (60%)	II	Fåhraeus *et al.* (1988); Brooks *et al.* (1992); Busson *et al.* (1992a); Smith & Griffin (1992)
Thymic epithelium	Thymic carcinoma	Case report	+	–	+	II	Leyvraz *et al.* (1985)
Gastric epithelium	Gastric carcinoma	90	+	–	–	I	Imai *et al.* (1994a)
Smooth muscle	Leiomyosarcoma	100	+	+	ñ	IV	Lee *et al.* (1995b)

Viral antigen expression was demonstrated by immunohistochemistry and/or reverse transcriptase–polymerase chain reaction.
HRS, Hodgkin and Reed-Sternberg

Figure 5. Ugandan patients with Burkitt's lymphoma involving the jaw

From WHO (1969); left, mandibular tumour; right, maxillary tumour

Figure 6. Orbital Burkitt's lymphoma in a Ugandan child
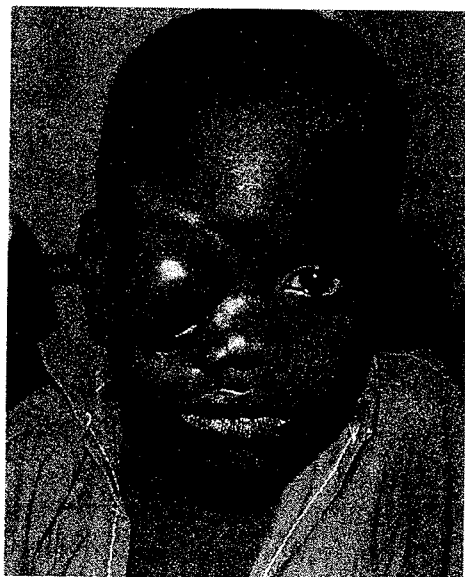
From WHO (1969)

reported no case of intussusception in Uganda among over 500 cases. In contrast, involvement of the mesentery and omentum (i.e. extraluminal) is common in African cases of Burkitt's lymphoma, hence the rarity of Burkitt's lymphoma-associated intussusception in Africa. Ascites may be a manifestation of abdominal disease regardless of geographic location. Precise figures for the involvement of various intra-abdominal and

retroperitoneal structures at presentation are not available from Africa, as relatively few centres have adequate radiological facilities. The available data are based largely on the results of clinical examinations, sometimes supplemented by laparotomy, or by autopsy studies dating from the 1960s (O'Conor, 1961; Wright, 1964, 1970; Williams, 1975; see below).

Bone-marrow involvement is seen in some 7–8% of Ugandan patients at presentation and relapse but in about 20% of patients in Europe and the United States. An additional fraction of patients in Europe and the United States presents with a leukaemic syndrome, referred to as the French–American–British subtype 'L3' or acute B-cell Burkitt's lymphoma (Magrath & Ziegler, 1980).

Central nervous system involvement — including cerebral spinal fluid pleocytosis, cranial nerve palsy and paraplegia due to paraspinal disease — is relatively common in Africa, being found in about one-third of patients at presentation (Ziegler et al., 1970), but is much less common in regions of sporadic incidence (Magrath, 1997). Interestingly, cranial nerve involvement (usually due to direct infiltration by tumour cells) is frequently not associated with cerebral spinal fluid pleocytosis at presentation, but malignant cells are nearly always detectable in cerebral spinal fluid of patients with cranial nerve palsy at relapse (Magrath, 1991). The optic nerve is frequently compressed and the surrounding subarachnoid space involved, however, when an intraorbital tumour is present (Ifekwunigwe et al., 1966). Cranial nerves and meninges have been described as the only sites of disease at presentation (Osuntokun et al., 1973). Paraplegia is the presenting feature in 15% of Ugandan patients but in less than 1% of patients in the United States (Magrath, 1991, 1997). In Ugandan patients, the spine is quite frequently the only evident site of disease, such that lamininectomy is required to make a diagnosis (and to relieve spinal cord pressure). Intracerebral disease is very uncommon and usually occurs in patients who have had persistent or multiple relapsed cranial nerve palsy or cerebral spinal fluid pleocytosis (Wright, 1970; Magrath et al., 1974); however, presentation with raised intracranial pressure from parenchymal brain infiltration is observed, albeit very uncommonly (Odeku et al., 1973; Osuntokun et al., 1973). Other sites of disease that are occasionally observed include the salivary glands, thyroid, breast (in pubertal girls or lactating women), testis, bone, pleura and heart, with involvement of the pericardium or, infrequently, cardiac muscle (Burkitt, 1970a; Aderele et al., 1975; Durodola, 1976; Magrath, 1991, 1997). Interestingly, pharyngeal involvement, peripheral lymphadenopathy and splenic involvement are rare in the African patient (although there is frequently splenomegaly from malaria) and rather more common in patients in regions of sporadic disease, although both splenic and lymph-node involvement are significantly more frequently observed in cases of Burkitt-like lymphoma.

2.1.1.2 Gross pathology

Burkitt's lymphoma, unlike follicular lymphomas and diffuse large B-cell lymphomas, arises predominantly at extranodal sites. The kidneys are a frequent site of disease in sub-Saharan Africa, being the organ involved in 80% of cases at autopsy; the ovaries are frequently bilaterally involved in young females, representing the site seen in

81% of autopsies in females. The adrenals are the third most commonly involved intra-abdominal organ, are more often involved at autopsy than the liver or spleen and are about as frequently involved in children as the jaw (58%). Bowel involvement is common, but other organs are involved less frequently. The lung parenchyma is very rarely involved, but serosal infiltration of the pleura and peritoneum, resulting in effusion, is frequent. Wright (1964, 1970) never observed involvement of the thymus. Fatal Burkitt's lymphoma is usually accompanied by widespread dissemination.

Grossly, the tumour is fleshy, creamy and soft. Areas of necrosis and haemorrhage are seen only in very large tumours. The tumours can locally infiltrate surrounding tissues and may spread via the lymphatics or blood vessels. In the central nervous system, choroid plexus involvement is not uncommon and is usually associated with cerebro-spinal fluid pleocytosis. Haematogenous dissemination may result in parenchymal involvement of the brain and spinal cord, in which case cerebrospinal fluid pleocytosis may be difficult to detect.

2.1.1.3 Histological characteristics

Burkitt's lymphoma (small non-cleaved-cell lymphoma, Burkitt's type) is classified as a non-Hodgkin's lymphoma and is characterized by a monomorphic cytoarchitecture composed of medium-sized cells (between those of large B-cell lymphoma and small-cell lymphocytic lymphoma). These cells do not have the characteristics of plasmacytoid cells or mature lymphocytes; they have a high nucleus to cytoplasm ratio, a round or oval nucleus with a coarse or 'open' chromatin pattern and usually two to five readily discernible nucleoli. A few cells may have a single large nucleolus, but when more than a few such cells are present the tumour is probably a Burkitt-like lymphoma. The cyto-plasm is intensely basophilic, staining strongly with methyl-green pyronine (pyronino-philic) because of the abundant free ribosomes, which are readily visible on transmission electron microscopy. Intracytoplasmic lipid vacuoles which stain with oil-red O and Sudan black are usually apparent on imprint or smear cytology (Berard et al., 1969). His-tological sections often reveal the presence of tingible body macrophages scattered among the tumour cells, giving rise to a 'starry sky appearance' in which nuclear debris from apoptotic tumour cells is discernible. This appearance is not, however, patho-gnomonic of Burkitt's lymphoma and may be seen in other lymphomas (O'Conor, 1961).

Burkitt-like (small non-cleaved, non-Burkitt's) lymphomas are similar in appearance to Burkitt's lymphoma, but are distinguishable by a greater degree of pleomorphism, a fraction of the cells being similar in size to those of large-cell or centroblastic lympho-mas, and a higher frequency of cells with a single large nucleolus in the neoplastic popu-lation. This entity may simply represent the borderline between Burkitt's lymphoma and large-cell lymphoma.

Burkitt's lymphoma is invariably of B-cell origin, the presence of surface immuno-globulin being first shown in 1967 (Klein et al., 1967), and has the immunophenotypic characteristics of a subset of germinal-centre cells; hence, the cells do not or very uncommonly express terminal deoxyribonucleotide transferase. B-Cell lineage markers such as CD19, CD20, CD22 and CD79a and surface immunoglobulin are always

demonstrable. The surface immunoglobulin is usually IgM, but IgG and IgA are occasionally present and kappa or lambda immunoglobulin light chains are nearly always detected. Other surface antigens that are expressed in most Burkitt's lymphomas include CD10 and CD77, but CD23 and CD5 are absent (Harris et al., 1994). Burkitt's lymphoma cells express low levels of HLA class I adhesion and activation molecules such as CD54, CD11a/18 and CD58 (Masucci et al., 1987; Billaud et al., 1989; Andersson et al., 1991). Some EBV-negative Burkitt's lymphomas of American origin synthesize and secrete immunoglobulin (Benjamin et al., 1982).

### 2.1.2 Descriptive epidemiology

2.1.2.1 Historical aspects

Burkitt's lymphoma was first identified in Africa, where there is every reason to believe that it has existed for millennia. Its presence prior to its description by Europeans is attested to by wooden masks depicting jaw and orbital tumours (Pulvertaft, 1965). It seems probable that the environmental factors relevant to its pathogenesis, with the possible exception of HIV, were relatively constant prior to the lifestyle changes brought about by the technological revolution of this century. The first known medical description is that of Sir Albert Cook who, with his brother, established the first mission hospital in Uganda in 1897. Cook's meticulous records were analysed many years later by Davies et al. (1964a,b), who reproduced in their report a drawing made by Cook in 1910 of a malignant jaw tumour in a child. Subsequently, a number of expatriate pathologists working in Africa noted that facial 'sarcomas' and lymphomas occurred at high frequency in African children. Most of these tumours were probably Burkitt's lymphomas.

Smith and Elmes (1934) described a series of 500 malignant tumours collected in Lagos, Nigeria, which included 16 jaw tumours recorded as sarcomas, three of which were in children, and 10 'round-cell sarcomas of the orbit', all in children under 10 years of age. More than a decade later, Davies (1948) observed that neoplasms of the reticuloendothelial system occurred at high frequency in Uganda, and Edington (1956; Edington & Giles, 1968) working in Ghana, then known as the Gold Coast, commented on the relatively high frequency of maxillary lymphosarcoma in children. Thijs (1957) reported from the Belgian Congo that 74 of 145 children with malignant tumours had lymphosarcoma. Interestingly, jaw tumours accounted for only 11 of the latter cases. De Smet (1956), also working in the Belgian Congo, mentioned in his report on children with lymphosarcoma that multiple organ sites, including the maxilla, orbit, abdomen and thyroid, were frequently involved.

Denis Burkitt and his pathologist colleagues, Davies and O'Conor noted, like De Smet, that children with jaw tumours often had histologically similar tumours at multiple organ sites, particularly in the abdomen (Burkitt, 1958; O'Conor & Davies, 1960). They subsequently demonstrated that the tumour occurred at high frequency in a broad belt across Africa, extending approximately 15° N and S of the equator, with a southern prolongation on the eastern side of the continent (Burkitt, 1962a,b,c; Figure 7). While Burkitt was initially under the impression that the tumour he was studying was a

sarcoma, O'Conor and Davies, in a review of the malignant tumours of children in the Kampala Cancer Registry in Uganda, recognized, as had Thijs, that malignant lymphoma accounted for some 50% of all childhood malignant tumours in the registry (O'Conor & Davies, 1960; O'Conor, 1961).

Figure 7. Distribution of Burkitt's lymphoma in Africa
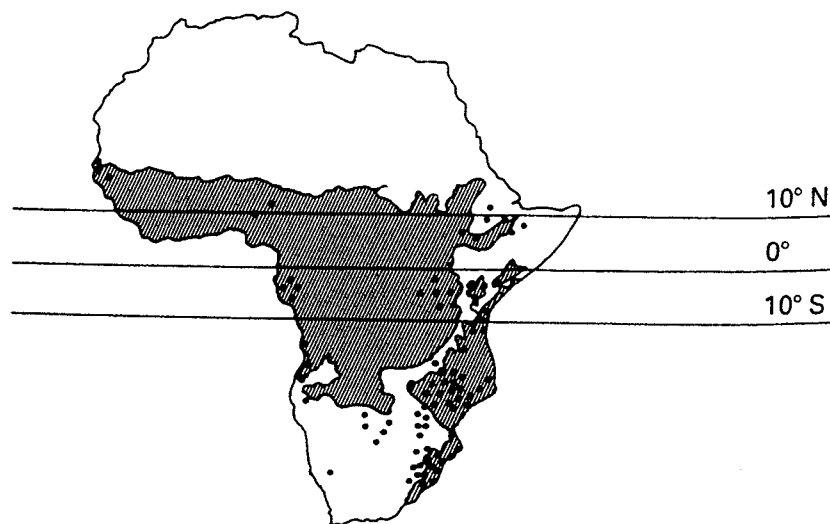
From Haddow (1963)

The shaded area represents the area in which, on climatological grounds, Burkitt's lymphoma might be expected to occur. The black points show the distribution of the series of cases compiled by D. Burkitt. The method used was to fill in any degree-square in which the condition had been recorded, irrespective of the number of cases.

Soon after these observations in Africa, O'Conor, Wright (who had also worked in Uganda) and others reported histologically indistinguishable tumours in children in Europe and the United States (Dorfman, 1965; O'Conor et al., 1965; Wright, 1966). At about the same time, strong similarities between the distribution of Burkitt's lymphoma and that of yellow fever were seen (Burkitt & Davies, 1961; Haddow, 1963), and it was therefore suggested that Burkitt's lymphoma might be caused by a vectored virus.

2.1.2.2   Incidence

Estimates made some 30 years ago suggested a relatively high incidence rate of Burkitt's lymphoma in Africa, with about 5–10 cases per 100 000 children below the age of 16 years and a peak incidence between five and 10 years of age. In Nigeria, the incidence in 1960–63 was reported to be 22 per 100 000 in 5–9-year-old boys and 10 per 100 000 in girls (Edington & MacLean, 1964). Williams (1975) found that Burkitt's lymphoma accounted for 682 of 1325 tumours in a hospital-based tumour registry in Ibadan, Nigeria, in 1960–72.

Burkitt's lymphoma now accounts for 30–70% of childhood cancers in equatorial Africa. In the United States, the incidence of Burkitt's lymphoma is 2–3 per million children per year. Recent estimates of incidence rates worldwide range from zero, which

may be due to differences in nomenclature or diagnostic practices, to 3.6 per 100 000 per year.

2.1.2.3 *Climatic determinants*

The very high frequency of Burkitt's lymphoma in equatorial Africa and its climatically determined distribution, first described in the early 1960s, focused attention on the possibility of a causal association with an environmental agent. The evidence that malaria is such an agent is based largely on the similarity of the distribution of holo-endemic malaria and African Burkitt's lymphoma and on associations between rates of parasitaemia and the likelihood of developing Burkitt's lymphoma (see section 2.1.4.1).

The peri-equatorial distribution of patients in Africa with a clinical syndrome consistent with Burkitt's lymphoma was established by the initial investigations of Burkitt and several colleagues throughout Africa and on responses on questionnaires administered in a large number of hospitals on the continent (Burkitt, 1962a,b,c; Haddow, 1963; Burkitt, 1985). These investigators observed that the tumour occurred in a belt across equatorial Africa, with a prolongation to the south-east (Mozambique and Natal) (see Figure 7). Within this region, however, the tumour was rare or absent in a number of densely populated areas (Burkitt, 1963, 1969, 1970a). These included south-west Uganda, Rwanda and Burundi, the highlands of Kenya and the United Republic of Tanzania, the islands of Pemba and Zanzibar and some parts of Zaire. Several sharply delineated transitions from high to low incidence were observed, the majority between river valleys or lake shores and highland regions. In Nigeria, the relative rarity of the tumour in the arid (30 in [76 cm] of rainfall per year between the months of June and September) but densely populated northern region, Kano, contrasted with its much greater prevalence in wetter (up to 400 in [10 m] of rainfall per year), more sparsely populated regions less than 400 miles [640 km] to the south. Interestingly, this pattern applied to West Africa in general, the tumour being common throughout the southern parts of these countries (Accra in Ghana being an exception) but rare in the north. With some exceptions (e.g. the islands of Pemba and Zanzibar, Kinshasa, Brazzaville and Lambarene), the distribution was clearly determined by altitude in East Africa and by rainfall in West Africa. Thus, in East Africa, the tumour did not occur at any notable frequency above 1500 feet (450 m), or in regions where the mean temperature fell below 15.5 °C in any month (Haddow, 1963), and in West Africa the tumour did not in occur in regions in which the annual rainfall was less than 50 cm.

Burkitt and Wright (1966) showed in their series of histologically proven cases that the incidence of the tumour was much higher in the northern, eastern and central regions of Uganda. In Kigezi, in the south-west, for example, the incidence of 0.6 per 100 000 was estimated to be one-twentieth of that in the north-west. The low-incidence areas are highlands over 5000 feet (1500 m) above sea level, e.g. Kigezi, which continues westwards into the high central plateau that forms much of Rwanda and Burundi and extends into north-western Tanzania. Less than 4% of the cases in the Kampala registry occurred in children living in the south-western districts, which accounted for 25% of the population of Uganda; however, some 20% of the population of the central region of Uganda,

Buganda, is comprised of peoples from the low-incidence south-western region (Rwanda, Burundi and Kigezi), and about 19% of the cases of Burkitt's lymphoma recorded at Mulago Hospital up to the time of the report occurred in these immigrant peoples. Moreover, the average age of patients in the immigrant groups was 16.5 years, while that of patients from high-incidence areas was 8.1 years. Some of the immigrants with Burkitt's lymphoma had been living in the area for fewer than three years. The average age of patients from low-incidence areas was also higher than that of patients from high-incidence areas. These data strongly suggested that differences in the geographical distribution of the tumour were not a consequence of genetic differences among the tribes of Uganda but were related to environmental differences, being entirely consistent with the previously described barriers of temperature (altitude) and rainfall.

Haddow (1963) subsequently refined the map of the distribution of Burkitt's lymphoma in relation to climatic parameters and pointed out that the high-frequency regions were those in which the rainfall was more than 20 in (50 cm) per year (Figure 7). This finding is consistent with the hypothesis that Burkitt's lymphoma is caused by a vectored virus, since investigators at the East African Virus Research Institute in Entebbe, which Haddow directed, had shown that the yellow fever virus cannot replicate in mosquito vectors when temperatures fall below 60 °F (15 °C), accounting for the virtual absence of yellow fever in people living above 5000 feet (1500 m). In addition, adult mosquitoes do not survive in dry weather, but rely upon drought-resistant eggs in regions prone to dry spells. In West Africa, such regions were defined as those in which the annual rainfall was less than 20 in (50 cm). By using maps in which the distribution of Burkitt's lymphoma was superimposed on isotherms and isohets, it was possible to determine that only 5% of cases of Burkitt's lymphoma fell outside these limits of temperature and rainfall.

The distribution of Burkitt's lymphoma was also shown (Burkitt, 1963) to be very similar to that of a mosquito-vectored arbovirus disease, o'nyong nyong fever, in Uganda (Shore, 1961). Thus, it seemed highly probable that insect vectors were in some way involved in transmission of the disease. Tumour-free areas were readily explained on the basis of the absence of the vector, the vectored microorganism or both.

Wright and Roberts (1966), who studied 324 histologically proven cases of Burkitt's lymphoma seen in Uganda between 1959 and 1964 and 425 cases of other types of lymphoma seen during the same period, showed that the remarkably precise climatic determinants of the distribution of Burkitt's lymphoma did not apply to the distribution of other types of lymphoma in Uganda, which varied, instead, solely with the density of the population. Similar data were reported from northern Nigeria by Edington (1978) in respect of other malignant lymphomas.

Dalldorf et al. (1964) reported on the distribution of Burkitt's lymphoma in Kenya. The lowest incidence was found in the Kalenjin tribe, living in the highland belt (above 5000 feet [1500 m]), and the highest incidence in coastal or lake-shore dwelling tribes (below 5000 feet). There was no significant difference in the incidence of squamous-cell carcinoma among these tribal groups. These authors also reported on exposure to natural radiation, to arboviruses (by serology) and to malaria. Children of the Kalenjin tribe had

a variable rate of splenomegaly, this region being considered to be free of malaria in the recent past, in contrast to coastal tribes and the Luo, in regions where malaria was considered to be holoendemic. The prevalence of malaria in these tribes was confirmed by the rates of sickle-cell trait (negatively correlated) and glucose-6-phosphate dehydrogenase deficiency (positively correlated) (Bienzle et al., 1981).

Eshleman (1966) reported on 31 cases of Burkitt's tumour seen at the Shirati Hospital in the United Republic of Tanzania. All of the patients came from lowland areas, less than 4500 feet (1350 m) above sea level, although the other patients seen at the hospital came from the Mara region with a height above sea level varying from 3700 to 5500 feet (1110–1650 m).

Goma (1965) carried out a survey of the environment in Uganda within a two-mile zone of 21 huts in which cases of Burkitt's lymphoma had occurred. He found permanent or semi-permanent water nearby and usually dense vegetation — ideal breeding conditions for mosquitoes. Goma easily trapped mosquitoes from the vicinity of the huts and was able to identify 42 different species. Williams (1967) pointed out that, among the large variety of arthropods known to transmit human diseases, only Mansonia and Anopheles species had similar distributions in Uganda to Burkitt's lymphoma.

More recent data (Kitinya & Lauren, 1982) confirm the relationship between the incidence of Burkitt's lymphoma and altitude. On Mount Kilimanjaro in northern Tanzania, the relative frequency of Burkitt's lymphoma (2.2% of all malignancies) was less than that in the coastal and low-lying regions of Uganda and Tanzania. Moreover, on the slopes of Mount Kilimanjaro, cases of Burkitt's lymphoma occurred predominantly in the sparsely populated areas below 1000 m, although some cases were found in densely populated areas up to 1500 m. As in earlier studies in East Africa, the average age of patients from this low-incidence area was higher (16.4% were over 20 years of age) than that of patients from high-incidence areas (in Uganda, for example, only 5% of patients were over 20), while the frequency of jaw tumours was lower (22%, compared with 55% or more in Uganda).

2.1.2.4   Time–space clustering

Pike et al. (1967) first reported on time–space clustering of Burkitt's lymphoma in the West Nile District of Uganda between 1961 and 1965. They concluded that patients whose dates of onset were close tended to live close together. This finding was highly statistically significant and suggested an 'epidemic' character.

Williams et al. (1969) provided further evidence of time–space clustering in the West Nile District, refining previously collected information and extending the study period to 1967. While the new data confirmed the results of the earlier analysis, the authors reported that an unpublished analysis conducted in the East and West Mengo districts in central Uganda did not show a similar phenomenon.

Baikie et al. (1972) subsequently reviewed the evidence for clustering of Burkitt's lymphoma. Of particular interest is a cluster observed in Bwamba County, in a low-incidence area in the south-west of Uganda, close to the border with the Congo. The only seven cases known to have occurred in the county were diagnosed between October 1966

and December 1968. Two of these cases were in a brother and sister, and five occurred during a six-month period from July to December 1968. No other clusters, apart from those in the West Nile, were observed in either Uganda or Tanzania (Brubaker *et al.*, 1973).

2.1.2.5 *Familial cases*

X-Linked lymphoproliferative syndrome, characterized by an abnormal response to EBV, predisposes males to the development of lymphomas, some of which may resemble Burkitt's lymphoma. Familial Burkitt's lymphomas, apparently unrelated to this syndrome and in some cases associated with a familial predisposition to other tumours, such as chronic myeloid leukaemia and nasopharyngeal carcinoma, have also been described.

Brubaker *et al.* (1980) reported the occurrence of Burkitt's lymphoma in North Mara District, Tanzania, in five families in which several members were afflicted, sometimes with another malignancy. The families in which multiple cases of Burkitt's lymphoma had occurred included two brothers and a half-brother and two brothers. In two other families, there were cases of chronic myeloid leukaemia and of Burkitt's lymphoma, in young siblings in one family and in a father and a son in the other; in another family, one woman who had nasopharyngeal carcinoma had a son with Burkitt's lymphoma. Naso-pharyngeal carcinoma and chronic myeloid leukaemia are both rare tumours in the North Mara region.

Stevens *et al.* (1972) described two siblings with Burkitt's lymphoma, one of whom had Burkitt's leukaemia, in the United States. Joncas *et al.* (1976) described Burkitt's lymphoma in first-degree cousins, a young woman aged 16 and a young man of 17, in a large French-Canadian family. Two of the siblings of one of the patients with Burkitt's lymphoma had nasopharyngeal carcinomas, and an additional first-degree cousin had a plasmacytoma. Several siblings and cousins had low IgA values. Poulsen *et al.* (1991) described a case of EBV-negative Burkitt's lymphoma of the breast in two sisters aged 18 and 21 years in Denmark.

Familial Burkitt's lymphoma has also been described in three families in Papua–New Guinea (Winnett *et al.*, 1997). The first family had three cases of Burkitt's lymphoma between 1964 and 1965 in full brothers. In the second, two cases of Burkitt's lymphoma occurred between 1983 and 1984 in a full brother and sister; and in the third family, three cases arose between 1980 and 1983 in two sons and one daughter of one man and his two wives.

2.1.3 *Epidemiology of Burkitt's lymphoma in association with EBV*

The EBV was identified in an attempt to confirm the hypothesis that African Burkitt's lymphoma is caused by a vectored virus. Epstein (1985) examined tumour samples sent by Burkitt by electron microscopy, as the viral culture systems available at the time gave negative results. No virus was found, however, despite the most exhaustive searches, until he and his colleagues, Achong and Barr, succeeded in growing cell lines from the tumours. They believed that the virus could grow in cells that did not have host defences,

as is the case for avian tumour virus. Viral particles with a morphology consistent with that of the herpes family were identified in the first grid square (Epstein *et al.*, 1964). Subsequently, antigenic relationships with other herpesviruses, including the Lucké virus, Marek's disease virus and infectious bovine rhinotracheitis, were demonstrated by immunological methods (Ono *et al.*, 1970; Stevens *et al.*, 1971; Evans *et al.*, 1972). Evidence that this virus — EBV, as it came to be known — is frequently associated with Burkitt's lymphoma was based initially on serological evidence, made possible by the development of immunofluorescence tests for viral antibodies by Werner Henle and Gertrude Henle (1966). This test was later shown to reflect the level of VCA in the cells; cells that gave positive results in immunofluorescence assays were shown to contain viral particles by electron microscopy (Henle & Henle, 1967; Epstein & Achong, 1968). It was found, however, that control sera also generally showed anti-EBV antibodies, and the late viral antigens detected by the fluorescence antibody tests were rarely, if ever, detected in fresh tumour cells, although they were detected in a small percentage of cells in cell lines derived from tumours. Confirmation that viral genomes are present in every tumour cell therefore had to await the development of fluorescence tests for latent antigens and in-situ hybridization techniques.

zur Hausen and Schulte-Holthausen (1970) were the first to detect EBV DNA, using a nucleic acid hybridization technique, in a cell line in which viral particles could not be demonstrated. This result was then confirmed by Nonoyama and Pagano (1971). These results strongly suggested that viral DNA is present in cells in which late viral antigens or viral particles cannot be detected, indicating that the virus must be able to latently infect cells, i.e. remain within the cell without replicating viral particles.

2.1.3.1 *Case series*

Differences in the frequency of the association of Burkitt's lymphoma with EBV, as determined by the presence of EBV DNA (or RNA) or EBV antigens, are found in different regions of the world, as reflected in differences in serology. Table 7 gives the results found in case series in endemic and non-endemic areas of the world with respect to the association between Burkitt's lymphoma and markers of infection with EBV.

(a) *African patients*

Some 95% of African Burkitt's lymphomas are associated with EBV, as demonstrated by the presence of either EBNA or EBV DNA in the tumour cells, and such patients have higher geometric mean titres of antibodies against EBV-associated antigens. For some time, investigators had recognized the presence of complement-fixing antibodies in the sera of patients with Burkitt's lymphoma (Armstrong *et al.*, 1966). Pope *et al.* (1969) subsequently identified an EBV-associated complement-fixing antigen in soluble extracts of EBV-transformed lymphoblastoid cell lines and the Raji cell line, then thought to be 'virus free'. Reedman and Klein (1973) identified these antigens at the cellular level with an anticomplement immunofluorescence test in the nuclei of over 90% of the cells of EBV-transformed lymphoblastoid cell lines, regardless of whether they produced virus or not, and in a similar fraction of cells from two Burkitt's lymphoma biopsy samples. Comparison with other antibodies against EBV in 52 sera used to demonstrate the

antigen showed that the 32 with anti-VCA and complement-fixing antibodies also showed anticomplement immunofluorescence. Five of six sera which had no anti-VCA but had complement-fixing antibodies gave positive or equivocal results for anticomplement immunofluorescence, and 14 sera that had no anti-VCA and no complement-fixing antibodies gave negative results. A variety of control cells also gave negative results. This complement-fixing antigen became known as the EBV nuclear antigen (EBNA).

Table 7. Association between Burkitt's lymphoma and EBV in endemic and non-endemic regions

Country	No. of cases	Association with EBV	Reference
Endemic areas of Africa			
	23	EBV DNA in 22 (low level in 1 case)	Nonoyama et al. (1973)
	13	11 EBNA-positive	Reedman et al. (1974)
	27	EBV DNA in 26; 25 EBNA-positive	Lindahl et al. (1974)
Cameroon and Gabon	10	EBV DNA in 10	Prévot et al. (1992)
Ghana	4	EBV RNA identified	Dambaugh et al. (1979)
	23	EBV DNA in 23	Shiramizu et al. (1991)
Uganda	27	EBNA-positive; satisfactory biopsies	Olweny et al. (1977)
	15	EBV DNA in 14; all EBNA-positive	
	5	All EBNA-positive; IF on extracted DNA	Luka et al. (1978)
Malawi	44	42 EBER-1- or BamHI W-positive by ISH	Labrecque et al. (1994)
Non-endemic areas			
Americas			
Argentina	16	EBER-1 in 4	Drut et al. (1994)
Argentina, Chile	27	EBV DNA in 13 (48%)	Gutiérrez et al. (1992)
Brazil	12	EBV DNA in 7 (58%)	
Brazil	24	EBER-1 in 17 (71%)	Bacchi et al. (1996a)
Brazil	54	47 EBER-1 positive (87%)	Araujo et al. (1996)
Cuba	7	6 VCA and EA(R)-positive	Riverend et al. (1984)
USA	4	No EBV DNA	Pagano et al. (1973)
USA	1	EBV DNA present	Gravell et al. (1976)
USA	12	EBV DNA in 5	Andersson et al. (1976);
	20	EBV DNA in same 5	Ziegler et al. (1976)
USA	3	EBV DNA in 3	Judson et al. (1977)
USA	32	EBV DNA in 12 (38%)	Shiramizu et al. (1991)
Asia			
China	18	EBER-1 in 5 (28%)	Chan et al. (1995a)

Table 7 (contd)

Country	No. of cases	Association with EBV	Reference
Non-endemic areas (contd)			
Egypt	41	30 EBER-1-positive (73%)	Anwar *et al.* (1995)
India	2	EBNA-positive	Venkitaraman *et al.* (1983)
Japan	14	2 EBNA-positive	Miyoshi (1983)
Japan	7	EBV DNA in 4	Okano *et al.* (1992)
Europe			
France	67	EBV DNA in 24%	Philip (1985)
Germany	1	EBV DNA present	Bornkamm *et al.* (1976)
Germany	1	EBNA-positive	Kachel *et al.* (1980)
Italy	15	EBV DNA in 1/12	Tirelli *et al.* (1984)
Sweden	1	EBV DNA present	Biberfeld *et al.* (1981)
Turkey	15	EBV DNA in 14	Çavdar *et al.* (1994)
Oceania			
Australia	1	EBNA-negative	Roeser *et al.* (1977)

EBNA, EBV nuclear antigen; EBER, EBV-encoded RNA; IF, immunofluorescence; ISH, in-situ hybridization

Nonoyama *et al.* (1973) reported that 22 of 23 biopsy samples from African patients with Burkitt's lymphoma contained EBV DNA as ascertained by nucleic acid hybridization, most containing 15–113 genome copies per cell (average, 38). Reedman *et al.* (1974) subsequently examined biopsies from 19 African Burkitt's lymphomas by complement-fixation tests and anticomplement immunofluorescence for EBV-associated antigens. Extracts from 12 of these tumours reacted in complement fixation tests with EBV-seropositive sera but not with EBV-seronegative sera. Eleven of 13 biopsy samples showed anticomplement fluorescence, and the reactive antibodies could be absorbed out with lymphoblastoid cell lines. In all cases, the fluorescence was coarsely granular and present in almost all the tumour cells. The remaining biopsy samples were considered not to contain EBV. None of 13 samples from tumours other than Burkitt's lymphoma showed anticomplement immunofluorescence.

Lindahl *et al.* (1974) reported on the correlation between the presence of EBNA and the detection of EBV DNA by nucleic acid hybridization with complementary RNA probes. Twenty-six of 27 histologically confirmed cases of African Burkitt's lymphoma were shown to contain 10–101 viral genomes per cell (average, 39) by hybridization, and 25 of these 26 cases also contained EBNA.

Magrath *et al.* (1975) reported on a patient who presented with Hodgkin's disease and then Burkitt's lymphoma. The patient had a high titre of anti-VCA (> 1:320) but not anti-EA at the time of presentation with Hodgkin's disease. The anti-VCA titres continued to rise, albeit at a slow pace, over the ensuing months, to > 1:1250, and those of anti-EA and anti-EBNA increased rapidly to 1:1250 shortly before presentation of widespread

Burkitt's lymphoma nine months later. Anti-membrane antigen antibodies, although continuously present, did not change in titre in association with the onset of Burkitt's lymphoma. This case demonstrates that antibody titres to EBV are raised many months before the onset of Burkitt's lymphoma, consistent with the results of the prospective study in the West Nile District, and also that rapid changes in EA titres may occur at the time of tumour growth.

Olweny et al. (1977) examined the expression of EBNA in 34 patients with Burkitt's lymphoma in Uganda and 25 patients with other malignancies (predominantly other lymphomas). All 27 biopsy samples from cases of Burkitt's lymphoma considered to be in satisfactory condition contained EBNA. Fourteen of 15 of these samples examined by nucleic acid hybridization were shown to contain EBV DNA, with a mean genome copy number of 39. The one case in which fewer than two genome copies (the level of detection of the test) were found was serologically negative for EBV antibodies and was in a patient who had lived in a malaria-free highland area before migrating two months before the diagnosis of Burkitt's lymphoma to a malaria-endemic region. None of the 25 non-Burkitt's lymphoma biopsy specimens contained EBNA, and all 15 subjected to nucleic acid hybridization showed no EBV DNA. The results of EBV serology were consistent with those reported previously, the anti-VCA titre being fourfold higher and anti-EA being detectable in 59% of the group with Burkitt's lymphoma.

Luka et al. (1978) examined five biopsy samples from Burkitt's lymphoma patients in Africa previously shown to contain EBNA by standard anticomplementary immunofluorescence, DNA extraction, separation on DNA–cellulose columns and fixation to chicken erythrocytes before immunofluorescence testing. All five samples contained EBV DNA.

Dambaugh et al. (1979) studied four Burkitt's lymphoma biopsy samples from Accra, Ghana, and demonstrated RNA homologous to at least 3–6% of the DNA of EBV; they also identified the general regions of the EBV genome from which the RNA was transcribed.

Prévot et al. (1992) studied tissues from 12 cases of Burkitt's lymphoma and 12 cases of non-Burkitt's lymphoma from Cameroon and Gabon by in-situ hybridization with a DNA probe derived from the BamHI W repeat region of EBV. All 10 samples from Burkitt's lymphoma considered technically satisfactory were shown to contain EBV DNA, and all 12 of the non-Burkitt's lymphoma samples gave negative results.

Shiramizu et al. (1991) studied tissues from 23 cases of Burkitt's lymphoma from Ghana by hybridization with a probe derived from the BamHI K fragment of the EBV genome. All 23 samples showed the presence of EBV DNA, which was confirmed with probes from the terminal repeat region of EBV.

Labrecque et al. (1994) in Malawi used in-situ hybridization on material obtained from Burkitt's lymphomas by aspiration biopsy, with two probes: EBER-1 and the BamHI W fragment of EBV. Of 66 cases of suspected Burkitt's lymphoma, 44 were confirmed cytopathologically, and 42 showed the presence of EBV DNA with EBER-1 and/or BamHI W. Of the 22 cases considered not be Burkitt's lymphoma, only one contained EBV.

(b)   Non-African patients

Some 10–30% of European and American tumours appear to be associated with EBV, while 50–90% of tumours in developing countries are EBV-associated. The fact that only a proportion of cases are associated with EBV should be borne in mind when interpreting the results of serological studies, as the available data indicate that only patients with EBV present in the tumour cells have raised antibody titres to EBV. Thus, serological results reflect the fact that these series include both EBV-associated and EBV-negative cases. Furthermore, age may be an important factor in the frequency of association with EBV.

Goldberg and Drut (1986) reviewed their 16-year experience of Burkitt's lymphoma in Argentina; 73% of patients had elevated antibody titres to EBV. Drut *et al.* (1994) examined 16 Argentine patients with Burkitt's lymphoma aged 2–8 years for EBV by PCR and in-situ hybridization (using a NotI/PstI fragment). Four cases gave positive results in both tests, 70–90% of the cells giving a signal in in-situ hybridization. Interestingly, all of these cases were seen in the same year, 1984. Gutiérrez *et al.* (1992) reported 39 cases of Burkitt's lymphoma from Argentina, Chile and Brazil, mostly in children, the age range being 1–28, with only two patients over 15 years of age. Overall, 51% of these cases (48% from Argentina and Chile and 58% from Brazil) were EBV-associated, as determined by Southern blotting. The EBV in these tumours was shown with the EBV terminal-repeat region probe to be monoclonal.

Bacchi *et al.* (1996a) reported the results of in-situ hybridization for EBER-1 in 24 cases (mostly in children, but some ages unknown) of Burkitt's lymphoma from Campinas and São Paulo in Brazil. Seventeen (71%) gave positive results, the signal being present in virtually all of the neoplastic cells of each case. Only one of these patients had jaw involvement. Araujo *et al.* (1996) described 54 children (median age, six years) with Burkitt's lymphoma, four of whom had mandibular tumours, in Bahia, in tropical Brazil and reported the results of in-situ hybridization with an EBER-1 probe. Forty seven (87%) of the cases showed the presence of EBV DNA. Interestingly, the fraction of positive cases appeared to be related to age: 39 of 41 patients under nine years of age and six of 11 aged nine years or more had EBER-1-positive tumours. Essentially 100% of the cells showed positive results. LMP-1 was not expressed in the tumours, except in a few cells in proximity to ova of *Schistosoma mansoni* in two cases. Of 27 EBV-positive tumours tested for EBV subtype, 20 (74%) were type 1 and the remaining seven (26%) were type 2. This result is intermediate between that of African Burkitt's lymphoma (50% type 1) and North American Burkitt's lymphoma (10% type 2) (Goldschmidts *et al.*, 1992).

Riverend *et al.* (1984) described seven children with Burkitt's lymphoma in Cuba. Five of the patients had high antibody titres to VCA (1:640–1:5120) and to EA(R) (1:10–1:2560), one had low titres to these antigens (1:20 and 1:5, respectively) and one had negative titres. Interestingly, three of the patients with high antibody titres had jaw tumours.

In spite of the serological evidence that Burkitt's lymphoma in the United States is also EBV-associated, Pagano *et al.* (1973) failed to detect hybridizable EBV DNA in

tumour-infiltrated tissue from four cases of Burkitt's lymphoma; however, Gravell *et al.* (1976) detected EBV DNA in an American patient with Burkitt's lymphoma.

Andersson *et al.* (1976) and Ziegler *et al.* (1976) studied an additional 12 American patients with Burkitt's lymphoma. Nine of the patients had low anti-VCA titres, but two had titres of 1:320 and 1:160 and one gave a negative result. Five of the tumours had EBV DNA that was readily detectable by liquid hybridization studies; the remainder had less than one genome copy per cell. These data suggested that the majority of American Burkitt's lymphomas lack EBV DNA. This suggestion was confirmed by Shiramizu *et al.* (1991), who studied tissues from 32 cases of Burkitt's lymphoma in the United States by hybridization with a probe derived from the *Bam*HI K fragment of the EBV genome. Twelve samples (38%) showed the presence of EBV DNA, which was confirmed with probes from the terminal repeat region of EBV. Judson *et al.* (1977), however, described four patients with Burkitt's lymphoma living within 50 km of each other in the United States. All showed anti-EBV antibodies similar to those observed in African patients (e.g. anti-VCA titre > 1:640 in all cases). Similarly, viral DNA and EBNA were found in tumour samples from three of the patients; the fourth was not tested.

Chan *et al.* (1995a) reported on the association between EBV and Burkitt's lymphoma in a Chinese population in Hong Kong. Among 18 patients who ranged in age from 4 to 85 years (median, 35.5), five aged 4, 6, 18, 57 and 58 years showed EBER-1 by in-situ hybridization in essentially all tumour cells. There were no cases of jaw tumour. Two cases (a T cell-rich large B-cell lymphoma and an anaplastic large-cell lymphoma) out of 54 of non-Hodgkin's lymphoma also showed positive results, the Reed-Sternberg-like cells found in one case being positive.

Anwar *et al.* (1995) found positive results by in-situ hybridization in 30 of 41 patients with Burkitt's lymphoma in Egypt.

Venkitaraman *et al.* (1983) reported on two Indian cases of Burkitt's lymphoma in children aged 5 and 11 years. Their anti-VCA titres were 1: 640 and 1:80; those of anti-EBNA were 1:1280 and 1:320, and those of anti-EA(R) were 1:80 and < 1:5, respectively. The presence of EBV in the tumour cells was not assessed.

Miyoshi *et al.* (1978) reported a case of EBNA-positive Burkitt's lymphoma in a person aged 29 years in Okayama, Japan, and referred to an earlier EBV-negative case (Tanaka *et al.*, 1976; Miyoshi *et al.*, 1977). Miyoshi (1983) subsequently described 14 patients (age range, 4–52 years), including these two cases, with Burkitt's lymphoma, five of which were jaw tumours. Two were EBV-seronegative, nine had anti-VCA titres of 1:40–1:1280, and three were not tested. The anti-EBNA titres in the six tested ranged from 1:20 to 1:80. Four of these patients had bone-marrow involvement at the time of presentation. Two of 14 tumours tested for EBNA by anticomplement immunofluorescence showed positive results. Okano *et al.* (1992) reported on seven patients in Hokkaido, Japan, aged 4–39 years. Five had anti-VCA titres of ≥ 1:320, including three cases with anti-VCA titres of 1:2560 and one with a titre of 1:320, with correspondingly high titres of antibodies to EA; two had titres of 1:40 and 1:80 and no anti-EA antibodies.

Four of these seven cases were EBNA-positive and three of the six tested showed EBV DNA by nucleic acid hybridization.

Philip (1985) mentioned the unpublished results of G. Lenoir showing that 16 of 67 Burkitt's lymphomas in Caucasian patients were EBNA-positive; 42 of the patients were from Lyon, France, of whom 6% had EBNA. Bornkamm et al. (1976) studied a single patient among a series of lymphoma patients in Germany and detected EBV DNA by reassociation kinetics. A single case of EBV-associated Burkitt's lymphoma that was EBNA-positive, with high titres to anti-VCA and anti-EA (1:2056), was reported in a 34-year-old woman in Germany (Kachel et al., 1980). Among 15 patients, five of whom were 15 years of age or less, with Burkitt's lymphoma reported in north-east Italy, only one was serologically positive for EBV among 12 patients tested (Tirelli et al., 1984). Biberfeld et al. (1981) reported an EBV-associated case in a 25-year-old man in Sweden. Çavdar et al. (1994) found EBV DNA by Southern blotting in 14 of 15 tumours examined in Turkey.

Roeser et al. (1977) studied a single Australian case of Burkitt's lymphoma and found the patient to be EBV-seronegative; no EBNA was found in the tumour cells.

In summary, the frequency of EBV association in Burkitt's lymphoma varies with geographical location. In most developed countries, some 20% or less of tumours are positive in most series, while in equatorial Africa about 95% are positive. In temperate regions in South America, such as Argentina and Chile, the rate of EBV association is lower than in tropical regions in the north of the continent; however, differences in the climate and in socioeconomic circumstances may account for the apparent differences in EBV association. If age at EBV infection is a primary determinant of association, socio-economic or lifestyle factors are likely to be of paramount importance. Too few data are available to assess the importance of age as a determinant of EBV status in countries in which the frequency of EBV-negative cases is sufficiently high to make such an investigation worthwhile.

2.1.3.2   Case–control studies

Because the prevalence of antibodies to EBV changes with age (see section 1.4), cases and controls should be closely matched for age; however, in most of the studies described below, the closeness of age-matching is not stated.

(a)   African patients

Henle et al. (1969) compared antibody titres to EBV (using an indirect immuno-fluorescent test for IgG antibodies to Burkitt's lymphoma tumour cells) in sera from Burkitt's lymphoma patients and various comparison groups, including 94 'recent onset' cases from East Africa (58 from Nairobi, Kenya and 36 from Kampala, Uganda). The comparison groups [selection criteria unspecified] comprised 62 children who were matched to cases for age, sex and tribe, 72 siblings and neighbours aged 1–15 years, 62 children from 'regions of high Burkitt's lymphoma incidence', 50 children from 'regions of low Burkitt's lymphoma incidence', 113 children aged six months to five years from 'well-baby' clinics in Nairobi and 130 patients without cancer from the paediatric wards

of Kenya Hospital, Nairobi. All of the Burkitt's lymphoma patients had EBV antibody titres ≥ 1:10, 87.2% having titres of ≥ 1/160. The geometric mean titre (GMT) was 1:326 (1:382 in Kenya, 1:243 in Uganda). In the 'control' groups, 18% had no antibody (13% in age-, sex- and tribe-matched controls), and only 14% had titres ≥ 1:160 (11% in age-, sex- and tribe-matched control [odds ratio, 54]). The GMT in the control children was 1:37 (1:36 in age-, sex- and tribe-matched controls).

Klein et al. (1970) investigated the presence of anti-EBV antibody in sera from 19 confirmed cases of Burkitt's lymphoma and 27 controls [selection criteria unspecified, but probably not matched in any way to the cases], using intracellular immuno-fluorescence in fixed smears of EBV-carrying lymphoblastoid (P3J) cell lines (anti-EBV), blocking of membrane fluorescence or immunoprecipitation against a soluble antigen from an EBV-carrying cell line. Of the 19 patients with confirmed Burkitt's lymphoma, 15 were highly reactive in all three tests, while only three of the 27 controls were; 19 of the controls had low titres in all three tests.

Henle et al. (1971b) compared the anti-EA antibody titres in sera from 156 Burkitt's lymphoma patients recruited from hospitals in Kampala, Uganda, and Nairobi, Kenya, with those in sera from 200 control children from the West Nile District of Uganda. The controls were selected to include children with low or absent titres of anti-VCA titre [but presumably were not matched to the cases]. At each level of anti-VCA titre, more of the Burkitt's lymphoma cases were anti-EA-positive. [The Mantel-Haenszel odds ratio (stratified for anti-VCA titre) associated with positive anti-EA is 19 (95% confidence interval [CI], 9.6–39)].

Hirshaut et al. (1973) studied cases of Burkitt's lymphoma from Africa (as well as from the United States, see below) with respect to anti-EBV antibody (measured by immunofluorescence and immunodiffusion) and compared them with age- and sex-matched controls from the same district and parents and siblings. When tested by immunofluorescence, EBV antibody was present in all 21 cases tested and in 21/27 of the age- and sex-matched controls, 76% of cases and 12% of controls having titres > 1:640 (odds ratio, 26; [95% CI, 5.3–120]). When tested by immunodiffusion, 67 of 73 cases and 19/30 controls gave positive results (odds ratio, 6.5; [95% CI, 2.1–20]).

Nkrumah et al. (1976) examined sera from 141 patients with Burkitt's lymphoma in Ghana. For a subset of 75 patients, they compared the anti-VCA antibody titres with those of 54 siblings and 50 age- and sex-matched neighbourhood controls. The GMT of antibody in the cases was significantly higher (1:424; p < 0.001) than that in either siblings (1:56) or neighbours (1:62).

(b) Non-African patients

Levine et al. (1972) examined patients with histologically identified Burkitt's lymphoma in the United States for anti-VCA antibodies against EBV. Twenty-four of 29 cases gave positive results, in comparison with 31/57 age- and sex-matched controls, and the antibody GMTs were higher (1:94) than in controls (1:11) or in 26 patients with lymphoblastic leukaemia (1:16), but were not as high as in African Burkitt's lymphoma patients (1:3338). A subset of patients under eight years of age all had anti-VCA anti-

bodies, however with a GMT of 1:425 (in comparison with 1:2848 in 11 African children), which was markedly higher than that in the 34 controls (1:4), 62% of whom had no anti-VCA.

Hirshaut *et al.* (1973) compared the EBV antibody titres of 15 patients with Burkitt's lymphoma in the United States with those in 15 age- and sex-matched controls and 25 parents and siblings of the cases. As ascertained by immunofluorescence, 10 cases and seven controls had antibodies. Although two patients and one control had antibody levels ≥ 1:640, the GMT was not significantly different between the two groups (5.2 versus 4.4). When 16 cases and 16 controls were ascertained by immunodiffusion, seven cases and six controls were found to have anti-EBV antibodies.

The data of Levine *et al.* (1972) were extended by Ablashi *et al.* (1974), who studied a wider range of antibodies in 21 patients in the United States with Burkitt's lymphoma, and compared them with 10 control subjects. [It is not clear how the controls were selected; one was a relative of a case.] All 15 sera tested for anti-VCA, anti-EA, anti-EBNA and complement-fixing antibodies had titres of at least one of the antibodies, whereas only one of seven control sera did. Five of the case sera did not show anti-VCA antibodies, but three of these sera had antibodies against EBNA. The GMTs for the patients were 1:111 for VCA, 1:17 for EA, 1:4.2 for complement fixation and 1:59 for EBNA; those of controls were 1:24, 1:2.7, 1:2.6 and 1:1.5, respectively.

Gotlieb-Stematsky *et al.* (1976) reported on 16 children with Burkitt's lymphoma in Israel. Although some of the 10 Arab children did not have elevated antibody titres against EBV (only one had negative serology), elevated titres against VCA were observed in the Burkitt's lymphoma patients, four having titres ≥ 1:160 (GMT, 1:26), whereas controls matched for age, sex and ethnicity showed no such increase, except for one subject with a titre of 1:160 (GMT, 1: 5.6).

Çavdar *et al.* (1994) reported on 81 children, of a median age of five years, with Burkitt's lymphoma in Turkey. In 32 of these patients in whom antibody titres against EBV were examined, they were found to be high, with 100% positive for anti-EBNA and a GMT of 1:320 for VCA. In 311 healthy children [unmatched for age or sex], the GMT for VCA was 1:93. [The proportion that was anti-EBNA positive was not reported.]

2.1.3.3 *Cohort study*

Between February 1972 and September 1974, serum samples were collected from about 42 000 children aged four to eight years in four counties in the West Nile District of Uganda. As of November 1977, 13 incident cases of histologically confirmed Burkitt's lymphoma and one case of 'unclassified lymphoma' had developed among the cohort members. The interval between initial serum collection and diagnosis of Burkitt's lymphoma ranged from seven to 54 months. EBV was found in seven of nine tumours tested by nucleic acid hybridization and in eight of these tumours by testing for EBNA. For each case of Burkitt's lymphoma, five control subjects from the cohort matched for age, sex and locality to the index case were chosen for a nested case–control analysis (de Thé *et al.*, 1978a). The 14 Burkitt's lymphoma patients had significantly higher pre-diagnostic anti-VCA titres than control subjects (GMT, 425.5 versus 125.8), but no

difference was observed between cases and controls in the titres of anti-EA and anti-EBNA. No difference in anti-VCA antibody titres was seen before and after diagnosis in the Burkitt's lymphoma patients, but seven of them developed anti-EA(R) antibodies; only one patient had anti-EA(D) antibodies before developing Burkitt's lymphoma. Similar temporal changes were not observed in the controls. Antibody titres to herpes simplex virus, cytomegalovirus and measles were unchanged, and the malaria parasitaemia rates before development of the tumour did not differ in patients and controls. This study clearly demonstrated that antibodies to EBV are present months to years before the development of Burkitt's lymphoma.

In 1982, Geser et al. reported the final results of this study. Two additional EBV-associated, histologically confirmed cases were detected up to 1979, both of which had high anti-VCA titres before the onset of Burkitt's lymphoma. One had anti-EA antibodies and the other did not, both before and after onset of tumour; anti-EBNA antibodies were also found before and after development of the tumour in both patients. This study showed that anti-VCA titres can be elevated as long as six years before the onset of Burkitt's lymphoma and as early as three months after birth. The relative risk for developing Burkitt's lymphoma increased multiplicatively by a factor of 5.1 for each two-fold dilution in anti-VCA titre for all cases of Burkitt's lymphoma and by a factor of 9.2 when the analysis was confined to cases in which EBV DNA was present in the tumour.

2.1.4   Cofactors

2.1.4.1   Malaria

Morrow (1985) summarized the data that suggest that malaria is a cofactor in the development of Burkitt's lymphoma:

- The incidence of Burkitt's lymphoma correlates within countries and internationally with the incidence of malaria and with parasitaemia rates.
- The age at which peak levels of antimalarial antibodies are acquired (5–8 years) corresponds to the peak age incidence of Burkitt's lymphoma.
- Individuals who live in urban areas where malarial transmission rates are lower also have a lower incidence of Burkitt's lymphoma.
- In regions in which death rates due to malaria have declined, there is a corresponding decline in the incidence of Burkitt's lymphoma.
- The age at onset of Burkitt's lymphoma in immigrants from malaria-free areas to malarious areas is higher than that of the original inhabitants.
- There is an inverse relationship between the age at onset of Burkitt's lymphoma and the intensity of infection with Plasmodium falciparum.
- There is an apparently reduced incidence (though not statistically significant) of Burkitt's lymphoma in individuals with the sickle-cell trait, which also protects against malaria.
- There is some evidence for a seasonal variation in the onset of Burkitt's lymphoma and for time–space clustering (see section 2.1.2.4).

(a) Ecological studies

Dalldorf (1962) first suggested that malaria is relevant to the development of Burkitt's lymphoma. Subsequently, he examined the distribution of Burkitt's lymphoma in Kenya (Dalldorf et al., 1964). Because of marked variability in the endemicity of malaria in Kenya, primarily related to the suitability of various environments for breeding of the mosquito vector, he was able to show that the highest incidence of Burkitt's lymphoma occurred in areas where malaria was holoendemic, namely in the coastal and lakeside regions (see Figure 7). The major vectors in these areas are Anopheles gambiae and A. funestus. Dalldorf pointed out that malaria affects the reticuloendothelial system and as such could well influence the development of Burkitt's lymphoma. Infestation rates by malarial parasites rise rapidly during the first year of life and often persist up to the age of three. Similarly high rates of infestation occurred in Papua–New Guinea (ten Seldam et al., 1966), the only other region in the world where malaria was known to be holo-endemic and where the incidence of Burkitt's lymphoma was similarly high.

Burkitt (1969) also noted that high incidences of Burkitt's lymphoma occurred only in regions where malaria was holo- or hyperendemic (equatorial Africa, Papua–New Guinea and parts of Malaysia) and that the disease was rare in regions in which malaria eradication campaigns had been successful (the islands of Zanzibar and Pemba, Singapore, Sri Lanka, the West Indies and India) or showed a marked decrease in incidence in regions where malaria eradication had been undertaken only recently.

Not all areas believed in the 1960s to be lymphoma-free, however, were known to be malaria-free. Kinshasa, for example, and Lambarene appeared to have a markedly lower incidence of Burkitt's lymphoma than surrounding areas (Kafuko & Burkitt, 1970); however, similar anomalies of distribution apply to known vectored viral diseases, such as yellow fever, and could be related to the paucity of suitable mosquito breeding grounds (e.g. in many urban regions) or even differences in exposure to mosquitoes related to differences in life style.

Interestingly, ten Seldam et al. (1966) were not convinced that similar climatic determinants of the distribution of Burkitt's lymphoma pertained in the territories of Papua–New Guinea, largely because of their observation that four of their 35 cases occurred in highlands above 5000 feet (1500 m). Booth et al. (1967) found, however, that the distribution of the 37 cases they reported (many of which had been reported by ten Seldam et al.) was consistent with that observed in Africa. Thus, 34 of their cases came from the coastal regions or plains immediately adjacent to the coast, and only three came from the highlands. They also remarked that the capital (Port Moresby) appeared to be tumour-free. They equated this with the six dry months of the year in Port Moresby and drew a parallel with the situation in Accra, Ghana. They estimated that the incidence of Burkitt's lymphoma in the highlands of New Guinea, where 40% of the people live, was 1 per 442 000 children, as compared with 1 in 29 000 in children living in the coastal region — a difference of some 14- or 15-fold. These figures, are, however, based on rather small numbers of observed cases. Tefuarani et al. (1988) later estimated the incidence of Burkitt's lymphoma in Papua–New Guinea on the basis of 109 cases. The incidence of all childhood tumours was 36.5 per 100 000 (based on a total of 680 cases) per annum,

and that of Burkitt's lymphoma, representing 16% of all tumours, was about 6 per 100 000 — closely similar to that in Africa.

Kafuko and Burkitt (1970) summarized the available information on the influence of malaria on the risk for developing Burkitt's lymphoma. They stated that the disease is not common in any area where malaria transmission occurs for less than six months in the year. They quoted an unpublished report by P.J. Cook and D.P. Burkitt on the frequency of Burkitt's lymphoma in relationship to gastric cancer, liver cancer, Kaposi's sarcoma and epithelioma at the site of a chronic tropical ulcer in a large number of hospitals in Uganda, Kenya and Tanzania, who found that the hospitals with the highest relative fraction of Burkitt's lymphoma were all in highly malarious areas. A survey of spleen size and malarial parasite rates was carried out in Uganda between 1963 and 1966 by testing children in over 100 schools and conducting 86 mass surveys; the degree of malarial endemicity was calculated according to accepted criteria based on parasitaemia rates in children of various ages. A close correlation was found between malarial endemicity and the incidence of Burkitt's lymphoma, although no statistical analysis was performed. In addition, it was shown that the parasite density index was highest in the 0–4-year-old age group and higher in the 0–10-year-old age group than in older individuals. Thus, the peak incidence of Burkitt's lymphoma corresponds to the age range in which malarial infestation rates are highest. It was also noted that adults who emigrate to malarious regions from malaria-free regions develop an intense parasitaemia not seen in the resident adults, who have acquired immunity. This is consistent with the greater incidence of Burkitt's lymphoma in immigrants than in the regions from which they come.

Morrow et al. (1976) and Morrow (1985) studied the incidence of Burkitt's lymphoma in various counties in the Mengo districts of central Uganda. On the basis of 130 cases from East and West Mengo, 100 with a confirmed histological diagnosis and 11 with a typical clinical syndrome of Burkitt's lymphoma, seen at Mulago Hospital between 1959 and 1968, they recorded a marked variation in the incidence of Burkitt's lymphoma which, they stated, corresponded to the recorded incidence rates of malaria in those regions. They observed a gradual decline in the incidence rate of Burkitt's lymphoma throughout this period, in spite of improved case ascertainment, as evidenced by a tripling of the number of other cancers reported from the Mengo districts to the Kampala Cancer Registry during the same period. The decline was particularly noticeable among the Ganda tribe, native to this region, which they suggested was due to the greater availability and use of chloroquine from Government and private dispensaries, with a consequent decline in the incidence of severe malarial infection. They showed that immigrants into Mengo from highland regions with low malarial prevalence who developed Burkitt's lymphoma were significantly older (median, 12 years) at the onset of Burkitt's lymphoma than patients from meso-endemic Mengo (median, 8 years; $p < 0.008$), while patients from Mengo were significantly older than patients from hyper- or holoendemic regions at lower altitude (median, 6 years; $p < 0.04$). These observations were considered to be consistent with the hypothesis that Burkitt's lymphoma is most likely to occur within a few years of a first intense infection with malaria. The authors also showed a seasonal variation in the onset of Burkitt's lymphoma, more cases occurring in the first half of the year. No time–space clustering was observed.

In Ghana, Biggar *et al.* (1981) showed a significant difference in malaria parasitaemia (*P. falciparum*) rates in urban (1.4%) and rural populations (22%), accompanied by similar differences in antimalarial antibody titres. Persons who had taken chloroquine for treatment of suspected malaria had a lower antibody frequency and lower titres than those who did not use chloroquine. This difference correlated with the distribution of Burkitt's lymphoma in Ghana (Biggar & Nkrumah, 1979).

Morrow (1985) reported a significant correlation ($p < 0.001$ by Spearman rank coefficient) between malaria parasitaemia (*P. falciparum*) rates and the incidence of Burkitt's lymphoma in various districts in Uganda. The parasitaemia rates ranged from 7.9% (in Ankole) to 75.2% (in Madi), and the incidence of Burkitt's lymphoma in children aged 0–14 from 0 (0.09 in Ankole) to 6.0 (in Lango) per 100 000. He also suggested that differences in vectorial capacity, i.e. the rate of potentially infective contacts per person by a vector, and the consequent levels of parasitaemia may account for differences in the likelihood that Burkitt's lymphoma will occur. In holoendemic areas, the peak age of prevalence and of the density of falciparum parasitaemia is two to three years, whereas the maximal level of antimalarial and non-antimalarial immunoglobulin occurs two to five years later, coinciding with the peak age of incidence of Burkitt's lymphoma in these regions (Molineaux & Gramiccia, 1980). No differences have been reported in the levels of malarial antibodies between patients and controls.

(b)   Relationship between Burkitt's lymphoma and sickle-cell trait

Since sickle-cell trait (AS haemoglobin) was shown to protect substantially against severe *P. falciparum* malaria (Allison, 1963), several attempts have been made to determine whether children with AS haemoglobin are less likely to develop Burkitt's lymphoma than others.

Williams (1966) compared the haemoglobin electrophoretic patterns of 100 children of the Yoruba tribe in Nigeria who had Burkitt's lymphoma (78% AA, 17% AS and 5% SC, SS, C or AC haemoglobin) with those of 331 similarly aged control patients from the same hospital (68% AA). Children with AA haemoglobin were more susceptible to Burkitt's lymphoma ($p = 0.03$). In another study of children over five years of age from a single Yoruba village near Ibadan, Nigeria, 66% had AA haemoglobin, 26% AS and 8% SC, SS, CC or AC, and there was no significant difference between patients and village controls (Gilles, 1963). Pike *et al.* (1970) conducted a case–control study in Uganda in which the controls were matched for age, sex, tribe and place of residence. Although the AS haemoglobin type appeared to be protective, the results were not statistically significant.

Studies in Uganda are difficult to conduct owing to the marked variation in the frequency of AS haemoglobin disease in different districts. Nkrumah and Perkins (1976) studied 112 patients with Burkitt's lymphoma, using the patients' nearest neighbours of the same age, sex and tribe as controls. Once again, no statistically significant difference in the frequency of patients with AS and other variations from AA haemoglobin was observed. The issue of whether individuals with variant haemoglobins are protected against the development of Burkitt's lymphoma therefore remains unanswered, largely

because of the lack of large enough studies. If there is a protective effect, it is presumably a modest one.

(c) Intervention study

Geser *et al.* (1989) undertook a study to determine whether suppression of malaria in the North Mara District of Tanzania by distributing chloroquine regularly to a cohort of children below the age of 10 years would result in a decrease in the incidence of Burkitt's lymphoma. This trial provided confirmation of the relationship between malaria prevalence rates and the incidence of Burkitt's lymphoma. Thus, before the trial (1964–76), all 85 cases of Burkitt's lymphoma in North Mara occurred in the lowlands (near Lake Victoria), with a high malarial parasitaemia rate (28–48%), and none occurred in the high plateau (over 1500 m) bordering Kenya, with a low malarial parasitaemia rate (5–24%). The prevalence of both malaria (parasitaemia rates of 11 and 13% in 1977 and 1978, with corresponding reductions in antimalarial fluorescence antibody titres) and Burkitt's lymphoma fell transiently during and after the period of chloroquine administration (1977–82), the latter to the lowest level ever recorded in the region: 0.5 per 100 000 in 1979 and 1981 in comparison with 2.6–6.9 per 100 000 before the trial. After 1979, the prevalence of malaria rapidly rose again to pre-trial levels, apparently because of problems in chloroquine distribution, although the incidence of Burkitt's lymphoma remained low until approximately two years after the distribution of chloroquine was stopped, when it reached a high of 7.1 in 1984. Interestingly, the prevalence of parasitaemia in South Mara, where chloroquine distribution was not conducted, rose throughout the trial from the pre-trial level of 23–28% to a high of 57% in 1985. Chloroquine resistance was not reported in the area until 1982 (Draper *et al.*, 1985). As anticipated, 90% of malaria detected was due to *P. falciparum*, the remainder being due to *P. malariae*. The authors reported some evidence of a trend towards a lower incidence of Burkitt's lymphoma in North Mara before the trial, although this did not reach statistical significance, whereas the reduction in the incidence of Burkitt's lymphoma between 1964 and 1982 was highly significant ($p < 0.001$).

2.1.4.2 Euphorbia tirucalli *and other medicinal plants*

Another possible cofactor in the pathogenesis of Burkitt's lymphoma in Africa is the plant *Euphorbia tirucalli*, which is used quite widely in equatorial Africa for medicinal purposes. Phorbol esters present in this plant have been reported to increase the ability of EBV to transform B lymphocytes and to increase the likelihood that a chromosomal translocation will develop in transformed cells (see section 4.2.4). Epidemiological information relevant to this issue is limited, although the distribution of *Euphorbia* in Africa has been examined.

Osato *et al.* (1987, 1990) found *Euphorbia tirucalli* around almost all houses, fields and reservoirs in villages surrounding Lake Victoria and in other high-incidence regions in Kenya and Tanzania. The plant was reported to be uncommon in areas in these countries in which Burkitt's lymphoma is uncommon.

van den Bosch *et al.* (1993) reported this plant to be frequent within the 'lymphoma belt' in equatorial Africa and to be used significantly more commonly in the homes of patients from Malawi with Burkitt's lymphoma than in control patients. A number of other plants of this family (Euphorbiaceae) and a variety of medicinal plants were also common. The tobacco plant was also significantly more commonly used in the homes of patients with Burkitt's lymphoma than those of controls, but use of other plants was equally distributed (Ito *et al.*, 1983).

van den Bosch *et al.* (1993) mentioned three patients who developed Burkitt's lymphoma after being treated for an illness with an extract of *Terminalia sericea*.

2.1.5   *Molecular epidemiology*

The identification of specific chromosomal abnormalities in patients with Burkitt's lymphoma (see section 4.2.1.1) permitted the examination of differences in the translocation-dependent structural alterations in *c-myc* in different regions of the world (Pelicci *et al.*, 1986; Shiramizu *et al.*, 1991; Gutiérrez *et al.*, 1992). In some tumours, the chromosomal breakpoint is quite distant from *c-myc* — often as much as several hundred kilobases upstream (in t(8;14)) or downstream (in variant translocations). This is the most frequent location of the breakpoint in African Burkitt's lymphoma, occurring in 75% of tumours, and is observed in about 50% of Brazilian tumours. Sometimes the breakpoint is close to the gene, i.e. within its 5′ flanking region, arbitrarily defined as extending from the upstream *Hind*III restriction enzyme site to exon 1. This is found in approximately half of the tumours occurring in Chile and Argentina (Gutiérrez *et al.*, 1992). Of tumours in North America, 60% have the breakpoints within the gene, i.e. within exon 1 or intron 1, and only 9% outside the *Hind*III fragment (Shiramizu *et al.*, 1991). Intron and exon breakpoints separate the coding region of *c-myc* from its major promoters, and transcripts are initiated from regions within the first intron. This is a marked difference from African Burkitt's lymphomas in which the *c-myc* gene is grossly intact, although point mutations in regulatory and coding regions are nearly always observed, implying different mechanisms leading to *c-myc* deregulation. Factors that are relevant to the induction of specific chromosomal breakpoint locations have not been identified but are likely to be environmental.

Interestingly, there is an apparent geographical association between the frequency of breakpoints outside the *c-myc* gene and the fraction of tumours associated with EBV (Magrath, 1997). In the United States, for example, only 15–20% of tumours are EBV-associated and only 9% have a breakpoint outside *c-myc*. In Africa, 95% of tumours are EBV-associated and 75% have breakpoints outside *c-myc*. Tumours from South American countries are intermediate in both respects. The nature of this relationship is uncertain, since, for example, non-African tumours with breakpoints outside *c-myc*, which occur at highest frequency (in countries for which data are available) in Brazil are not necessarily EBV-associated.

2.2 Non-Hodgkin's lymphomas other than Burkitt's lymphoma

2.2.1 Pathology

Non-Hodgkin's lymphomas are a numerous, heterogeneous group of malignancies that originate from lymphocytes. They can develop either from within organized lymphoid tissues, such as lymph nodes, or from other sites. Many lymphoid neoplasms pass through both solid tumour and circulating (leukaemic) phases.

Classification of non-Hodgkin's lymphomas is a complex and evolving process, and a thorough description of these diseases and their diagnostic criteria is beyond the scope of this volume. Several schemes of nomenclature have been proposed and used in different parts of the world, and many lymphoma entities that are recognized clinically and pathologically have been described by different names in these classifications. Lymphomas are divided into B-cell and T-cell neoplasms, according to their immunophenotypic characteristics. Within each group, there are numerous specific disease entities, many of which are associated with specific karyotypic abnormalities. Neoplasms of putative NK cell origin are provisionally grouped with the T-cell malignancies.

The International Lymphoma Study Group proposed in 1994 (Harris *et al.*, 1994) a 'Revised European–American Lymphoma (REAL) Classification' that attempted to link the major classification systems then in use in Europe and the United States. These included the Kiel classification, widely used in Europe (Stansfeld *et al.*, 1988), and the Working Formulation used in clinical trials in the United States (Anon., 1982). Table 8, adapted from Harris *et al.* (1994), is a list of non-Hodgkin's lymphoid neoplasms recognized by the International Lymphoma Study Group. The disease entities mentioned in section 2.2.2 are named in most cases according to the REAL classification; the pathology of Burkitt's lymphoma, a B-cell neoplasm, is presented in section 2.1.1. Hodgkin's disease, which is also considered within the REAL classification, is discussed separately in section 2.3.1.

2.2.2 Epidemiology

2.2.2.1 Descriptive epidemiology

Non-Hodgkin's lymphoma is estimated to account for 2.5% of all cancer cases worldwide (Pisani *et al.*, 1997). The incidence varies approximately sixfold, the highest reported rates being seen in whites in the United States and the lowest in Southeast Asia, India and sub-Saharan Africa (Parkin *et al.*, 1997). The incidence rises steeply with age. In the United States, the age-adjusted rates for 1990–94 were 9.2 cases per 100 000 annually among people under 65 years of age and 73.5 per 100 000 for those aged 65 and over (Ries *et al.*, 1997). Males are at higher risk, having incidence rates approximately 50–100% higher than females in most countries (Parkin *et al.*, 1997). The rates have been increasing steadily throughout the world, for reasons that are largely unexplained (Hartge *et al.*, 1994). Immunodeficiency of various etiologies, including HIV infection (see section 2.2.3), iatrogenic immunosuppression (see section 2.2.3) and congenital immunodeficiency (see section 2.2.4), is associated with a greatly increased risk, but the etiology

Table 8. The Revised European–American Lymphoma classification of non-Hodgkin's lymphomas

B-Cell neoplasms

I.   Precursor B-cell neoplasm: Precursor B-lymphoblastic leukaemia/lymphoma

II. Peripheral B-cell neoplasms

1. B-Cell chronic lymphocytic leukaemia/prolymphocytic leukaemia/small lymphocytic lymphoma
2. Lymphoplasmacytoid lymphoma/immunocytoma
3. Mantle-cell lymphoma
4. Follicle-centre lymphoma, follicular
5. Marginal zone B-cell lymphoma
   Extranodal (MALT-type +/− monocytoid B cells)
6. Provisional entity: Splenic marginal zone lymphoma
   (+/− villous lymphocytes)
7. Hairy-cell leukaemia
8. Plasmacytoma/plasma-cell myeloma
9. Diffuse large B-cell lymphoma[a] (centroblastic)
   Subtype: Primary mediastinal (thymic) B-cell lymphoma
10. Burkitt's lymphoma (small noncleaved cell)
11. Provisional entity: High-grade B-cell lymphoma, Burkitt-like[a]

T-Cell and putative NK-cell neoplasms

I.   Precursor T-cell neoplasm: Precursor T-lymphoblastic lymphoma/leukaemia

II. Peripheral T-cell and NK-cell neoplasms

1. T-Cell chronic lymphocytic leukaemia/prolymphocytic leukaemia
2. Large granular lymphocyte leukaemia
   T-cell type
   NK-cell type
3. Mycosis fungoides/Sezary syndrome
4. Peripheral T-cell lymphomas, unspecified[a]
   Provisional cytological categories: Medium-sized cell, mixed medium and large cell, large cell, lymphoepithelioid cell
5. Angioimmunoblastic T-cell lymphoma
6. Angiocentric lymphoma
7. Intestinal T-cell lymphoma (+/− enteropathy associated)
8. Adult T-cell lymphoma/leukaemia
9. Anaplastic large-cell lymphoma, CD30^{+}, T- and null-cell types
10. Provisional entity: Anaplastic large-cell lymphoma, Hodgkin's-like

From Harris *et al.* (1994)

[a]These categories are thought likely to include more than one disease entity.

of most cases of non-Hodgkin's lymphoma remains unknown. As immunodeficiency-associated lymphomas differ in a number of respects, the remainder of this section addresses only lymphomas in individuals with no evidence of prior immunocompromise.

2.2.2.2 Case reports and case series

Non-Hodgkin's lymphomas are pathologically diverse, as noted above, and their association with EBV differs accordingly.

(a) B-Cell non-Hodgkin's lymphoma

Although the B cell is the usual target in latent infection, the rate of detection of EBV was relatively low in three large series of B-cell lymphomas. Hamilton-Dutoit and Pallesen (1992) found that four of 105 B-cell lymphomas expressed LMP-1 and only one expressed EBNA-2. Hummel et al. (1995b) found EBER transcription in 54 (26%) of 208 tumours; in half of the positive cases, this was localized to non-neoplastic bystander cells. Of 27 cases with EBER in the tumour cells, more than 80% of tumour cells in 17 cases expressed EBER. d'Amore et al. (1996) found EBER transcription in 25 (6.5%) of 386 tumours, but only 6 (2%) had more than 10 EBER-positive tumour cells per medium-power ($\times$ 200) field on light microscopic examination. The three studies showed no consistent association between histological subtype and the presence of EBV (Table 9).

Primary central nervous system lymphoma, which is nearly always of B-cell origin, has been of particular interest since the first description by Hochberg et al. (1983) of an EBV-containing tumour detected by Southern blot, as EBV is almost universally present in brain lymphomas secondary to immunodeficiency (see section 2.2.3). In the series of central nervous system lymphomas reported by Murphy et al. (1990) and by DeAngelis et al. (1992), nearly half of the tumours were shown to contain EBV DNA by in-situ hybridization or PCR. These two initial reports were not, however, confirmed by many other studies of DNA (Bashir et al., 1990; Nakhleh et al., 1991; Geddes et al., 1992) and RNA (MacMahon et al., 1991; Chang et al., 1993a; Bashir et al., 1994; Bergmann et al., 1995) by in-situ hybridization, in which EBV was detected in no more than 12% of cases (Table 10).

B-Cell lymphoma of mucosa-associated lymphoid tissue (MALT), which is found in the stomach and elsewhere, warrants attention because of the association between gastric adenocarcinoma and EBV (see section 2.5). Liu et al. (1995) found one of 16 Japanese cases of gastric MALT lymphoma to contain EBER-1 by in-situ hybridization, and 20% of the tumour cells contained EBV. An additional case showed no EBV in the primary gastric tumour, but an EBER-positive tumour was found in a regional lymph node. d'Amore et al. (1996) found rare EBER-1/2-positive tumour cells in three of 28 MALT tumours in Denmark. Lee et al. (1997) found no EBER-1-positive cases among eight gastric MALT tumours in the Republic of Korea. A similarly low frequency of EBV-positivity has been found in MALT tumours outside of the stomach: using PCR, Diss et al. (1995) found EBV DNA in three of 36 parotid MALT tumours in the United

Kingdom; in the one case with EBV DNA detected by in-situ hybridization, less than 5% of tumour cells contained EBER.

Table 9. Presence of EBV in B-cell non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	Histology	No. of cases	No. with EBV	Tumour cells with EBV
Hamilton-Dutoit & Pallesen (1992)	Denmark	LMP-1, IHC	Diffuse large cell	37	0	
			Immunoblastic	54	3	< 50%
			Small non-cleaved cell	10	0	
			Anaplastic large cell	4	1	
Hummel et al. (1995b)	Germany	EBER-1/2, ISH	Low-grade	65	3	Variable
			Diffuse large cell	39	3	
			Immunoblastic	28	4	
			Small non-cleaved cell	36	7	
			Anaplastic large cell	16	3	
			Other high grade	24	7	
		LMP, IHC	Low grade	65	1	Low
			Diffuse large cell	39	2	
			Immunoblastic	28	2	
			Small non-cleaved cell	36	0	
			Anaplastic large cell	16	3	
			Other high grade	24	3	
d'Amore et al. (1996)	Denmark	EBER-1/2, ISH	Low grade	154	9	Few
			Diffuse large cell	74	3	
			Immunoblastic	21	1	
			Small non-cleaved cell	24	3	
			Anaplastic large cell	7	0	
			Other high grade	106	9	

All in non-immunocompromised individuals
EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein

(b) Angiocentric T-cell lymphoma

Sinonasal T-cell lymphoma, which encompasses entities also referred to as lethal midline granuloma and midline reticulosis, is the non-Hodgkin's lymphoma most strongly associated with EBV. Series in Asia (Harabuchi et al., 1990; Ho et al., 1990; Chan et al., 1994a; Ko & Lee, 1994; Lee et al., 1994a; Peh et al., 1995; Harabuchi et al., 1996), South America (Arber et al., 1993), Europe (O'Leary & Kennedy, 1995; Dictor et al., 1996; Kanavaros et al., 1996) and the United States (Weiss et al., 1992a; Davison et al., 1996) consistently showed the presence of EBV in the great majority of cases, EBV being present in most tumour cells (Table 11). CD56-positive tumours, which are suggested to be of NK-cell derivation, were uniformly EBV-positive, whereas CD56-negative tumours were more frequently EBV-negative (Chan et al., 1994a; Harabuchi

et al., 1996; Kanavaros *et al.*, 1996). EBV-positive tumours consistently expressed the transforming EBV protein LMP-1 (Harabuchi *et al.*, 1996; Kanavaros *et al.*, 1996), and EBV terminal repeat sequences have been shown to have monoclonal or biclonal infection (Ho *et al.*, 1990; Harabuchi *et al.*, 1996).

Table 10. Presence of EBV in primary central nervous system non-Hodgkin's lymphoma tissue in HIV-negative cases

Reference	Study area	Detection method	No. of cases	No. with EBV
Hochberg *et al.* (1983)	USA	EBV DNA, SB	1	1
Bashir *et al.* (1990)	USA	BamHI V, ISH	10	0
Murphy *et al.* (1990)	UK	BamHI W, ISH	24	11
Rouah *et al.* (1990)	USA	EBV DNA, ISH	5	2
		EBV DNA, PCR	7	2
Bignon *et al.* (1991)	France	EBV DNA, PCR	9	1
MacMahon *et al.* (1991)	USA	EBER-1, ISH	14	1
Nakhleh *et al.* (1991)	USA	BamHI W, ISH	17	2
Geddes *et al.* (1992)	UK	BamHI W, ISH	43	2
DeAngelis *et al.* (1992)	USA	EBV DNA, PCR	13	7
Chang *et al.* (1993a)	USA	EBER-1, ISH	27	1
Bashir *et al.* (1994)	USA	EBER-1, ISH	9	0
Bergmann *et al.* (1995/96)	Germany	EBER, ISH	36	0

BamHI V and BamHI W, DNA restriction fragments; EBER, EBV-encoded RNA; ISH, in-situ hybridization; PCR, polymerase chain reaction; SB, Southern blot

In contrast, EBV is much less frequently found in sinonasal B-cell lymphoma. Chan *et al.* (1994a) and Peh *et al.* (1995) found one EBV-positive case among ten and nine tumours respectively, and Kanavaros *et al.* (1996) found one among 10 tumours; a somewhat higher frequency was found by Weiss *et al.* (1992a) (Table 11).

B-Cell lymphomas of the nasopharynx, tonsil and tongue ('Waldeyer's ring') less frequently contain EBV, the frequency ranging from 0 to 13% in six series (Chan *et al.*, 1994a; Ko & Lee, 1994; Lee *et al.*, 1994a; O'Leary & Kennedy, 1995; Peh *et al.*, 1995; Kanavaros *et al.*, 1996), with a slightly higher frequency in the report of Weiss *et al.* (1992a) (Table 11). T-Cell tumours of Waldeyer's ring have a more variable association with EBV: 0/8 and 3/6 tumours were found to contain EBV in the two largest series (Ko & Lee, 1994; Kanavaros *et al.* 1996).

Lymphomatoid granulomatosis, which resembles sinonasal lymphoma histologically, is another condition in which EBV is highly prevalent. In eight series, EBV was detected in the majority of cases (Katzenstein & Peiper, 1990; Medeiros *et al.*, 1991; Guinee *et al.*, 1994; Tsang *et al.*, 1994; Myers *et al.*, 1995; Nicholson *et al.*, 1996; Takeshita *et al.*, 1996; Wilson *et al.*, 1996b); in two other series it was detected at lower frequency (Sabourin *et al.*, 1993; Kobashi *et al.*, 1996) (Table 12). The diagnostic classification of

Table 11. Presence of EBV in sinonasal and Waldeyer's ring non-Hodgkin's lymphomas

Reference	Study area	Detection method	Immuno-phenotype	Sinonasal		Waldeyer's ring		Tumour cells with EBV	Comments
				No. of cases	No. with EBV	No. of cases	No. with EBV		
Harabuchi et al. (1990)	Japan	BamHI W, ISH	T Cell	5	5			80–90%	
		EBNA, IFA	T Cell	5	5				
Ho et al. (1990)	Hong Kong	BamHI W, SB	T Cell	7	7				EBV mono- or biclonal
			B Cell	3	2				
Weiss et al. (1989a; 1992a)	USA	BamHI W, ISH	T Cell	3	3			Uniform	
			B Cell	5	2	10	2		
Arber et al. (1993)	Peru	EBER-1, ISH	T Cell	11	11			Many	
			B Cell	2	1				
			Indeterminate	1	1				
Chan et al. (1994a)	Hong Kong	EBER-1/2, ISH	T Cell	30	25	1	0	Most	21/21 CD56$^+$ and 4/9 CD56$^-$ contained EBV
			B Cell	10	1	20	0		
Ko & Lee (1994)	Republic of Korea	EBER, ISH	T Cell	10	7	8	0		
			B Cell			12	1		
Lee et al. (1994a)	Republic of Korea	EBER-1/2, ISH	T Cell	12	12	2	2	Many	
			B Cell	1	0	16	2		
O'Leary & Kennedy (1995)	Ireland	EBER-1/2, ISH	T Cell	8	6			Many	
			B Cell	3	0	10	2		
Peh et al. (1995)	Malaysia	EBER-1, ISH	T Cell	10	9	3	2	70–100%	
			B Cell	9	1	7	0		
Davison et al. (1996)	USA	EBER-1, ISH	T Cell	30	29			Majority	
Dictor et al. (1996)	Sweden	EBER-1, ISH	T Cell	12	12			50–100%	

Table 11 (contd)

Reference	Study area	Detection method	Immuno-phenotype	Sinonasal		Waldeyer's ring		Tumour cells with EBV	Comments
				No. of cases	No. with EBV	No. of cases	No. with EBV		
Harabuchi *et al.* (1996)	Japan	EBER-1, ISH	T Cell	16	16			> 50%	EBV monoclonal; 9/9 CD56$^+$; includes 6 cases from Harabuchi *et al.* (1990)
		LMP-1, IHC		9	9			> 50%	
Kanavaros *et al.* (1996)	France and Greece	EBER-1/2, ISH	B Cell	17	16	6	3	> 50%	11/11 CD56$^+$ and 1/3 CD56$^-$ contained EBV
				10	1	22	1		
		LMP-1, IHC	T Cell	17	16	6	3	1–50%	
			B Cell	10	0	22	0		

All in non-immunocompromised individuals

EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; SB, Southern blot

Table 12. Presence of EBV in lymphomatoid granulomatosis tissue

Reference	Study area	Detection method	Immunophenotype	No. of cases	No. with EBV	Tumours with EBV	Comments
Katzenstein & Peiper (1990)	USA	EBV DNA, PCR		29	21		
Medeiros *et al.* (1991)	USA	*Bam*HI W, PCR	T Cell	5	3		
		EBV, SB	T Cell	7	2		
Sabourin *et al.* (1993)	France	EBER-1/2, ISH	T Cell	6	2	Numerous	
			B Cell	1	0		
		LMP, IHC	T Cell	6	2		
			B Cell	1	0		
Guinee *et al.* (1994)	USA	EBER-1, ISH	T and B cell	10	10	Only in B cells	B cells monoclonal, T cells polyclonal
Tsang *et al.* (1994)	Hong Kong	EBER-1/2, ISH, IHC	NK Cell	15	10	Most	
Myers *et al.* (1995)	USA	EBER-1, ISH	T Cell	6	0		
			T and B cell	11	10	Only in B cells	
Kobashi *et al.* (1996)	Japan	EBER-1/2, ISH	NK Cell	9	3	Most	EBV mono- or biclonal
		LMP-1, IHC	NK Cell	9	2		
Nicholson *et al.* (1996)	UK	EBER-1, ISH	T and B cell	7	4	Only in B cells	
Takeshita *et al.* (1996)	Japan	EBER-1, ISH	T and B cell	3	2	Some to many	1 only in B cells
			B Cell	9	4		
			NK Cell	2	2		
		LMP, IHC	T and B cell	3	2		1 only in B cells
			B Cell	9	4		
			NK Cell	2	1		
Wilson *et al.* (1996b)	USA	EBER-1, ISH	T and B cell	4	4	Only in B cells	

All in non-immunocompromised individuals

*Bam*HI W, DNA restriction fragment; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IFA, immunofluorescence assay; IHC, immuno-histochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot; NK, natural killer

this disorder has recently undergone revision. Guinee et al. (1994) found that despite a predominance of T cells in these tumours, a minor population of monoclonal B cells is present within a polyclonal population of T cells. Furthermore, in all 10 cases, EBV was present in the B-cell population only. Myers et al. (1995), Nicholson et al. (1996) and Takeshita et al. (1996) reported a similar restriction of EBV to B cells in tumours of mixed immunophenotype. Kobashi et al. (1996) found monoclonal or biclonal EBV by terminal-repeat fragment analysis in three of three EBV-positive tumours.

(c)   Other peripheral T-cell lymphomas

These entities have been reviewed by Pallesen et al. (1993). Other peripheral T-cell lymphomas commonly contain EBV (Table 13). In 12 series from North America, Europe and Asia, the frequency of EBV positivity ranged from 18 to 70% in all but one study (Herbst et al., 1991b; Lee et al., 1991; Kanavaros et al., 1992; Ott et al., 1992; Weiss et al., 1992b; Korbjuhn et al., 1993; Tsang et al., 1994; Zhou et al., 1994; Lopategui et al., 1995; d'Amore et al., 1996; Hirose et al., 1996). Hamilton-Dutoit and Pallesen (1992) found only 10% positivity, but used an assay with low sensitivity. The presence of EBV in tumour cells varies, some tumours showing its presence uniformly and others in only a fraction of cells. Terminal-repeat assays have been used to confirm the mono- or oligoclonality of latent EBV episomes (Ott et al., 1992; Hirose et al., 1996).

There is some variation by histological subtype. Most angioimmunoblastic lymphomas contain EBV (Ott et al., 1992; Weiss et al., 1992b; Zhou et al., 1994; d'Amore et al., 1996; Hirose et al., 1996), although in one study tumour cells containing EBV were rare (Tsang et al., 1994). Most cases of anaplastic large-cell lymphoma do not contain EBV (Herbst et al., 1991b; Hamilton-Dutoit & Pallesen, 1992; Kanavaros et al., 1992; Ott et al., 1992; Zhou et al., 1994; Lopategui et al., 1995; d'Amore et al., 1996). Pleomorphic and other tumour types, such as lymphoepithelioid and T-zone tumours, show an intermediate frequency, about one-third of the reported cases containing EBV (Hamilton-Dutoit & Pallesen, 1992; Ott et al., 1992; Korbjuhn et al., 1993; Tsang et al., 1994; Zhou et al., 1994; d'Amore et al., 1996; Hirose et al., 1996; Table 13).

Enteropathy-associated T-cell lymphoma is a distinct entity which has been investigated for the presence of EBV by EBER in-situ hybridization in four studies. Pan et al. (1993) detected EBV in four of 11 cases in the United Kingdom; in the cases with EBV, over 80% of tumour cells contained EBER, and the EBV was shown to be monoclonal by terminal-repeat analysis. These findings were not confirmed in other studies. Korbjuhn et al. (1993) found EBV in two of 10 cases in Germany; in both cases, less than 20% of the tumour cells contained EBER. Similarly, Ilyas et al. (1995) found EBV in none of seven cases in the United Kingdom, and Walsh et al. (1995) found EBV in only one of 16 cases in Ireland.

EBV was detected infrequently in low-grade cutaneous lymphomas (mycosis fungoides and Sézary syndrome) in three studies in which EBER-1/2 was analysed by in-situ hybridization. Anagnostopoulos et al. (1996) found EBER in four of 42 cases in Germany, but less than 1% of the tumour cells contained EBV. Angel et al. (1996) in the

Table 13. Presence of EBV in peripheral T-cell lymphoma tissue

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Herbst *et al.* (1991b)	Germany	*Bam*HI W, PCR	Anaplastic large cell	18	5		
		LMP, IHC		18	2	50–90%	
Lee *et al.*(1991)	Taiwan	EBV DNA, SB		6	4		Paediatric series
Hamilton-Dutoit & Pallesen (1992)	Denmark	LMP-1, IHC	Pleomorphic	50	7	< 50%	
			Angioimmunoblastic	4	1		
			Anaplastic large cell	19	0		
			Other	9	0		
Kanavaros *et al.* (1992)	Netherlands	EBV DNA, PCR, ISH	Anaplastic large cell	3	2	50%	
		LMP, IHC		11	1	Variable	
Ott *et al.* (1992)	Germany	*Bam*HI W, SB	Pleomorphic	14	5	Variable	EBV mono- or biclonal
			Angioimmunoblastic	14	8		
			Anaplastic large cell	8	3		
			Other	10	2		
Weiss *et al.* (1992b)	USA	EBER-1, ISH	Angioimmunoblastic	23	19	Variable	Mostly B cells
Korbjuhn *et al.* (1993)	Germany	EBER, ISH	Pleomorphic	81	38	1–100%	
Tsang *et al.* (1994)	Hong Kong	EBER-1/2, ISH	Pleomorphic	7	3	≥ 90%	
			Angioimmunoblastic	6	0		Rare EBER-positive cells in 4 cases
			Other	2	0		
Zhou *et al.* (1994)	China	EBER ISH	Pleomorphic	27	18	≥ 50%	
			Angioimmunoblastic	6	4		
			Anaplastic large cell	2	0		
			Other	4	2		
		LMP-1, IHC	Pleomorphic	26	13		
			Angioimmunoblastic	6	4		
			Anaplastic large cell	2	0		
			Other	4	2		

Table 13 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Lopategui et al. (1995)	USA and Hong Kong	EBER-1, ISH LMP-1, IHC	Anaplastic large cell	15 15	3 0	25–90%	
d'Amore et al. (1996)	Denmark	EBER-1/2, ISH	Pleomorphic	67	24	< 10–> 50%	
			Angioimmunoblastic	13	11		B cells also positive
			Anaplastic large cell	9	0		
			Other	21	0		
		LMP-1, IHC	Pleomorphic	24	8		
			Angioimmunoblastic	11	2		
			Other	5	2		
Hirose et al. (1996)	Japan	EBER, ISH	Pleomorphic	10	4	Few to extensive	
			Angioimmunoblastic	9	7		B cells also positive; EBV mono- or biclonal
			Other	4	4		
		LMP-1, IHC	Pleomorphic	10	3		
			Angioimmunoblastic	9	1		
			Other	4	0		

All in non-immunocompromised individuals

BamHI W, DNA restriction fragment; EBER, EBV-encoded RNA; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot

United Kingdom and d'Amore *et al.* (1996) in Denmark found no EBV in 25 and 13 cases, respectively.

2.2.2.3 Cohort studies

The association between abnormal EBV seroreactivity and the risk for subsequent non-Hodgkin's lymphoma has been examined in two studies. Mueller *et al.* (1991) performed a nested case–control study based on sera from four banks established between 1964 and 1974 containing specimens from over 240 000 people in Norway and the United States, 104 of whom had developed non-Hodgkin's lymphoma an average of 63 months after their serum had been collected. They were compared with 259 controls matched for age, sex, ethnic group and date of serum collection. Immunofluorescence assays showed that elevated levels of IgG (titre, ≥ 1:320) and IgM (titre, ≥ 1:5) antibodies against EBV VCA were associated with relative risks for non-Hodgkin's lymphoma of 2.5 (95% CI, 1.1–5.7) for IgG and 3.2 (95% CI, 1.3–7.5) for IgM. Low titres (≤ 1:5) of antibody to EBNA were associated with a decreased risk for lymphoma, although the effect was significant only for cases that developed within five years of serum collection. No differences were reported in subgroup analyses of follicular versus diffuse, large-cell versus mixed- and small-cell, or low- versus intermediate- versus high-grade lymphomas. [The Working Group noted that no T versus B immunophenotyping or tissue analyses for EBV markers were reported, and that the relative risks may have been attenuated by the inclusion of all types of non-Hodgkin's lymphoma.]

Lehtinen *et al.* (1993) performed a nested case–control study of 39 000 Finnish adults from whom serum was collected between 1968 and 1972. Eleven subjects who developed non-Hodgkin's lymphoma 1–12 years after serum collection were compared with 22 controls matched for age, sex, municipality and date of phlebotomy. Enzyme immunoassays showed no significant differences in antibodies to VCA, EA or EBNA. [The Working Group noted that immunofluorescence is the standard method for detecting EBV antibodies and the relevance of the authors' enzyme immunoassay is uncertain.]

2.2.3 Human immunodeficiency virus as a cofactor

HIV and HHV8 also play a role in the pathogenesis of EBV-associated non-Hodgkin's lymphoma. The monograph on HHV8 in this volume covers its interaction with EBV; this section deals only with HIV.

Lymphomas in HIV-infected patients are nearly always of B-cell origin. Under the Working Formulation classification (Rosenberg, 1982), they include tumours of the small non-cleaved cell (diffuse large B cell in the REAL classification), large cell diffuse and immunoblastic types; anaplastic large cell and unclassified high-grade types are also observed. Like other immune-deficient conditions, HIV infection is associated in particular with primary central nervous system lymphoma. The association of EBV with HIV-related lymphomas varies.

2.2.3.1 Primary central nervous system lymphomas

Unlike central nervous system lymphomas in immunocompetent individuals, those found in association with HIV infection nearly always contain detectable EBV. In nine reported series, EBV was detected in all cases in five studies (MacMahon *et al.*, 1991; Chang *et al.*, 1993a; Cinque *et al.*, 1993; Bashir *et al.*, 1994; Arribas *et al.*, 1995) and in the majority of cases in the remaining four studies (Bashir *et al.*, 1990; DeAngelis *et al.*, 1992; Morgello, 1992; Bergmann *et al.*, 1995; Table 14). In-situ hybridization has been used to show that most or all of the tumour cells contained EBV RNA (MacMahon *et al.*, 1991; Chang *et al.*, 1993a; Cinque *et al.*, 1993; Bashir *et al.*, 1994). LMP expression is more variable; nine of 21 tumours reported by MacMahon *et al.* (1991) and 13 of 19 reported by Bergmann *et al.* (1995/96) were shown by immunohistochemistry to contain LMP.

Table 14. Presence of EBV in HIV-associated primary central nervous system non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	No. of cases	No. with EBV	Tumour cells with EBV
Bashir *et al.* (1990)	USA	*Bam*HI V, ISH	5	4	Variable
MacMahon *et al.* (1991)	USA	EBER-1, ISH	21	21	Uniform
		LMP, IHC	21	9	Many cells
DeAngelis *et al.* (1992)	USA	*Bam*HI W, PCR	13	11	
Morgello (1992)	USA	EBNA-1, PCR	12	6	
Chang *et al.* (1993a)	USA	EBER-1, ISH	5	5	Uniform
Cinque *et al.* (1993)	Sweden and Italy	EBER, ISH	16	16	40–90%
Bashir *et al.* (1994)	USA	EBER-1, ISH	5	5	Most
Arribas *et al.* (1995)	USA	LMP, IHC	6	6	Variable
Bergmann *et al.* (1995/96)	Germany	EBER, ISH	19	15	
		LMP, IHC	19	13	

*Bam*HI V and *Bam*HI W, DNA restriction fragments; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction

2.2.3.2 Systemic non-Hodgkin's lymphomas

EBV is found in a large fraction of systemic tumours. In 13 series of 10 or more patients, the frequency of EBV positivity ranged from 38 to 79% (Subar *et al.*, 1988; Borisch-Chappuis *et al.*, 1990a,b; Boyle *et al.*, 1991; Guarner *et al.*, 1991; Borisch *et al.*, 1992a; Ballerini *et al.*, 1993; Carbone *et al.*, 1993a,b; Hamilton-Dutoit *et al.*, 1993a; Shibata *et al.*, 1993; Raphael *et al.*, 1994; Bacchi *et al.*, 1996b; Carbone *et al.*, 1996; Table 15). In many of the tumours, the majority of the cells contained EBV (Borisch-Chappuis *et al.*, 1990a,b; Guarner *et al.*, 1991), although in several studies the fraction of EBV positivity was somewhat variable (Borisch *et al.*, 1992a; Carbone *et al.*, 1993a;

Table 15. Presence of EBV in HIV-associated systemic non-Hodgkin's lymphoma tissue

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Subar et al. (1988)	USA	EBV DNA, SB	Diffuse large cell	4	1		
			Immunoblastic	2	2		
			Small non-cleaved cell	10	3		
		EBNA, IFA	Diffuse large cell	3	1	80%	
			Immunoblastic	1	1		
			Small non-cleaved cell	4	2		
Borisch-Chappuis et al. (1990a,b)	Germany	BamHI W, ISH	Diffuse large cell	1	1	> 50%	
			Immunoblastic	2	1		EBV-positive tumour, T immunophenotype
			Small non-cleaved cell	9	4		
			Anaplastic large cell	1	0		
			Other high grade	1	1		
Boiocchi et al. (1990)	Italy	BamHI W, SB	Immunoblastic	2	1		EBV monoclonal
			Small non-cleaved cell	3	0		
Boyle et al. (1991)	Australia	EBNA-1, PCR	Immunoblastic	12	6		
			Small non-cleaved cell	7	3		
			Other high grade	1	1		
Guarner et al. (1991)	USA	EBV DNA, ISH	Diffuse large cell	4	2	> 80%	
			Immunoblastic	2	1		
			Small non-cleaved cell	6	6		
			Other	2	0		
MacMahon et al. (1991)	USA	EBER-1, ISH	NR	7	3		
Neri et al. (1991)	USA	BamHI	Diffuse large cell	1	1		EBV monoclonal in 10/10
			Immunoblastic	1	1		
			Small non-cleaved cell	5	5		
			Other	3	3		

Table 15 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Borisch *et al.* (1992a)	Switzerland	EBER-1, ISH	Diffuse large cell	3	3	Variable	
			Immunoblastic	8	8		
			Small non-cleaved cell	2	0		
			Anaplastic large cell	1	0		
		LMP, IHC	Diffuse large cell	3	1		
			Immunoblastic	8	3		
			Small non-cleaved cell	2	0		
			Anaplastic large cell	1	0		
Ballerini *et al.* (1993)	USA	EBV DNA, SB and EBNA-1, IHC	Diffuse large cell	4	1		EBV monoclonal
			Immunoblastic	4	4		
			Small non-cleaved cell	16	5		
Carbone *et al.* (1993a)	Italy	EBV DNA, ISH	Immunoblastic	4	1	Variable	
			Small non-cleaved cell	9	4		
			Anaplastic large cell	9	7		
Carbone *et al.* (1993b)	Italy	EBER-1/2 or *Bam*HI W, ISH	Diffuse large cell	6	1		
			Immunoblastic	7	3		
			Small non-cleaved cell	15	6		
			Anaplastic large cell	12	10		
		LMP, IHC	Diffuse large cell	6	0		
			Immunoblastic	7	3		
			Small non-cleaved cell	15	0		
			Anaplastic large cell	12	9		
Hamilton-Dutoit *et al.* (1993a)	France and Denmark	EBER-1, ISH or *Bam*HI W, SB LMP-1, IHC	Immunoblastic	30	22	30–90%	
			Small non-cleaved cell	19	11		
			Immunoblastic	30	16		
			Small non-cleaved cell	19	3		

Table 15 (contd)

Reference	Study area	Detection method	Histological type	No. of cases	No. with EBV	Tumour cells with EBV	Comments
Shibata et al. (1993)	USA	EBNA-1, PCR and EBER-1, ISH	Diffuse large cell	11	6		EBV monoclonal
			Immunoblastic	20	17		
			Small non-cleaved cell	28	16		
Raphael et al. (1994)	France	BamHI W, SB or EBER, ISH	Diffuse large cell	3	0		
			Immunoblastic	9	8		
			Small non-cleaved cell	16	8		
			Anaplastic large cell	1	0		
			Other high grade	3	1		
Bacchi et al. (1996b)	Brazil	EBER-1, ISH	Diffuse large cell	11	6	40–100%	1 EBV-negative tumour T immunophenotype
			Immunoblastic	4	3		
			Small non-cleaved cell	5	2		
Carbone et al. (1996)	Italy	EBER-1/2, ISH	Small non-cleaved cell	11	4	25–75%	EBV monoclonal
			Anaplastic large cell	5	4		
		LMP, IHC	Small non-cleaved cell	10	0		
			Anaplastic large cell	5	3		

BamHI V and BamHI W, DNA restriction fragments; EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; IFA, immunofluorescence assay; IHC, immunohistochemistry; ISH, in-situ hybridization; LMP, latent membrane protein; PCR, polymerase chain reaction; SB, Southern blot; NR, not reported

Hamilton-Dutoit *et al.*, 1993a; Bacchi *et al.*, 1996b; Carbone *et al.*, 1996). Immuno-blastic lymphomas (classified as diffuse large-cell lymphomas in the REAL classification) tend to be associated with more advanced immunosuppression, and these tumours more commonly contain EBV than other diffuse large-cell and small non-cleaved-cell lymphomas (Ballerini *et al.*, 1993; Hamilton-Dutoit *et al.*, 1993a; Raphael *et al.*, 1994); Carbone *et al.* (1993a,b; 1996) also noted a higher frequency of EBV positivity in anaplastic large-cell tumours. EBV monoclonality has been consistently observed by terminal-repeat sequence analysis (Boiocchi *et al.*, 1990; Neri *et al.*, 1991; Ballerini *et al.*, 1993; Shibata *et al.*, 1993; Carbone *et al.*, 1996).

2.2.4 Congenital immunodeficiency syndromes

Patients with X-linked lymphoproliferative syndrome (see section 4.2) are at increased risk for non-Hodgkin's lymphoma, which occurs in up to 25% of affected individuals (Purtilo, 1981; Sullivan & Woda, 1989). Wiskott-Aldrich syndrome, ataxia telangiectasia and other primary immunodeficiency syndromes are also associated with greatly increased risks for lymphoma (Filipovich *et al.*, 1992; Schuster *et al.*, 1995).

Patients with X-linked lymphoproliferative syndrome have a specific defect in their control of infection with EBV. EBV was found in all of 82 lymphomas associated with this condition (Sullivan & Woda, 1989). Several case reports of lymphomas associated with Wiskott-Aldrich syndrome (Okano *et al.*, 1984; Nakhleh *et al.*, 1991; Nakanishi *et al.*, 1993), ataxia telangiectasia (Saemundson *et al.*, 1981; Mselati *et al.*, 1983) and severe combined immunodeficiency (Garcia *et al.*, 1987) have confirmed the near universal presence of EBV in lymphomas associated with congenital immunodeficiency, although one EBV-negative case has been reported (Okano *et al.*, 1984; Table 16).

Table 16. Prevalence of EBV-associated lymphomas arising in children with primary immunodeficiency

Reference	Study area	Detection method	Primary immunodeficiency	No. of cases	No. with EBV
Sullivan & Woda (1989)	USA and Europe	Immunofluorescence	X-linked lymphoproliferative	82	82
Okano *et al.* (1984)	Japan	Immunofluorescence and EBV DNA	Wiskott-Aldrich	2	1
Nakanishi *et al.* (1993)	Japan	EBV DNA	Wiskott-Aldrich	1	1
Nakhleh *et al.* (1991)	USA	EBV DNA, in-situ hybridization	Wiskott-Aldrich	1	1
Saemundsen *et al.* (1981)	Turkey	EBV DNA	Ataxia telangiectasia	1	1
Mselati *et al.* (1983)	France	Serology	Ataxia telangiectasia	2	2
Garcia *et al.* (1987)	USA	EBV DNA	Severe combined	1	1

2.3 Hodgkin's disease

2.3.1 *Pathology and clinical features*

Histologically, Hodgkin's disease is characterized by mononuclear Hodgkin cells and their multinucleated variants, the Reed-Sternberg cells, together abbreviated as HRS cells. HRS cells are embedded in a background of abundant reactive cells, including lymphocytes, plasma cells, histiocytes and eosinophils (Lukes & Butler, 1966). Typically, HRS cells account for only a small proportion of cells in an affected lymph node, rarely amounting to more than 2% of the total cell population. The Rye classification distinguishes four major types of Hodgkin's disease: nodular lymphocyte predominant, nodular sclerosis, mixed cellularity and lymphocyte-depleted (Lukes & Butler, 1966; Herbst & Niedobitek, 1993; Harris *et al.*, 1994). It is now accepted that lymphocyte-depleted Hodgkin's disease represents a separate tumour entity probably derived from germinal-centre B cells, and this is considered separately from the other three 'classical' forms of Hodgkin's disease. Lymphocyte-depleted Hodgkin's disease is encountered only rarely at primary diagnosis of Hodgkin's disease. It is usually seen as recurrent Hodgkin's disease in patients in whom nodular sclerosis or mixed cellularity Hodgkin's disease had been diagnosed previously. Moreover, many cases diagnosed as lymphocyte-depleted Hodgkin's disease are now reported as CD30-positive anaplastic large-cell lymphomas. There is thus increasing evidence to suggest that Hodgkin's disease is not a single entity but rather a heterogeneous group of diseases. This prompted inclusion of Hodgkin's disease in the Revised European–American Lymphoma (REAL) classification (Harris *et al.*, 1994).

The nature and clonal origin of HRS cells are a matter of controversy: most of the constituent cells of the lymph node have been proposed as HRS precursor cells at some stage (Herbst *et al.*, 1996a). HRS cells are characterized immunophenotypically by the expression of lymphocyte-activation antigens, such as CD25, CD30 and CD70 (Herbst *et al.*, 1993; Harris *et al.*, 1994). In a proportion of cases of Hodgkin's disease, HRS cells express B-cell antigens, and in a number of cases antigens characteristic of T cells can be demonstrated (Kadin *et al.*, 1988; Schmid *et al.*, 1991; Herbst *et al.*, 1996a); however, in most cases, no lineage-specific antigens are seen (Herbst *et al.*, 1993). Most recent studies indicate that HRS cells in many (but not all) cases are derived from B cells (Schmid *et al.*, 1991; Küppers *et al.*, 1994), and a germinal-centre origin has been proposed (Kanzler *et al.*, 1996).

The clinical presentation of Hodgkin's disease varies in different geographical locations. In this section, the clinical features seen in the western world are described briefly. Hodgkin's disease usually arises as a unifocal lesion in cervical lymph nodes. Contiguous spread of the tumour to adjacent lymph nodes gives rise to palpably enlarged nodes. With spread of the tumour through lymphatic channels, other organs are involved, the preferential sites of involvement including the spleen and distant lymph nodes. Subsequently, as the disease becomes more aggressive, other organs are involved, including the liver and the kidneys (Kaplan, 1980).

The presence of Hodgkin's disease at each of 17 sites of potential nodal involvement was determined in 719 patients in the United States. The predominant sites of lymph

node involvement were mediastinal (59%), left neck (58%) and right neck (55%), and at least one of these sites was involved in 92% of the patients (Mauch *et al.*, 1993). The spleen was involved in 27% of patients; the epitrochlear, popliteal and mesenteric lymph nodes were not commonly involved. Abdominal lymph node involvement without splenic involvement was rare, and the risk for abdominal lymph node involvement increased with increasing splenic size.

Bone-marrow involvement in Hodgkin's disease is indicative of extensive tumour infiltration and is associated with systemic symptoms including leukopenia, anaemia, thrombocytopenia and elevated levels of alkaline phosphatases. Other organs occasionally involved in Hodgkin's disease include the skin, subcutaneous tissue and breast. Involvement of the central nervous system is relatively rare, except through invasion and extension of the epidural space for enlarged para-aortic nodes. Hilar and mediastinal adenopathy may predispose to pleural involvement, with pleural effusions and pericardial involvement (DeVita *et al.*, 1993).

2.3.2   Epidemiology

2.3.2.1   Descriptive epidemiology

The distinguishing epidemiological feature of Hodgkin's disease characteristically seen in most western populations such as the United States is the bimodal age–incidence curve (Figure 8). In such populations, very few cases occur among children; a rapid increase in incidence among teenagers peaks at about age 25; the incidence then decreases to a plateau through middle age, after which the rates increase with age to a second peak. There is an excess among males, which is pronounced at older ages. MacMahon (1957) proposed that the bimodality results from the overlap of two disease distributions with peaks at different ages. He further suggested that Hodgkin's disease in young adults is caused by a biological agent of low infectivity, while the cause among the elderly is probably similar to those of other lymphomas (MacMahon, 1966).

In 1971, Correa and O'Conor noted a different age pattern among poor populations, with an initial peak in childhood only among boys, relatively low rates among young adults, followed by a late peak among those of advanced age. They further described an intermediate pattern, contrasting data for rural and urban Norwegians in the 1960s (see Figure 8). The shift from a 'developing' to an 'intermediate' pattern in parallel with economic development has been noted by others (Glaser, 1990; Hartge *et al.*, 1994). An intermediate pattern of the occurrence of Hodgkin's disease was seen in African Americans in the late 1970s (Olisa *et al.*, 1976; Cozen *et al.*, 1992), which is less evident in recent data (Parkin *et al.*, 1997).

Currently, essentially all of the majority populations in Europe and North America have a well-defined 'developed' pattern of incidence of Hodgkin's disease. The peak of occurrence in young adulthood varies within this set of countries, being high in Canada, France, Switzerland and the United States and lower in southern Europe. The pattern within eastern European countries is variable. In contrast, the pattern in Asia and Africa is generally 'intermediate' or 'developing' (Parkin *et al.*, 1997).

Figure 8. Age-specific incidence rates of Hodgkin's disease per 100 000 population of each sex in (a) Cali, Colombia (1962–66), (b) Connecticut, United States (1960–62), (c) rural Norway (1964–66) and (d) urban Norway (1964–66)
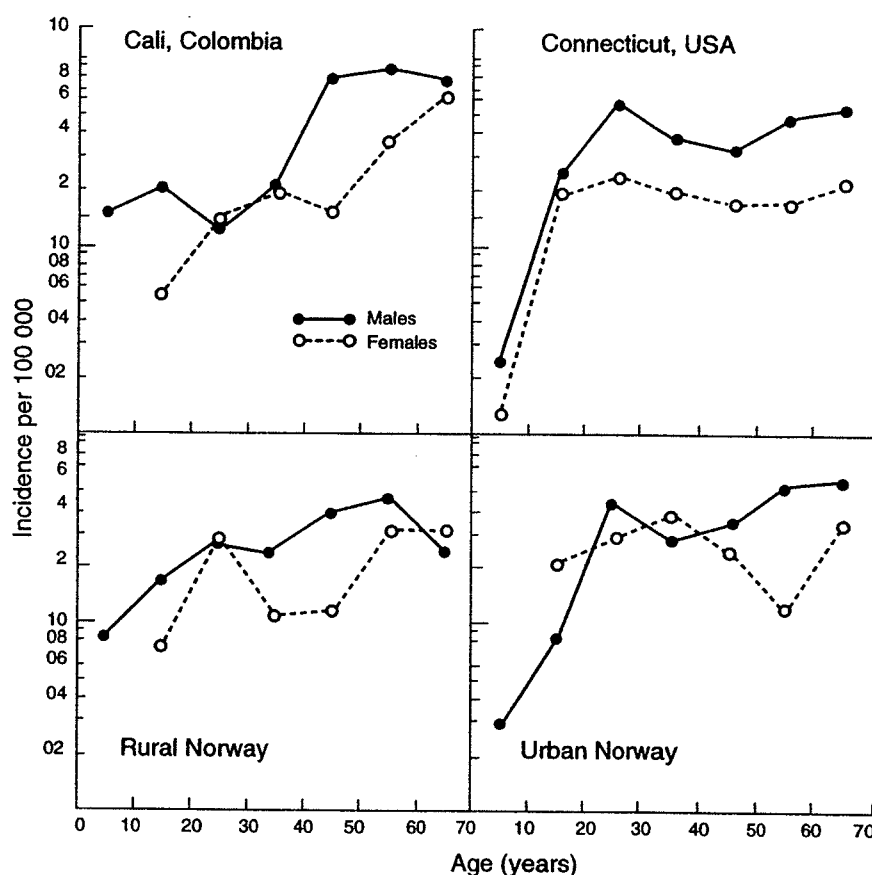
From Correa and O'Conor (1971)

Alexander *et al.* (1991a,b) used a large, population-based registry of leukaemia and lymphoma covering about half of the United Kingdom during a five-year period to evaluate the characteristics of over 1800 cases of Hodgkin's disease by area-based indices of socioeconomic status and population density. They reported that significantly more of the 486 cases diagnosed in people under the age of 25 occurred in areas of higher socioeconomic status (relative risk [RR], 1.2), and there was a significant trend to increased incidence in areas closer to 'built-up areas' (mutually adjusted). For cases among people aged less than 35 years at the time of diagnosis, there was a significantly positive association with social class, while a negative association was found for cases grouped by ages 35–49 and 50–79, the trend for the latter being significant.

Descriptive studies have shown that the association between higher social class and Hodgkin's disease in young adults is specific for the nodular sclerosis subtype. Henderson *et al.* (1979) computed the incidence rates of Hodgkin's disease of specific histological types in Los Angeles County (United States) in 1972–75 by social class. They reported that the incidence of the nodular sclerosis type was directly related to social class; there was no consistent association for the other histological types. These data were confirmed and extended through 1985 by Cozen *et al.* (1992), who also found

an increase in incidence between 1972 and 1985 only among cases of the nodular sclerosis subtype. They further reported that the risk pattern for Hodgkin's disease of mixed cellularity was quite distinct and negatively associated with social class. This latter finding fits the general observation that cases of Hodgkin's disease occurring in economically developing populations (Mueller, 1987) and among groups of lower social class in developed populations (Hu *et al.*, 1988) are predominantly of the mixed cellularity and lymphocyte depletion subtypes.

These data are consistent with the rates in the United States in 1969–80 (Glaser, 1987): thus, the incidence rates for young adults were positively correlated with community-level indicators of social class and the incidence of the nodular sclerosis subtype increased in parallel with regional indices of social class.

There is a consistent body of evidence that the risk for Hodgkin's disease occurring from early childhood through middle age is associated with factors in the childhood environment that influence the age at infection with a virus such as EBV. The pattern of association with these factors varies with the age at diagnosis. The data have been reviewed (Jarrett *et al.*, 1996; Mueller, 1996).

Because Hodgkin's disease in childhood occurs primarily in developing countries, the children at risk for the disease would appear to be of lower social class and thus to be infected earlier. This has been a consistent finding in the few case–control studies that have been reported (Sobrinho-Simões & Areias, 1978; Gutensohn & Shapiro, 1982; Bogger-Goren *et al.*, 1983). A shift occurs in young adults (generally defined as between 15 and 40 years of age), who have been found quite consistently to be of higher social class than expected, as measured by occupation or educational attainment (Cohen *et al.*, 1964; Gutensohn & Cole, 1977; Abramson *et al.*, 1978; Serraino *et al.*, 1991). In addition, there is an inverse association between risk for Hodgkin's disease and number of siblings and also with birth order (Gutensohn & Cole, 1977, 1981; Bernard *et al.*, 1987; Bonelli *et al.*, 1990). The young adult patients also lived in less crowded conditions during childhood. Among the oldest patients, however, no consistent association with social class was seen (Abramson *et al.*, 1978; Gutensohn, 1982).

As is generally recognized for most haematopoietic malignancies, Jews are at somewhat higher risk for Hodgkin's disease than non-Jews (MacMahon, 1960). In a study of cases occurring in the 1950s in Brooklyn, New York, United States, MacMahon (1957) reported that older, but not younger, Jews were at increased risk. In a population-based case–control study conducted in Boston–Worcester (United States) in the 1970s, however, Jewish people of all ages in the population were at particularly high risk (Gutensohn & Cole, 1981). Bernard *et al.* (1984, 1987) also noted an excess of Jewish cases in their population-based studies in Yorkshire, United Kingdom. Similarly, an increased incidence among Jews was documented in Los Angeles County, United States (Cozen *et al.*, 1992).

In summary, there is evidence that the risk for Hodgkin's disease in young adulthood through middle age is associated with higher education, higher social class, fewer siblings, less crowded housing and early birth rank. All of these factors lead to susceptibility to late infections with the common childhood infections. As in the model of

paralytic polio, such late infections tend to be more severe than those in younger children; however, social class does not appear to predict the occurrence of Hodgkin's disease in the late decades of life, when primary infection with EBV is unlikely.

2.3.2.2 Association with EBV

(a) Case reports and case series

There have been numerous case reports of Hodgkin's disease developing in close association with serologically documented primary infection with EBV (Kaplan, 1980) but with no direct evidence of the presence of the virus in the tumour itself.

(i) Infectious mononucleosis

Poppema et al. (1985) described a case of mixed cellularity Hodgkin's disease that developed after heterophil-negative infectious mononucleosis. The patient had been followed clinically by multiple node biopsies and serology during this period and was described as having a clinical and serological picture consistent with chronic EBV infection. The investigators demonstrated the presence of EBNA in HRS cells from involved lymph nodes.

(ii) Serology

Few data are available on the relationship between EBV serology and EBV status. Ohshima et al. (1990) reported a single case of an eight-year-old boy with EBV-positive Hodgkin's disease, as determined by Southern blot and PCR. His titres were 1:1280 anti-VCA IgG, 1:10 anti-VCA IgA, 1:10 anti-VCA IgM, 1:160 anti-EA and 1:40 anti-EBNA, consistent with an active EBV infection. In an overlapping series of 107 cases, Brousset et al. (1991) and Delsol et al. (1992) concluded that there was no association between the presence of EBV and a serological pattern of reactivation, which they defined as > 1:640 anti-VCA, > 1:40 anti-EA and > 1:160 anti-EBNA; however, only one of 35 EBV-negative and none of 16 EBV-positive cases had this rather extreme pattern. Levine et al. (1994) assessed the relationship of the EBV status of 39 cases with serological results published previously. No differences in anti-VCA or anti-EA titres were seen between EBV-positive and EBV-negative cases. When the sera of 19 of these cases were subsequently tested for anti-EBNA-1 and anti-EBNA-2, none of the 5 EBV-positive cases showed elevated titres (> 1:320) of EBNA-1, whereas 5 of 14 EBV-negative cases did so. Conversely, 2 of the 5 positive cases and 2 of the 14 negative cases had elevated titres (> 1:80) of EBNA-2. Neither of these differences was statistically significant (Mueller, 1997).

In preliminary reports from two groups, specimens from 54 patients with Hodgkin's disease who had reported a history of infectious mononucleosis were tested for the presence of the EBERs. They were found in only one (Mueller, 1997).

(iii) Viral nucleic acid and protein

With the advent of highly sensitive molecular probes, dozens of case series have now been published on the detection of genomic segments, transcripts and viral products of EBV in a substantial proportion of biopsy samples from patients with Hodgkin's disease.

Many of these reports (restricted to HIV-1-negative cases) are summarized in Table 17. Weiss *et al.* (1987, 1988) and Staal *et al.* (1989) first reported the detection of monoclonal EBV genome in Hodgkin's disease tissue, Staal *et al.* noting that the number of viral episomes per cell was low. Weiss *et al.* (1989a), Uccini *et al.* (1989) and Anagnostopoulos *et al.* (1989) all reported detection of the viral genome within HRS cells (and their variants) themselves. In 1990, Wu *et al.* reported detection of RNA EBER transcripts in HRS cells. Pallesen *et al.* (1991a) and Herbst *et al.* (1991a) reported that the EBV in Hodgkin's disease has a restricted latent phenotype of LMP-1 expression without detectable EBNA-2, as in nasopharyngeal carcinoma. These findings have been replicated in dozens of populations by a large number of laboratories.

EBV nucleic acid has also been found in a fraction of small lymphocytes in both EBV genome-positive and -negative tissue from patients with Hodgkin's disease (Masih *et al.*, 1991; Weiss *et al.*, 1991; Herbst *et al.*, 1992; Khan *et al.*, 1992; Ambinder *et al.*, 1993; Bhagat *et al.*, 1993; Chang *et al.*, 1993b; Jiwa *et al.*, 1993b), and at a low frequency in normal lymph nodes (Niedobitek *et al.*, 1992a).

Consistency of viral marker status of individual patients: EBV status is consistent among anatomically distinct sites involved at diagnosis. Vasef *et al.* (1995) evaluated 14 cases with two to five involved sites for the concordance of EBV status among patients. All of the biopsy samples from eight patients were EBV genome-negative and all those from six patients were EBV genome-positive. In five of the latter cases, the investigators analysed the 3' end of the *LMP-1* gene at all sites of disease by PCR. In three patients, all of the sites had a 30-base pair deletion; in the other two cases, there was discordance in the presence of this deletion, some sites having the germ-line configuration.

EBV status appears to be stable if not identical over time. Delsol *et al.* (1992) reported that EBV status was consistent in subsequent biopsy samples at relapse (range, 14–126 months) in 12 cases, of which seven were initially EBV-positive. Two EBV-positive cases showed substantial reduction or loss of staining for LMP-1 in their later biopsies. Coates *et al.* (1991a) found that sequential biopsy samples from three EBV-positive patients (age range, 2–10 years) contained EBV at about the same level as in the initial sample. Boiocchi *et al.* (1993a) reported the same clonal EBV genome in each of two patients from whom two or three biopsy samples had been taken over a period of up to 11 months. Brousset *et al.* (1994) evaluated 12 cases of relapsed Hodgkin's disease for consistency of EBV status: five showed no EBV at either initial diagnosis or relapse, while seven that were initially EBV-positive remained positive at relapse. For two of the latter, additional assays — including terminal repeat polymorphism by Southern blot and LMP-1 polymorphism sequencing by PCR — were carried out on tissue taken at both diagnosis and relapse. In one case, the EBV genome appeared to be identical in sequential biopsy samples taken nine years apart. In the other case, a monoclonal episome was detected by Southern blot in the initial sample but not in that taken at relapse eight years later. The authors noted that the latter finding may be due to a low viral load that cannot be detected by Southern blot. Both samples from this patient contained an identical mutation in EBV *LMP-1*.

Table 17. Detection of the EBV genome or gene products in tissues from HIV-1-negative cases of Hodgkin's disease

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Weiss *et al.* (1987, 1988)	21	4	SB, slot–blot	More mixed cellularity than nodular sclerosis types positive; no cytomegalovirus; overlaps with next study
Weiss *et al.* (1989a)	16	3	SB, slot–blot, ISH	
Staal *et al.* (1989)	28	8	SB	More mixed cellularity than nodular sclerosis types positive
Uccini *et al.* (1989)	32	6	SB, ISH	
Boiocchi *et al.* (1989)	17	7	SB	
Herbst *et al.* (1989)	39	5	SB	Overlaps with next study
Anagnostopoulos *et al.* (1989)	42	7	SB, ISH	
Uhara *et al.* (1990)	31	8	PCR, ISH	More mixed cellularity than nodular sclerosis types positive
Bignon *et al.* (1990)	16	8	PCR	Mostly young adults
Wu *et al.* (1990)	8	6	ISH (EBER)	EBV-positive cases from Staal *et al.* (1989)
Herbst *et al.* (1990)	198	114	PCR, ISH	No variation by age or sex
Libetta *et al.* (1990)	34	15	SB	No variation by age
Uccini *et al.* (1990)	20	3	SB, ISH	None contained VCA or EA; all positive cases were of mixed cellularity type
Ohshima *et al.* (1990)	7	2	SB, PCR	Serology available for one case: high titre of anti-VCA and EA IgG, positive for anti-VCA IgM and IgA
Pallesen *et al.* (1991a)	84	40	mAb to LMP-1 and EBNA-2	Positive only for LMP-1; more mixed cellularity than nodular sclerosis types positive
Gledhill *et al.* (1991)	35	11	PCR	8 of 8 analysed were of EBV type 1; no association with histology and little with age

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Brousset et al. (1991)	54	16	ISH ('mRNA')	More mixed cellularity than nodular sclerosis types positive; no correlation with serology (one case seronegative); results later interpreted as detection of EBV DNA (Delsol et al., 1992)
Herbst et al. (1991a)	47	32 with DNA 18 with LMP	PCR, mAb to LMP-1, EBNA-2 and gp350/250	All LMP-1-positive positive by PCR; no variation by histology
Knecht et al. (1991)	48	38	PCR (semi-quantitative)	About two-thirds of cases had numerous HRS; no association with histology or proportion of HRS
Masih et al. (1991)	52	30	PCR, slot–blot, SB	More mixed cellularity than nodular sclerosis types positive, 43% of controls with hyperplastic lymph nodes were EBV-positive; two of six tested were clonal
Pallesen et al. (1991c)	96	47	mAb to LMP-1, ZEBRA, EA, VCA, MA	Includes cases from Pallesen et al. (1991a); no cytomegalovirus; three positive for LMP-1 also positive for ZEBRA; none positive for EA, VCA or MA
Vestlev et al. (1992)	66	27	Follow-up of cases from above	LMP-1-positive cases more likely to be of mixed cellularity type, male, less likely to have mediastinal involvement; LMP-1 status not associated with prognosis
Jarrett et al. (1991)	95	43	SB	48 cases selected for age and histological type; includes cases from Gledhill et al. (1991); positivity highest among children and older adults; 30 of 30 samples tested were of EBV type 1
Weiss et al. (1991)	36	14	PCR, ISH (EBER-1)	More mixed cellularity than nodular sclerosis types positive; some background B and T cells positive

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Coates *et al.* (1991a)	55	9	ISH	More cases of nodular sclerosis (7/24) than of mixed cellularity (2/16) positive; no difference in mean age of EBV-positive and EBV-negative cases; variation in amount of virus between cases; 3 patients ≤ 10 years with sequential biopsies positive at same level in all specimens; in 8 other cases, EBV found only in non-neoplastic cells
Brocksmith *et al.* (1991)	57	33	SB, PCR	More mixed cellularity than nodular sclerosis types positive; no variation with age
Delsol *et al.* (1992)	107	37	ISH, mAb to LMP-1, EBNA-2	Overlaps with Brousset *et al.* (1991a); more mixed cellularity types positive; all 12 cases tested at diagnosis and relapse remained concordant for EBV; one LMP-1-positive case became LMP-negative and one LMP-1-positive case became intermediate; no correlation with serology or short-term prognosis; all 13 positive cases EBNA-2-negative
Khan *et al.* (1992)	33	12	ISH (EBER)	In six cases, EBER localized to non-neoplastic small lymphocytes only
Herbst *et al.* (1992)	46	26	ISH (EBER-1/2); mAb to LMP-1	18 positive for LMP-1 (all EBER-positive); EBER-positive small lymphocytes found in 39 cases at low levels, 3 at high levels
Fellbaum *et al.* (1992)	187	66	PCR	More mixed cellularity types positive; no association with survival
Murray *et al.* (1992b)	46	22	mAb to LMP-1	Positivity and proportion of LMP-positive HRS cells increased with 'histological grade'
Ambinder *et al.* (1993)	36	20	ISH (EBER-1); mAb to LMP1	All cases < 15 years; 11 of 11 cases from Honduras and 9 of 25 from USA positive; no cytomegalovirus; EBV-positive small lymphocytes also seen in 9 positive cases; more mixed cellularity than nodular sclerosis types positive in US series

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Boyle et al. (1993)	12	3	PCR, ISH	Positivity associated with younger age
Brousset et al. (1993)	35	3	ISH (ZEBRA, EBER-1/2), mAb to LMP-1, SB	All patients had relapsed; all consistent in EBV status at sequential biopsies; in 2 cases analysed, EBV appeared to be identical in both samples
Chang et al. (1993b)	32	30	ISH (EBER-1), mAb to LMP-1, MA	Patients from Peru; positivity by age: 19 of 19 ≤ 15 years, 5/6 15–39, 6/7 older; some small lymphocytes positive
Carbone et al. (1993c)	39	15	ISH, mAb to LMP-1, vimentin	More mixed cellularity than nodular sclerosis types positive; vimentin found in 24 cases localized to HRS cells, including all 11 LMP-1-positive cases
Deacon et al. (1993)	23	16	ISH (EBER-1/2), mAb to LMP-1	Transcription analysis demonstrated EBV latency pattern found in nasopharyngeal carcinoma
Jiwa et al. (1993b)	33	19	PCR, ISH (EBER-1/2), mAb to LMP, bcl-2, c-myc	Almost all cases nodular sclerosis type; some small lymphocytes also positive; 20 of 29 cases expressed bcl-2 and 30 of 32 expressed c-myc in HRS cells independently of EBV status
Niedobitek et al. (1993a)	116	33	ISH (EBER), mAb to p53	More mixed cellularity than nodular sclerosis types positive; 37 positive for p53 in HRS cells independently of histology, less frequent in EBV-positive (21%) than EBV-negative (36%) [$p = 0.12$]
Lin et al. (1993a)	23	16	PCR (EBER) (multiple gene loci)	2 of 10 reactive hyperplasia nodes were EBV-positive
Bhagat et al. (1993)	11	4	ISH (EBER-1), mAb to bcl-2	Most cases nodular sclerosis: 3 of 7 with t(14;8) and 5 of 6 without t(14;18) were bcl-2-positive; bcl-2 antibody reacted with HRS cells but also in majority of small lymphocytes; EBV-positivity not correlated with presence of t(14;18) or bcl-2

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Khan *et al.* (1993)	77	25	ISH (EBER), mAb to LMP-1	Cases selected on basis of histology and age; high positivity associated with mixed cellularity type (68%); some increase with age; some small lymphocytes positive; no cytomegalovirus or HHV6
Kanavaros *et al.* (1994)	22	12	ISH (EBER), mAb to LMP-1	Paediatric cases from Greece; more mixed cellularity than nodular sclerosis types positive
Gulley *et al.* (1994)	125	58	SB, ISH (EBER)	79 cases from USA, 31 from Mexico and 15 from Costa Rica; more mixed cellularity than nodular sclerosis types positive; Hispanic ethnicity associated with positivity (RR = 4.3)
Poppema & Visser (1994)	72	19	ISH (EBER-1/2), mAb to LMP-1, HLA-A2	Cases from Canada; more mixed cellularity types positive; no association with HLA-A2
Zarate-Osorno *et al.* (1994)	27	18	ISH (EBER-1)	Cases from Mexico; more mixed cellularity types positive
Quintanilla-Martínez *et al.* (1995)	50	35	mAb to LMP-1	Cases from Mexico; more mixed cellularity than nodular sclerosis types positive
Preciado *et al.* (1995)	41	22	SB; ISH (EBER), mAb to EBNA-2, LMP-1	Paediatric cases from Argentina; more mixed cellularity than nodular sclerosis types and more younger children (< 7) positive
Chan *et al.* (1995b)	23	15	ISH (EBER-1), mAb to BHLF1	Cases from Hong Kong; more mixed cellularity than nodular sclerosis types positive for EBER; none positive for BHLF1; least positivity among persons aged 15–49 years
Li *et al.* (1995b)	40	17	ISH (EBER-1), mAb to LMP-1	Cases from Japan; more mixed cellularity than nodular sclerosis types positive; positive cases more likely to have some positive small lymphocytes in background

Table 17 (contd)

Reference	No. of cases	No. with EBV genome or gene products	Method of detection	Comments
Vasef et al. (1995)	14	6	ISH (EBER-1, LMP-1)	Patients had two to five separate involved sites; all biopsy samples consistent for EBV status; three positive cases had identical LMP-1 genes; 2 patients had discordant LMP-1 genes at different sites
Leoncini et al. (1996) Kenya Italy	 92 65	 85 31	 ISH (EBER)	More mixed cellularity types positive in both series; positivity among cases of nodular sclerosis in Kenya higher than that in cases in Italy. In Kenya, more children ≤ 15 years (41 of 42) positive than adults > 15 years (44 of 50)
Weinreb et al. (1996a)	101	85	ISH (EBER-1/2), mAb to LMP-1	Cases from Kenya. Positivity varied by age: 100% in 53 paediatric cases, 67% in 48 adult cases
Tomita et al. (1996)	50	32	PCR, SB, ISH (EBER)	Cases from Japan; more mixed cellularity than other types, more older (> 40) than younger (< 40 years) and more males positive
Weinreb et al. (1996b)	277	196	ISH (EBER-1/2), mAb to LMP-1	Children in 10 countries; positivity 50–100%, more mixed cellularity type and more males positive [HIV status unknown]
Huh et al. (1996)	87	60	ISH, ISH (EBER, BHLF), mAb to LMP-1	Cases from Republic of Korea; positivity increased with stage; more mixed cellularity than nodular sclerosis positive; only 1 case positive for BamHI H left-frame transcript

Adapted from Mueller (1996)

HRS, Hodgkin and Reed-Sternberg; ISH, in-situ hybridization (for EBV genome probe, unless otherwise specified); SB, Southern blot; PCR, polymerase chain reaction; LMP-1, latent membrane protein 1; EBNA, EBV nuclear antigen; EBER, EBV-encoded RNA; mAb, monoclonal antibody; EA, early antigen; ZEBRA, Z EBV replication activator; gp350/250, envelope glycoprotein; VCA, viral capsid antigen; MA, membrane antigen

Relationship to clinical features: Few clinical or other molecular features appear to be related to EBV status. O'Grady *et al.* (1994) reported that EBV positivity in stage-I disease is associated with cervical neck node presentation, irrespective of histological subtype. This could be related to primary EBV infection in the oropharynx and would appear to strengthen the association between Hodgkin's disease and previous infectious mononucleosis. Similarly, Kapadia *et al.* (1995) reported a relatively high rate of EBV positivity in 8 of 12 cases of Hodgkin's disease occurring in Waldeyer's tonsillar ring. Primary Hodgkin's disease of the tonsils is rare, however, and recent studies have suggested that HRS cells in cases of Hodgkin's disease arising in association with infectious mononucleosis are predominantly EBV-negative (Mueller, 1997). EBV genome status has not been found to be an independent predictor of prognosis (Delsol *et al.*, 1992; Fellbaum *et al.*, 1992; Vestlev *et al.*, 1992).

Relationship to histology, age and ethnicity: Several factors appear to be predictive of EBV status. Viewing the data on the two most common subtypes overall (Table 17), cases of the mixed cellularity subtype generally occur at higher rates than those of nodular sclerosis. Given the difference in the age and sex distribution of these two subtypes of Hodgkin's disease (Medeiros & Greiner, 1995), the presence of EBV should have a U-shaped relationship with age, with the highest rates at the extremes of age and the lowest in young adulthood. The general consensus is that EBV infection of HRS cells is at best rare in cases of the nodular lymphocyte-predominant type, consistent with the notion that these are an entity distinct from the 'classical' forms of Hodgkin's disease (Pallesen *et al.*, 1991c; Shibata *et al.*, 1991a; Alkan *et al.*, 1995). Few cases of lymphocyte-depletion Hodgkin's disease have been reported in the literature, and their association with EBV is highly variable, consistent with the origin of such cases from Hodgkin's disease of either nodular sclerosis or mixed cellularity type and with the proposed overlap with CD30-positive anaplastic large-cell lymphomas (Zhou *et al.*, 1993; Bai *et al.*, 1994; Quintanilla-Martínez *et al.*, 1995; Herbst *et al.*, 1996a).

In studies of cases in developing countries, where patients generally present with more advanced disease, the rate of positivity is notably high, particularly among children. Gulley *et al.* (1994) analysed 125 cases from Costa Rica, Mexico and the United States by multivariate analysis and found that Hispanic ethnicity *per se* was an independent predictor of the presence of the EBV genome, with a RR of 4.3. Murray *et al.* (1992b) noted that the proportion of HRS cells containing LMP-1 in EBV-positive specimens increased in parallel with a less favourable histological subtype: 41% in one of 12 cases of lymphocyte-predominant disease, 49–78% in 12 of 24 cases of the nodular sclerosis type, 63–81% in six of seven cases of the mixed cellularity type and 89–97% in all three cases of lymphocyte-depletion Hodgkin's disease.

Glaser *et al.* (1997) analysed the data from a number of investigators and previously unpublished data by multivariate analysis, with a total series of 1546 HIV-1-negative cases of Hodgkin's disease, in order to identify characteristics that differentiate EBV-positive and EBV-negative cases. Cases of mixed-cell disease were significantly more likely to contain EBV than those of nodular sclerosis in all age groups: < 15 years, RR, 7.3; 15–49 years, RR, 13; ≥ 50 years, RR, 4.9. Patients under 15 years of age in developing countries were significantly more likely to have EBV than those from more deve-

loped countries [RR, 6.0]; similar results were not found for older individuals. Men aged 15–49 years, but not those in other age groups, were significantly more likely to have tumours containing the EBV genome (RR, 2.5). Overall, it appears that the presence of EBV is related to a less favourable host response.

HIV infection: Essentially all HIV-1-infected patients with Hodgkin's disease have a higher rate of EBV positivity: 8 of 10 (Moran *et al.*, 1992), 11 of 11 (Hamilton-Dutoit *et al.*, 1993b) and 14 of 18 (Tirelli *et al.*, 1995). In general, these patients present with advanced Hodgkin's disease and have a poor prognosis. [HIV-1-infected patients may be at increased risk for Hodgkin's disease (IARC, 1996).]

(b)   Case–control studies

(i)   Infectious mononucleosis

The association between history of infectious mononucleosis and Hodgkin's disease has been evaluated in several case–control studies (Table 18). A weak positive association is usually seen, particularly in young adults and patients with the nodular sclerosis subtype.

(ii)   Serology

In case–control studies of more than 2000 patients with Hodgkin's disease of all ages, the proportion who had IgG antibodies to VCA, indicative of prior infection, was similar to that of controls (Table 19); however, the cases consistently had higher mean antibody titres than controls, except in two studies involving paediatric patients (Lange *et al.*, 1978; Shope *et al.*, 1982). Similarly, in most studies, the cases had a higher prevalence of antibodies (as well as higher titres) against the EA complex of EBV, indicative of active viral replication (Table 20). Evans and Gutensohn (1984) found that subjects with a history of infectious mononucleosis had higher GMTs against VCA and against both EA(D) and EA(R) than did subjects who did not have such a history. This was true among both cases as a group and among the sibling controls as a group, although only sera in which antibody was present at the lowest dilution were included in calculating GMTs.

In eight studies, antibodies against EBNA were also evaluated (Table 21). Of these, three showed no higher levels in diagnosed cases than in controls. Lennette *et al.* (1993) and Merk *et al.* (1995) also tested for antibodies against specific components of the EBNAs, using recombinant proteins as targets. In a study of 20 Hodgkin's disease patients and 74 grouped controls (Lennette *et al.*, 1993), the cases had higher (non-significant) GMTs against EBNA-2A and EBNA-3C but not against EBNA-1 or EBNA-2B. In a later study of 61 untreated cases and 109 healthy EBV antibody-positive controls (Merk *et al.*, 1995) tested with the same assays, the cases had elevated titres against EBNA-1, EBNA-2A and EBNA-3C but not against EBNA-2B.

Chen *et al.* (1992b) developed an assay to test for immune response to recombinant full-length LMP-1. They found that 16 of 27 patients with Hodgkin's disease but only two of 26 EBV-seropositive controls and five of 22 patients with nasopharyngeal carcinoma had IgG antibodies against LMP-1. Lennette *et al.* (1995) developed a monoclonal,

Table 18. Results of case–control studies of the association between a history of infectious mononucleosis and the risk for Hodgkin's disease

Reference	Study population	No. of cases	No. of controls	Relative risk	Factors controlled
Henderson *et al.* (1979)	Population-based: Los Angeles County (USA), incident cases, 1972–73; neighbourhood controls	212	212	1.3 Nodular sclerosis, 1.5	Age, ethnicity, sex, neighbourhood
Gutensohn & Cole (1981)	Population-based: Eastern Massachusetts (USA), incident cases, 15–39 years, 1973–77; population controls	225	447	1.8 $p < 0.05$	Age, sex, family size, birth order, housing density in childhood
Gutensohn (1982)	As above, cases 40–54 years; population controls	53	106	1.3	Age, sex, family size, religion
Evans & Gutensohn (1984)	As above, cases 15–54 years; sibling controls	262	250	1.5	Family
Bernard *et al.* (1987)	Population-based: Yorkshire Health District (UK), incident cases, 1979–84; hospital controls [subjects appear to be ≥ 15 years]	248	489	1.0 Males 15–35 years, 4.9 $p = 0.04$	Sex, age, health district
Serraino *et al.* (1991)	Hospital-based: Pordenone (Italy), 1985–90, cases 15–77 years	152	613	8.2 Nodular sclerosis, 13.1 $p < 0.05$	Sex, education

Table 19. Results of case–control studies of the association between antibody titres against EBV capsid antigen and risk for Hodgkin's disease

Reference	Cases				Controls				RR
	No.	Prevalence	GMT	High titre[a]	No.	Prevalence	GMT	High titre[a]	
Goldman & Aisenberg (1970)	57	0.58	–	0.12	54	0.61	–	–	–
Johansson et al. (1970)	60	0.95	100	0.47	47	0.89	43	0.17	4.3
Levine et al. (1971)	63	–	367	0.35[b]	85	–	91	0.05	11
de Schryver et al. (1972)	17	–	60	0.24	63	–	23	0.17	1.5
Henle & Henle (1973b)	489	0.89	105	0.40	294	0.84	55	0.14	4.1
Henderson et al. (1973)	142	0.94	92	0.47	142	0.93	53	0.25	2.6
Langenhuysen et al. (1974)[c]	25	0.92	1580	0.88[d]	25	0.92	585	0.60	4.9
Hirshaut et al. (1974)	51	0.82	141	0.16[d]	45	0.89	–	0.09	1.9
Rocchi et al. (1975)	100	0.98	177	0.68	100	0.91	34	0.12	16
Gotlieb-Stematsky et al. (1975)	67	0.91	67	0.46	186	0.66	9	0.01	79
Hilgers & Hilgers (1976)	43	0.95	2420	–	43	0.95	401	–	1.9
Hesse et al. (1977)[e]	185	0.95	272	0.54	185	0.95	141	0.30	12
Evans et al. (1978)	67	1.00	146	0.31[e]	162	–	51	0.04	12
Lange et al. (1978)[f]	27	0.63	116[c]	–	71	0.61	101	–	–
ten Napel et al. (1980)[c]	15	0.94	3180	–	17	1.00	924	–	–
Mochanko et al. (1979)	37	1.00	69	0.27	40	0.83	44	0.07	4.6
Evans et al. (1980)	70	0.97	110	0.35[e]	70	0.84	30	0.03	17
Shope et al. (1982)[f]	15	0.73	103	0.20	24	0.58	73	0.25	0.8
Evans & Gutensohn (1984)	304	0.86	176	0.39[e]	276	0.87[g]	58	0.14	4.0
Lennette et al. (1993)	23	1.00	1359	–	75	1.00	303	–	–

Table 19 (contd)

Reference	Cases				Controls				RR
	No.	Prevalence	GMT	High titre[a]	No.	Prevalence	GMT	High titre[a]	
Merk et al. (1995)[c]	61	1.00	–	0.72[h]	109	1.00	–	0.14	16
Lenette et al. (1995)	113	–	878	–	30	–	199	–	–

From Evans and Gutensohn (1984), except for the last three references

RR, relative risk; GMT, geometric mean titre

[a] ≥ 1:160, unless otherwise noted

[b] > 1:640

[c] Sera collected before initiation of treatment; all other studies involve treated patients

[d] ≥ 1:640

[e] ≥ 1:320

[f] Children only

[g] Siblings of cases

[h] ≥ 1:1280

enhanced, indirect immunofluorescence assay for detecting antibodies against LMP 2A/2B. None of 113 Hodgkin's disease patients but 64% of patients with nasopharyngeal carcinoma had these antibodies.

Table 20. Results of case–control studies of the association between prevalence of antibodies against the early antigen complex of the EBV and risk for Hodgkin's disease

Reference	Cases				Controls				RR
	No.	Pre-valence[a]	High titre[b]	GMT	No.	Pre-valence	High titre	GMT	
de Schryver et al. (1972)	15	0.27	0.07	–	0	–	–	–	–
Henle & Henle (1973b)	458	0.30[c]	–	30	1718	0.03[c]	–	20	14
Rocchi et al. (1975)	100	0.50[c]	0.28	34	100	0.02	0	5	49
Gotlieb-Stematsky et al. (1975)	63	0.27	–	–	101	0	–	–	∞
Hilgers & Hilgers (1976)	43	0.91	–	52	43	0.81	–	9	2.4
Hesse et al. (1977)	176	0.84	0.26[d]	63	176	0.82	0.27	55	1.2
Evans et al. (1978)	42	0.21	–	–	11	0.09	–	–	2.7
Lange et al. (1978)[e,f]	17	0.53[c]	–	–	43	0.09	–	–	11
Evans & Gutensohn (1984)[g]	304	0.46	0.30[c]	13	276[h]	0.23	0.11	8	2.8
Lennette et al. (1993)[g]	23	0.39	–	20	75	0.04	–	5	15
Merk et al. (1995)[e]	61	0.25	0.11[i]	–	109	0.21	0.09	–	1.3

From Evans and Gutensohn (1984), except the last two references
RR, relative risk; GMT, geometric mean titre
[a] ≥ 1:5
[b] ≥ 1:40
[c] ≥ 1:10
[d] ≥ 1:160
[e] Sera collected before initiation of treatment
[f] Children only
[g] EA(D) only
[h] Siblings of cases
[i] ≥ 1:40

Table 21. Results of case–control studies of the association between proportion of high titres against the nuclear antigen complex of the EBV and risk for Hodgkin's disease

Reference	Cases			Controls			RR
	No.	High titre (%)	GMT	No.	High titre (%)	GMT	
Rocchi et al. (1975)	100	34[a]	18.9	100	0	6.2	∞
Hilgers & Hilgers	20[b]	–	186.0	20	–	169.0	–
(1976)	23[c]	–	128.0	23	–	198.0	–
Lange et al. (1978)[d]	28	–	13.0[e]	43[f]	–	42.8	–
Mochanko et al. (1979)	37	49[g]	58.2	40	14	36.2	[1.8]
Shope et al. (1982)[d]	15	13[g]	–	24	0	–	∞
Wutzler et al. (1983)	57	–	8.0	57	–	7.0	–
Lennette et al. (1993)	20	–	197.0	73	–	245.0	–
Merk et al. (1995)[h]	61	26[i]	–	109	17	–	[1.7]

Anticomplement immunofluorescence test (Reedman & Klein, 1973) used in all studies; numbers in square brackets were calculated by the Working Group.
[a] ≥ 1:40
[b] Mixed cellularity type
[c] Nodular sclerosis type
[d] Children only
[e] Before therapy
[f] Children and adults
[g] ≥ 1:80
[h] Untreated cases
[i] ≥ 1:1280

(c)   Cohort studies

(i)   Infectious mononucleosis

The risk for Hodgkin's disease after a diagnosis of infectious mononucleosis has been evaluated in six cohort studies (Table 22) involving nearly 42 000 young adults with serologically confirmed infectious mononucleosis. The expected number of cases of Hodgkin's disease was based on data for the general population in each study. Overall, there was about a threefold excess of Hodgkin's disease.

Miller and Beebe (1973) identified 2437 men in whom infectious mononucleosis had been diagnosed in 1944 while they were in the service of the United States Army. This cohort was traced for death through 1965. Diagnoses of Hodgkin's disease were based on information on death certificates. The two cases identified both died with Hodgkin's disease 20 years after the diagnosis of infectious mononucleosis.

Table 22. Results of cohort studies of persons with a history of infectious mononucleosis (IM) and subsequent Hodgkin's disease (HD)

Reference	Study population	No. of cases of IM	Follow-up period	No. of cases of HD observed	No. of cases of HD expected[a]	RR
Miller & Beebe (1973)	US Army during Second World War	2 437	1946–65	2	[1]	[2.0]
Connelly & Christine (1974)	Connecticut, USA	4 529	1948–68	5	[1]	[5.0]
Rosdahl et al. (1974)	Denmark	17 073	1940–70	17	6	2.8
Carter et al. (1977)	University students, USA	2 282	1949–74	3[b]	1.3	[2.3]
Muñoz et al. (1978)	Scotland and Sweden	9 454	1959–73	7	1.8	3.9
Kvåle et al. (1979)	Norway	5 840	1961–75	3[b]	[1]	[3.0]

RR, relative risk; numbers in square brackets calculated by the Working Group

[a] Expected number based on data for general population, except when taken from original paper

[b] After 12 months

Connelly and Christine (1974) identified 4529 residents of Connecticut (United States) in whom infectious mononucleosis was diagnosed between 1948 and 1964, from the records of the State Department of Health. This cohort was followed through 1968, with matching to the Connecticut Tumor Registry. Five cases (four female, one male) of Hodgkin's disease were diagnosed 3–10 years (median, 8 years) after the diagnosis of infectious mononucleosis.

Rosdahl et al. (1974) identified 17 073 Danes in whom infectious mononucleosis had been diagnosed at the Statens Seruminstitut in Copenhagen between 1940 and 1969. The records of the Danish Cancer Registry were screened for cancers diagnosed after an interval of 12 months to mid-1970. Seventeen of these people (one woman and 16 men) subsequently developed Hodgkin's disease between one and seven years (median, three years) after the diagnosis of infectious mononucleosis.

Carter et al. (1977) identified 2282 former university students in the United States in whom infectious mononucleosis was diagnosed between 1949 and 1969. Of these, three men had a subsequent diagnosis of Hodgkin's disease three, four and seven years after their infectious mononucleosis.

Muñoz et al. (1978) identified a cohort of 9454 persons in whom infectious mononucleosis had been diagnosed from laboratory records in either the city hospital in Aberdeen, Scotland, in 1959–1971 or at four public health laboratories in Sweden in 1952–70. The cohort was matched against local cancer registries for cancer occurring two years later than the diagnosis of mononucleosis. The seven cases (five female, two

male) of Hodgkin's disease were diagnosed 2–12 years (median, 2 years) after infectious mononucleosis.

Kvåle *et al.* (1979) identified 5840 persons with infectious mononucleosis diagnosed in nine laboratories in Norway in 1961–72 and matched them against the records of the Cancer Registry of Norway. Three male cases were diagnosed at least 12 months (one, two and four years) after diagnosis of mononucleosis.

(ii) *Serology*

Two case–control studies nested within cohorts included reports on EBV serology preceding a diagnosis of Hodgkin's disease. The first, by Mueller *et al.* (1989), consolidated the resources of five serum banks containing specimens from over 240 000 persons and identified 43 patients from whom blood had been drawn and stored for an average of 50.5 months before diagnosis of Hodgkin's disease. These were compared with 96 matched controls from whom blood had been drawn at the same time. All of the blood specimens were assayed for IgG, IgA and IgM antibodies against VCA and IgG antibodies against EA(D) and EA(R) and EBNA. As had been reported in the case–control studies, the cases had elevated IgG titres against VCA (RR, 2.6; 90% CI, 1.1–6.1), EA(D) (RR, 2.6; 90% CI, 1.1–6.1) and EA(R) (RR, 1.9; 90% CI, 0.90-4.0), with adjustment for IgM antibodies. In addition, a greater proportion of the cases than controls had elevated titres of IgA against VCA (RR, 3.7; 90% CI, 1.4–9.3; adjusted for IgM), and substantially fewer had IgM antibodies against VCA (RR, 0.22; 90% CI, 0.04–1.3). When all of the antibodies were controlled for simultaneously, the most significant findings were the prevalence of high titres of EBNA (RR, 6.7; 1.8–25) and an inverse association for IgM antibodies (RR, 0.07; 90% CI, 0.01–0.53). This finding of altered EBV antibody patterns before the diagnosis of Hodgkin's disease was generally stronger in blood specimens drawn at least three years before diagnosis than in those tested closer to the time of diagnosis and did not vary appreciably between the sexes, by age or by histological type.

Lehtinen *et al.* (1993) conducted a similar study in a cohort of 39 000 healthy Finnish adults who were followed-up for 12 years. Of these, six were subsequently found to have Hodgkin's disease. Although the data were not shown, the authors reported that the risk for Hodgkin's disease was associated with increased antibody responses to EBNA and to EA, consistent with the results of the previous study.

2.4 Nasopharyngeal carcinoma

2.4.1 *Clinical features and histopathology*

2.4.1.1 *Clinical features*

Nasopharyngeal carcinoma commonly arises from the fossa of Rosenmüller, a region of the nasopharynx rich in lymphoreticular tissue, and eustachian cushions, regardless of race or geographical area. Nasopharyngeal carcinoma may also arise in the roof of the nasopharynx and very rarely in the anterior and inferior walls of the nasopharynx (Simons & Shanmugaratnam, 1982).

Clinically, nasopharyngeal carcinoma may metastasize, particularly to cervical lymph nodes, and as a result is frequently identified only after dissemination. In several series, more than 50% of patients presented with cervical lymph node metastases (Ho, 1971; Sugano *et al.*, 1978; Levine & Connelly, 1985; Pathmanathan, 1997). The jugulodigastric lymph nodes are the most commonly affected cervical nodes (Stanley, 1997) and are found to be enlarged in 50–70% of patients. The presenting symptoms may thus be bilateral cervical node enlargement and also blood-stained nasal discharge, nasal obstruction, blood-stained sputum, tinnitus and hearing loss due to tumour involvement of the eustachian tube. Cranial nerves may be involved singly or in different combinations early or late in the disease; however, cranial nerve palsies, particularly involving cranial nerves III, IV, V and VI, IX and X are often seen. Intracranial extension of the tumour into the base of the skull may give rise to frequent headaches, which are occasionally unilateral, central or retro-orbital in distribution. The neurological signs and symptoms reflect the severity and extent of tumour invasion.

2.4.1.2 Histopathology

The microscopic appearance of nasopharyngeal carcinoma or of a metastatic nasopharyngeal carcinoma in lymph node material may be diagnostic and provide confirmation of a clinical diagnosis. When a biopsy sample from an early lesion shows no carcinoma cells, particularly in a patient with suggestive serological results (see below), additional curettings of the nasopharynx should be done and the tissue examined.

The WHO histopathological classification of nasopharyngeal carcinoma (Shanmugaratnam & Sobin, 1991) has been adopted by several investigators. It comprises (1) keratinizing squamous-cell carcinoma, (2) differentiated non-keratinizing carcinoma and (3) undifferentiated carcinoma. Keratinizing squamous-cell carcinomas are further divided into well, moderately or poorly differentiated squamous-cell carcinomas, depending on the presence of intercellular bridges and/or evidence of keratinization (pearl formation). Differentiated non-keratinizing carcinomas have organized cellular patterns but show no evidence of keratinization. The cells have vesicular nuclei with prominent nucleoli, well-defined cell margins, and show a stratified or pavemented pattern. A plexiform pattern may be seen, but the syncytial pattern of undifferentiated carcinomas is not apparent. Undifferentiated carcinomas include lymphoepitheliomas. They are composed of malignant epithelial cells arranged in a syncytial fashion with heavy infiltration of non-malignant lymphocytes. The malignant epithelial cells have vesicular nuclei and prominent nucleoli, and the cell margins are indistinct, consistent with the types described by Schminke (1921) and Regaud (1921). Occasionally, spindle-shaped tumour cells with hyperchromatic nuclei may be present.

2.4.2 Epidemiology

Nasopharyngeal carcinoma is a disease with a remarkable racial and geographical distribution. It constitutes 75–95% of all malignant tumours occurring in the nasopharynx in low-risk populations and virtually all of those in high-risk populations (Ho, 1971; Sugano *et al.*, 1978; Levine & Connelly, 1985).

2.4.2.1   *Descriptive epidemiology*

(a)   *International patterns*

Nasopharyngeal carcinoma is a rare malignancy in most parts of the world, where the age-standardized incidence rate for people of either sex is generally less than 1 per 100 000 persons per year (Parkin *et al.*, 1997). Table 23 lists the handful of populations that are known to deviate from this low-risk pattern, together with the age-standardized (world population) incidence rates for men and women separately. The overall incidence of nasopharyngeal carcinoma is elevated in China, with substantial variation between regions. In general, the incidence increases from the north to the south. Whereas the rates in Chinese men in the northernmost provinces are about 2–3/100 000 person-years, those residing in the southernmost province of Guangdong have rates of 25–40/100 000 person-years (National Cancer Control Office, Nanjing Institute of Geography, 1979; Yu *et al.*, 1981; Parkin *et al.*, 1997). High rates approaching those observed in southern China are seen among Inuits and other natives of the Arctic region (Albeck *et al.*, 1992; Nutting *et al.*, 1993). Intermediate to moderately high rates of nasopharyngeal carcinoma (3–10/100 000 person-years) are seen among many indigenous peoples of Southeast Asia, including Thais, Vietnamese, Malays and Filipinos (Parkin *et al.*, 1997). In Sabah, Malaysia, rates similar to those observed among the Inuits have been reported for the native Kadazans (Rothwell, 1979). Intermediate to moderately high rates of naso-pharyngeal carcinoma are also observed among Arabs in Kuwait and Algeria (Parkin *et al.*, 1997). Reviews of hospital series indicate that the rates of nasopharyngeal carci-noma are increased in the mainly Arab populations of Tunisia, Morocco, the Sudan and Saudi Arabia (Muir, 1971; Cammoun *et al.*, 1974; Hidayatalla *et al.*, 1983; Al-Idrissi, 1990). Migrants to Israel from North Africa (Morocco, Algeria and Tunisia) have inter-mediate rates of nasopharyngeal carcinoma which persist in their offspring born in Israel (Parkin & Iscovich, 1997).

The proportions of the histological types of nasopharyngeal carcinoma appear to vary with geographical location and race. In the low-risk areas of Japan, type-1 nasopharyn-geal carcinoma accounts for 12% of cases, whereas type 3 predominates in high-risk areas of Taiwan (Sugano *et al.*, 1978). In sub-Saharan Africa, which is a relatively low-risk area for nasopharyngeal carcinoma, type-3 nasopharyngeal carcinoma is also the predominant histopathological type (Cammoun *et al.*, 1974). Further details on the patho-logy of nasopharyngeal carcinomas are given in the proceedings edited by Muir and Shanmugaratnam (1967) and de Thé and Ito (1978).

(b)   *Migration*

Most Chinese living outside China originate from the provinces of Guangdong and Fujian in the south-east (Ho, 1959), a region with a high incidence of nasopharyngeal carcinoma (National Cancer Control Office, Nanjing Institute of Geography, 1979). Southern Chinese migrants, irrespective of the country to which they migrate, continue to have high rates of nasopharyngeal carcinoma (Worth & Valentine, 1967; King & Haenszel, 1973; Armstrong *et al.*, 1979; Gallagher & Elwood, 1979; Lee *et al.*, 1988;

Table 23. Populations at increased risk for nasopharyngeal cancer

Population	Age-standardized (world) incidence[a]		Reference
	Male	Female	
Chinese, Hong Kong	24.3	9.5	Parkin et al. (1997)
Chinese, Taipei	8.1	3.2	Chen et al. (1988a)
Chinese, Shanghai	4.5	1.8	Parkin et al. (1997)
Chinese, Tianjin	1.8	0.6	Parkin et al. (1997)
Inuits, Greenland	12.7	9.2	Albeck et al. (1992)
Inuits, Athabascans, Aleuts, Alaska	11.9	5.6	Nutting et al. (1993)
Thais, Chiang Mai	2.6	1.5	Parkin et al. (1997)
Vietnamese, Hanoi	10.3	4.8	Parkin et al. (1997)
Malays, Singapore	6.5	2.0	Parkin et al. (1997)
Filipinos, Manila	7.6	3.7	Parkin et al. (1997)
Kadazans, Sabah	15.9	8.7	Rothwell (1979)
Kuwaitis, Kuwait	2.3	0.6	Parkin et al. (1997)
Algerians, Sétif	8.0	2.7	Parkin et al. (1997)
Israeli Jews born in Morocco, Algeria or Tunisia	2.8	1.3	Steinitz et al. (1989)

[a] Per 100 000 person-years

Parkin et al., 1997), although succeeding generations of such immigrants in countries such as the United States (King & Haenszel, 1973; Buell, 1974; Yu et al., 1981) and Australia (Worth & Valentine, 1967) show continually declining rates. In contrast, Singapore-born Chinese do not have lower rates of nasopharyngeal carcinoma than Chinese born in China (Lee et al., 1988), perhaps because Chinese in Southeast Asia generally adhere to their traditional culture and customs while those in western countries gradually adopt the occidental way of life.

There have been reports that low-risk racial groups born and raised in high-risk areas have an increased risk for nasopharyngeal carcinoma. Buell (1973) reviewed deaths due to nasopharyngeal carcinoma in California, United States, and observed that white men born in the Philippine Islands or China had a higher risk than other white men, and Jews in Israel who were born in North Africa had higher rates of nasopharyngeal carcinoma than other Jews (Steinitz et al., 1989), the increase persisting in their children (Parkin & Iscovich, 1997). Men of French origin who were born in North Africa had a significantly higher rate of nasopharyngeal carcinoma than French men born in France (Jeannel et al., 1993).

(c)   Sex and age

In virtually all of the populations studied, the rates are higher among males than females. In most populations, the male:female ratio is 2–3:1 (Parkin et al., 1997). The

age distribution, however, shows interesting differences by population. In high-risk southern Chinese, the incidence in people of each sex increases steadily with age, reaching a peak at 45–54, and shows a definite decline at older ages (Armstrong et al., 1979; Yu et al., 1981; Parkin et al., 1997). The rates in China as a whole show no such decline after the age of 55 years; and the increase continues to at least 70–74 (National Cancer Control Office, Nanjing Institute of Geography, 1979). The distribution of age-specific rates in the low-risk populations studied, at least after age 20 years, is similar to the distribution of the overall rates in China (Balakrishnan, 1975; Burt et al., 1992; Parkin et al., 1997).

A peak in incidence is observed in adolescents of each sex in a number of populations at low to moderate risk for nasopharyngeal carcinoma. In the United States, a minor peak in the age group 10–19 years is seen in blacks (Burt et al., 1992; Parkin et al., 1997). In Sabah, Malaysia, Kadazan men have a secondary peak between the ages of 15 and 24 years (no data were available for Kadazan women; Rothwell, 1979). In India, the age distribution of 666 consecutive cases of nasopharyngeal carcinoma showed a peak at age 13–22 years, regardless of sex (Balakrishnan, 1975). When Balakrishnan (1975) pooled the incidence rates of nasopharyngeal cancer in 48 population groups published by Doll et al. (1970), he found a definite mode in young people of each sex between the ages of 10 and 19 years, which was not seen when similarly pooled data for cancers of the nose and nasal sinuses were plotted against age.

(d) Race and ethnicity

The high risk for nasopharyngeal carcinoma among Chinese is mainly confined to those residing in the southern provinces of Guangdong, Guangxi, Hunan and Fujian (see Figure 9; National Cancer Control Office, Nanjing Institute of Geography, 1979). The several distinct racial and ethnic groups that reside in this high-risk region have different rates of nasopharyngeal carcinoma. The highest rates are observed among the Tankas, a sub-ethnic group of Cantonese who inhabit the Pearl River Delta basin in central Guangdong. One feature that distinguishes the Tankas from other Cantonese is that they are seafaring people (either fishermen or sea transporters), who live on houseboats moored along the banks of the many branches of the Pearl River. The rates of naso-pharyngeal carcinoma among the Tankas are twice those among land-dwelling Cantonese (Ho, 1978; Li et al., 1985). In turn, the land-dwelling Cantonese (who comprise 98–99% of Cantonese) have a twofold higher rate of nasopharyngeal carcinoma than the Hakka and Chiu-Chau dialect groups, who live in north-east Guangdong (Yu et al., 1981; Li et al., 1985). The people of Fujian Province are culturally similar to the Chiu-Chau people in Guangdong Province, and so are their rates of nasopharyngeal carcinoma (National Cancer Control Office, 1980). It is interesting to note that the Hakkas (who rarely intermarry with other dialect groups) originated from northern China more than 500 years ago (Ho, 1959), but their rates resemble those of their Chiu-Chau neighbours and not those of their low-risk ancestors in the north. Even after they migrate to other parts of Southeast Asia, the Cantonese continue to have a twofold higher risk for nasopharyngeal carcinoma than the Hakkas, Chiu-Chaus and Fujianese (Armstrong et al., 1979; Lee et al., 1988).

Figure 9. Map of China, showing provinces where the risk for nasopharyngeal carcinoma is high
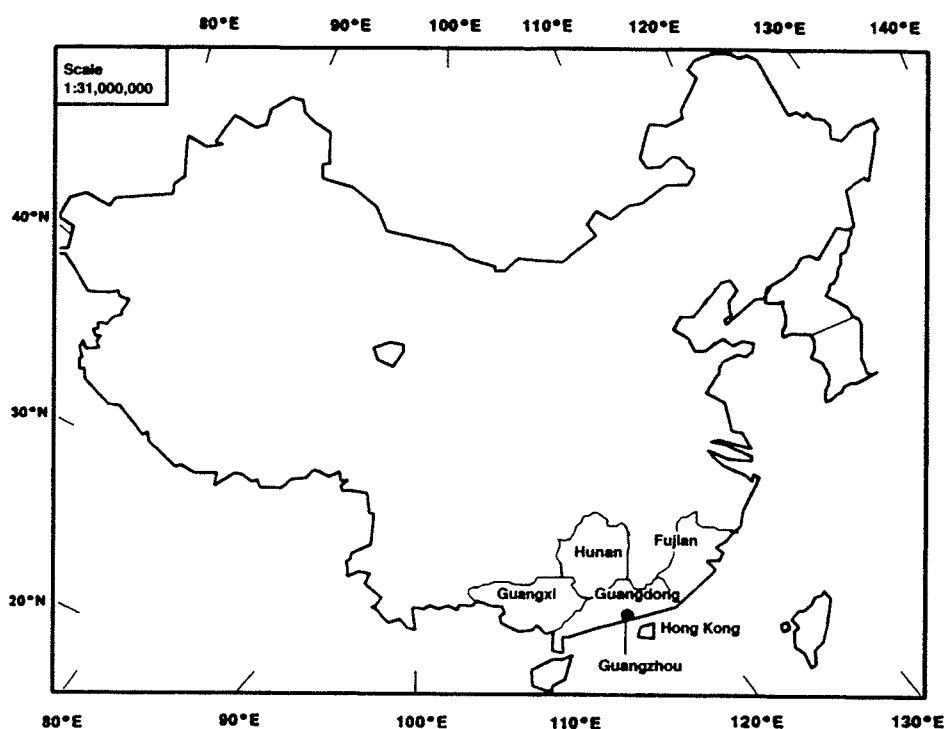
From National Cancer Control Office, Nanjing Institute of Geography (1979)

Two distinct racial groups inhabit the Autonomous Region of Guangxi. While the Zhuang people in western Guangxi have a rate of nasopharyngeal carcinoma that is one-fifth of that in Cantonese, the Han (the predominant race in China) people of eastern Guangxi, who are ethnically close to the Cantonese in Guangdong, have similar rates. The areas of Hunan Province that border both Guangxi and Guangdong to the north, not surprisingly have high rates of nasopharyngeal carcinoma. In addition, the Tujia and Miao minorities who inhabit the mountainous region of western Hunan have rates of nasopharyngeal carcinoma that approach those of the Cantonese (National Cancer Control Office, 1980).

In summary, the geographical variation in the incidence of nasopharyngeal carcinoma within southern China closely parallels the distribution of racial and ethnic groups inhabiting the region. The relatively high rates observed among the Hakkas who originated from low-risk northern China argues against genetic predisposition as a major cause of the varying risk patterns among these population groups. As these ethnic groups differ, however, in their customs and food habits, environmental factors inherent in their cultures may be responsible for the differences in susceptibility to nasopharyngeal carcinoma.

In Malaysia and Singapore, large numbers of Chinese and Indians live alongside the native Malays. As mentioned earlier, the Chinese and Malays have high rates; however, Indians in Southeast Asia have very low rates of nasopharyngeal carcinoma, comparable to those seen in whites in the United States (Armstrong *et al.*, 1979; Parkin *et al.*, 1997).

Different rates of nasopharyngeal carcinoma have also been reported among different ethnic groups inhabiting different parts of sub-Saharan Africa (Clifford, 1965; Schmauz & Templeton, 1972; Hidayatalla *et al.*, 1983).

(e) Socioeconomic status

Among the high-risk southern Chinese, individuals in the lower social strata have higher rates of nasopharyngeal carcinoma than those in the higher social strata (Geser *et al.*, 1978; Armstrong *et al.*, 1978; Yu *et al.*, 1981). Similar information for low-risk populations is scanty. Among white men living in rural or semi-rural counties in the United States, an inverse association is seen between years of schooling and the rate of mortality from nasopharyngeal carcinoma, while no clear trend is apparent among those living in predominantly urban counties (Hoover *et al.*, 1975).

(f) Urbanization

No difference in the risk for nasopharyngeal carcinoma has been noted between urban and rural residents in south China, or among Chinese in the Malaysian state of Selangor (Armstrong *et al.*, 1979). In metropolitan Hong Kong, local-born Cantonese have rates similar to those of people born and raised in the rural regions of Guangdong Province (Yu *et al.*, 1981). Urban residents in some low-risk populations, however, seem to have higher rates of nasopharyngeal carcinoma than their rural counterparts. In the United States, the mortality rate from this cancer in counties that are 100% urban is about twice that in counties that are 100% rural (Hoover *et al.*, 1975). In a comparison of the rates in urban and rural residents in 11 populations in Australia, Europe and Japan, seven populations had similar rates, while the urban rates were 1.3–2 times higher than those in rural areas in four populations (Muir *et al.*, 1987).

(g) Time trends

Early records showed that nasopharyngeal carcinoma was common among southern Chinese well over 50 years ago (Ho, 1978). Examinations of cancer registries in Malaysia between 1968 and 1977 and in Singapore between 1968 and 1987 showed little change in incidence in these southern Chinese populations (Armstrong *et al.*, 1979; Lee *et al.*, 1988, 1992). Similar data in Hong Kong, however, indicated a monotonic decline in the rates of nasopharyngeal carcinoma in people of each sex between 1974 and 1992 (the latest year for which complete incidence data were available). The male and female rates in 1974–77 were 32.9 and 14.4, respectively, while the corresponding figures in 1988–92 were 24.3 and 9.5, respectively (see Table 24). This decrease in the incidence of nasopharyngeal carcinoma in Hong Kong Chinese parallels a reduction in the use of Chinese-style salted fish (a human carcinogen, see IARC, 1993, and section 2.4.3.1(*a*)) since the mid- to late 1940s, as Hong Kong developed economically (Geser *et al.*, 1978; Yu *et al.*, 1986).

Data from the Connecticut Tumor Registry (United States) between 1935 and 1974 show relatively stable rates over the 40-year period (Levine *et al.*, 1980b). Similarly, an analysis of data collected from the nationwide Surveillance, Epidemiology and End

Table 24. Time trends in average annual incidence rates of nasopharyngeal carcinoma (per 100 000) in Hong Kong

Period	Average annual incidence	
	Males	Females
1974–77	32.9	14.4
1978–82	30.0	12.9
1983–87	28.5	11.2
1988–92	24.3	9.5

Based on data from *Cancer Incidence in Five Continents* (Waterhouse *et al.*, 1982; Muir *et al.*, 1987; Parkin *et al.*, 1992, 1997)

Results programme in the United States during 1973–86 showed no evidence of a change in incidence rates during the 14-year period (Burt *et al.*, 1992). A review of data on Canadian Inuits over a 25-year period (1949–74) also indicated no appreciable variation in rates over time (Schaefer *et al.*, 1975). In contrast, the rates for Chinese Americans have been declining steadily since 1950, such that the mortality rate from this cancer in men was halved between 1950–54 and 1970–79, from 12 to 6 per 100 000 person-years (Fraumeni & Mason, 1974; Levine *et al.*, 1987). This trend is likely to be the result of increased representation of local-born Chinese in the age groups at high risk for nasopharyngeal carcinoma as this lower-risk population ages, and increased migration from Taiwan and other intermediate- to low-risk regions in China since the 1950s. Prior to that time, virtually all Chinese Americans originated from Guangdong Province (Chinn *et al.*, 1969; Lai, 1988).

(h) Correlation with age-specific prevalence of EBV infection

Throughout China, there is little variation in the prevalence of infection or the age at primary infection with EBV (Zeng, 1985), although a more than 20-fold difference in risk exists within the country. Virtually all Chinese children are infected by the age of three to five, and no difference in serological profile has been observed between Chinese populations with drastically different rates of incidence of nasopharyngeal carcinoma (Gu & Zeng, 1978; Zeng, 1985). de Thé *et al.* (1975) compared the age-specific prevalence of antibodies to EBV VCA in three populations at low risk for nasopharyngeal carcinoma (Singapore Indian, Ugandan and French Caucasian) with that in Singapore Chinese, who have the highest incidence of nasopharyngeal carcinoma in the world (Parkin *et al.*, 1997). In comparison with Singapore Chinese, the age at primary infection with EBV is earlier in Ugandans and Singapore Indians and later in French Caucasians; yet the high risk for nasopharyngeal carcinoma is unique to Singapore Chinese.

2.4.2.2 Case series

(a) Antibodies in sera and throat washings

The association between nasopharyngeal carcinoma of the undifferentiated type and EBV was first revealed by Old et al. (1966). As seen in Figure 10, titres of IgG antibodies to VCA, EA and soluble complement-fixing antigens increase with clinical stage of disease (Henle et al., 1970; de Thé et al., 1975). IgA antibodies to VCA and EA were later found to be an outstanding feature of nasopharyngeal carcinoma (Wara et al., 1975; Henle & Henle, 1976b; Ho et al., 1976; Desgranges et al., 1977; Pearson et al., 1978b).

Figure 10. Titres of IgG antibodies to EBV in Chinese nasopharyngeal carcinoma (NPC) patients at different stages of the disease and, for comparison, in normal Chinese controls and Chinese patients with other tumours
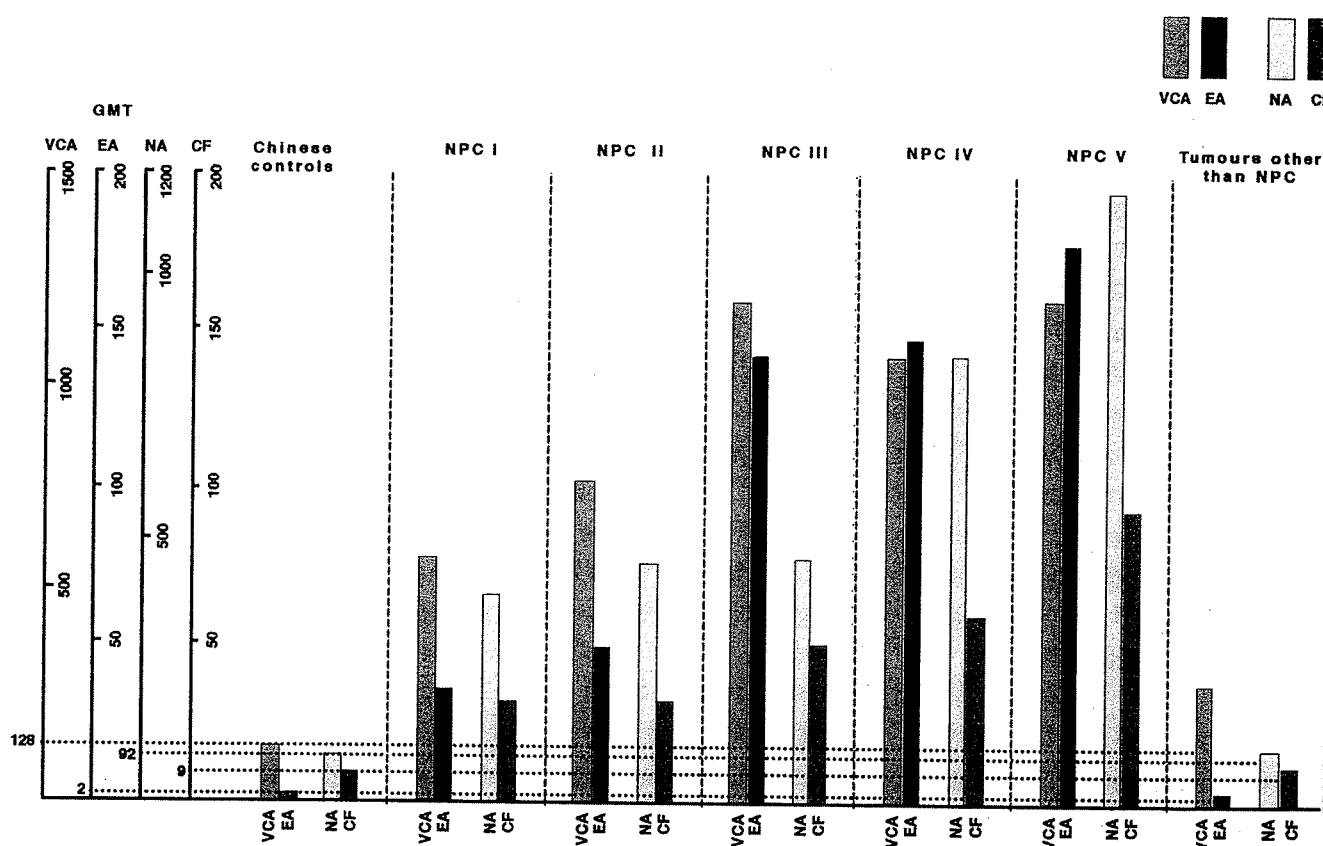
From de Thé et al. (1975b)

GMT, geometric mean titre; VCA, viral capsid antigen; EA, early antigen; NA, nuclear antigen; CF, complement-fixing antigens

Figure 11 shows the prevalence of IgG and IgA antibodies to VCA and EA in throat washings from patients with nasopharyngeal carcinoma and with other cancers. The titres of both complement fixing antibodies to a soluble EBNA (Sohier & de Thé, 1971) and antibodies to EBNA have been found to be elevated in nasopharyngeal carcinoma patients (de Thé et al., 1973; Baskies et al., 1979).

Figure 11. Titres of IgA and IgG antibodies to EBV in throat washings from patients with nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), infectious mononucleosis (IM) or other tumours and from normal individuals
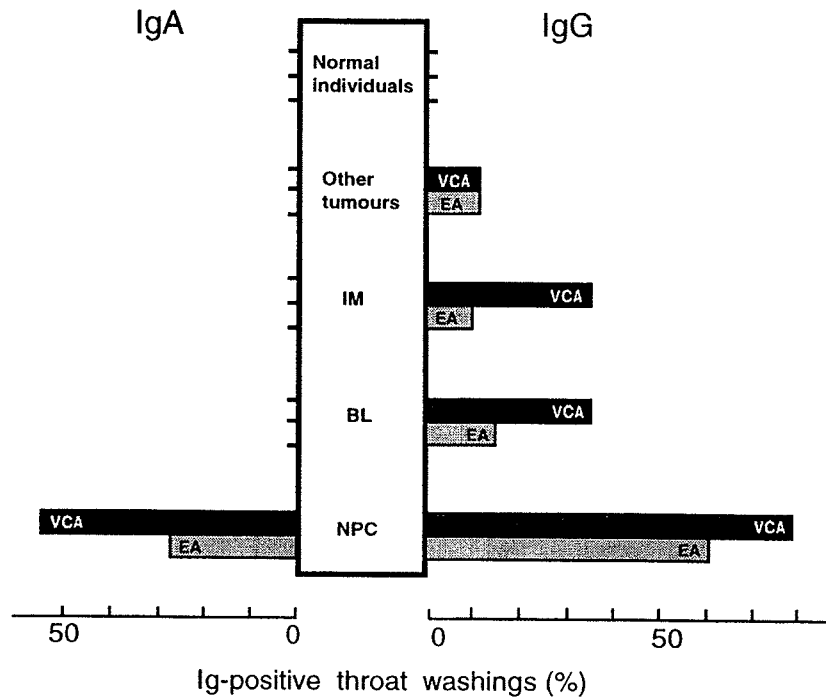
From Desgranges and de Thé (1978)
VCA, viral capsid antigen; EA, early antigen

Several studies have indicated that the EBV serological profile also differs between patients with different histological types of nasopharyngeal carcinoma. In one of the first studies of these histological subsets, patients with squamous-cell carcinoma were found to have lower EBNA titres but similar titres of IgG to VCA and EA (Shanmugaratnam *et al.*, 1979). Krueger *et al.* (1981) showed elevated titres of IgA to VCA and of IgG to EA and VCA in patients with type-2 or type-3 nasopharyngeal carcinoma, while two patients with type-1 squamous-cell carcinomas had normal titres of IgG to VCA. In a study of Malaysian patients of Chinese, Malay and other ethnic origins, patients with any form of nasopharyngeal carcinoma had elevated IgA titres to VCA (Sam *et al.*, 1989). EBV serology is useful in the identification of patients with occult nasopharyngeal carcinoma (Ho *et al.*, 1976), as shown by elevated titres of anti-IgA VCA; however, histopathological characterization of the tumour is essential for diagnosis, prognosis and treatment.

(b)   Nucleic acid markers in carcinoma cells

Nearly all cases of types-2 and -3 nasopharyngeal carcinoma had detectable EBV DNA sequences, as ascertained by DNA/DNA or c-RNA/DNA hybridization and DNA/-DNA reassociation kinetics in biopsy samples (zur Hausen *et al.*, 1970; Wolf *et al.*,

1973; Nonoyama & Pagano, 1973; Desgranges *et al.*, 1975; Pagano *et al.*, 1975). EBV EBNA was detected in touch smears of nasopharyngeal carcinomas (de Thé *et al.*, 1973; Huang *et al.*, 1974; Klein *et al.*, 1974; Zeng *et al.*, 1981).

Early studies by hybridization kinetics analysis revealed that the viral DNA found in Burkitt's lymphoma is homologous to that in nasopharyngeal carcinomas and that the latter have a relatively high copy number of the EBV genome (zur Hausen *et al.*, 1970; Nonoyama & Pagano, 1973). In another study, viral DNA and EBNA were detected in malignant epithelial cells rather than in the abundant infiltrating lymphoid cells (Wolf *et al.*, 1973). All samples of type-3 nasopharyngeal carcinoma from endemic areas and from areas of intermediate or low incidence contained EBV DNA (Desgranges *et al.*, 1975; Pagano *et al.*, 1975; Raab-Traub *et al.*, 1987). The detection of EBV DNA and EBERs has been useful in identifying carcinomas that have metastasized to lymph nodes when the primary tumour has not been identified (Ohshima *et al.*, 1991; Chao *et al.*, 1996). Type-1 nasopharyngeal carcinoma has been associated with a low copy number of EBV (Raab-Traub *et al.*, 1987): in some studies, EBV was not found in more than half of the cases of type 1 nasopharyngeal carcinoma from nonendemic areas, whereas cases from endemic areas all contained EBV (Pathmanathan *et al.*, 1995a).

Clonal EBV infection was demonstrated in biopsy samples from nasopharyngeal carcinomas in China and the United States (Raab-Traub & Flynn, 1986; Raab-Traub *et al.*, 1987; Pathmanathan *et al.*, 1995b), and also in dysplastic lesions of the naso-pharynx in individuals with elevated IgA titres in China, suggesting involvement of EBV prior to the carcinomatous state (Pathmanathan *et al.*, 1995b).

Viral replication is minimal in tumour cells, but the virus can be isolated afer grafting into nude mice (Trumper *et al.*, 1977).

(c) Viral gene expression in tumour specimens

Transcriptional expression of EBV latent genes in nasopharyngeal carcinoma cells has been studied by northern hybridization (Raab-Traub *et al.*, 1983; Gilligan *et al.*, 1990b, 1991; Brooks *et al.*, 1992; Busson *et al.*, 1992a; Chen *et al.*, 1992c; Karran *et al.*, 1992), by RT-PCR and, in some cases, by in-situ hybridization (Wu *et al.*, 1991; Cochet *et al.*, 1993).

BARFO, LMP-2, EBER and *EBNA-1* are always expressed in nasopharyngeal carcinoma cells (Fåhraeus *et al.*, 1988; Young *et al.*, 1988; Brooks *et al.*, 1992; Gilligan *et al.*, 1991; Sbih-Lammali *et al.*, 1996), whereas LMP-1 is expressed in a variable pro-portion of tumour cells (Fåhraeus *et al.*, 1988; Young *et al.*, 1988). The early genes *BALF 5, BZLF, BMFRI, EA(D)* and *BHRF* (*EA(R)*) are occasionally detected in a few cells. Early proteins such as ribonucleotide reductase, BZLF1, BMRF1 (EA(D)) and BHRF-1 (EA(R)) can be detected with monoclonal antibodies (Luka *et al.*, 1988; Lung *et al.*, 1989; Cochet *et al.*, 1993). The presence of EA(D), encoded by *BMRF1*, was not confirmed (Young *et al.*, 1989a), and the site of EA production, which is at the origin of the strong humoral response to that antigen by nasopharyngeal carcinoma patients, remains an open question.

2.4.2.3 Case–control studies

(a) Based on pre-diagnostic serological tests

Lanier *et al.* (1980a) compared the results of serological tests on seven native Alaskan patients with histologically confirmed nasopharyngeal carcinoma and eight controls matched for sex, age, race and residence for whom frozen sera could be found. Several sera were collected for each subject. The oldest samples were collected 22–120 months before diagnosis of nasopharyngeal carcinoma in cases and 3–90 months after the date of diagnosis of the cases in controls. IgA antibodies to VCA were detected in one case 22 months before and three months after diagnosis but in none of the controls. Two patients who showed no IgA antibodies to VCA on two occasions before diagnosis had raised titres at the time of diagnosis and in subsequent tests. All of the cases and controls had detectable titres of anti-EBNA: GMT, [60] in cases, [21] in controls; median titre, [80] in cases, [20] in controls.

(b) Based on serological tests at time of diagnosis

These studies are summarized in Table 25.

Patients with advanced nasopharyngeal carcinoma, whether Cantonese Chinese in Hong Kong, Maghrebian Tunisians or Caucasians in France, had higher IgG and IgA titres to VCA and EA than patients with other tumours or than normal subjects (de Thé *et al.*, 1978b).

Pearson *et al.* (1983b) compared the IgG and IgA anti-VCA antibody patterns of sera from 124 consecutive cases of nasopharyngeal carcinoma in the United States and in 278 sera obtained from relatives of the cases or of patients with benign diseases or cancer of the head and neck other than nasopharyngeal carcinoma. All cases and 90% of controls had detectable levels of IgG to VCA; IgA antibodies to VCA were detected in 69% of the cases (84% of undifferentiated carcinomas) and 9% of the controls, and IgG antibodies to EA were found in 76% of cases (86% of undifferentiated carcinomas) and 29% of controls.

Two case–control studies in Chinese populations examined the association between the prevalence of anti-VCA IgA antibodies in nasopharyngeal carcinomas and in population controls. Zheng *et al.* (1994a) reported an odds ratio associated with infection of 55 (95% CI, 11–280) adjusted for sex and age and foods associated with nasopharyngeal carcinoma. Chen *et al.* (1987) also found a significant increase in the odds ratio with increasing anti-VCA IgA titres. The odds ratio for positive versus negative subjects, adjusted for sex, age, marital status and consumption of foods associated with nasopharyngeal carcinoma, was 39 (statistically significant).

2.4.2.4 Cohort studies

Chan *et al.* (1991) identified seven cases of undifferentiated or poorly differentiated nasopharyngeal carcinoma in four Asians and three Caucasians (six male and one

Table 25. Case–control studies of nasopharyngeal carcinoma and EBV serology at time of diagnosis

Reference and region	Ethnicity	No of cases/No. of controls	Morphology	EBV marker	Measure	Results	Comments
de Thé et al. (1978b) Hong Kong, France, Tunisia	Chinese, Causasian, North African	132/150 selected cases of advanced nasopharyngeal carcinoma; controls in same age range	[92%] undifferen-tiated or poorly differentiated	IgG/VCA IgG/EA	Geometric mean titre	Significantly higher in cases	
Pearson et al. (1983b) Northern USA	Mainly Caucasian	124 /278 Controls: age-matched relatives, head-and-neck tumours other than nasopharyngeal carcinoma, benign disorders of head and neck	Undifferentiated, 85 Non-keratinizing, 11 Squamous, 26	IgG/VCA	Positive versus negative	Positive: 100% cases, 90% controls	Crude odds ratio [23], 95% CI [13–40]
				IgA/VCA	Positive versus negative	69% cases (84% undifferentiated), 9% controls	
				IgG/EA	Positive versus negative	76% cases (86% undifferentiated), 29% controls	[32] [18–57]
					Positive for both IgG EA and IgA VCA	62% of undifferentiated and non-keratinizing, 7% of squamous, 1% of normal	
Chen et al. (1988b) Taiwan	Chinese	205/205 neighbourhood controls matched on sex and age	Histologically verified	IgA/VCA	Positive versus negative	Odds ratio, 39, $p < 0.01$	
				Anti-EBV DNase	< 2 2 ≥ 4	1 12 62, $p < 0.01$	Adjusted for matching variables and dietary factors
Zheng et al. (1994a) China	Chinese	203/163 (blood available), neighbouhood controls matched on sex and age	Histologically confirmed but type not reported	IgA/VCA	Positive versus negative	Odds ratio, 55 (95% CI, 11–280)	Adjusted for matching variables and some dietary factors

Numbers in square brackets were calculated by the Working Group.
VCA, viral capsid antigen; EA, early antigen; CI, confidence interval

female) that developed in a cohort of over 240 000 individuals in Norway and the United States and for whom sera had been stored in four banks. Two controls per case were selected from the cohort and matched on bank, storage duration, age, sex and race. The intervals between serum collection and diagnosis of nasopharyngeal carcinoma were 26, 76, 122, 124, 134, 153 and 154 months. The GMTs of IgA antibodies to VCA, anti-EA(D), anti-EA(R) and anti-EBNA were similar in cases and controls. The odds ratio associated with high titres of IgA to VCA was 1.0 (95% CI, 0.1–11) and that for high anti-EBNA titres was 0.7 (95% CI, 0.1–5.3).

2.4.2.5   Mass serological surveys

These studies are summarized in Table 26.

Two screening surveys for EBV positivity in sera were conducted in Guangxi Autonomous Region, China. In the first, 148 029 subjects living in rural areas of Zangwu County were screened for IgA antibodies to VCA. Among the first 56 584 examined, 117 (0.2%) had positive results. These people then underwent thorough clinical investigation, which led to the diagnosis of 18 cases of nasopharyngeal carcinoma; two additional cases were diagnosed in the 10 months after the intervention (Zeng et al., 1980). The final number of antibody-positive subjects in the cohort was 3533 (prevalence, 2.4%), and 55 nasopharyngeal carcinomas were found in this group (Zeng et al., 1983a). Thus, the prevalence of IgA antibodies to VCA in the 91 445 subjects recruited after the interim report (Zeng et al., 1980) was 3.7%. In the second survey, individuals aged 40 years or more and resident in Wuzhou City in Zangwu County were examined. Among 20 726 people screened, 5.5% had IgA antibodies to VCA; 18 cases of nasopharyngeal carcinoma (1.6%) were detected among the latter (Zeng et al., 1985; Zeng, 1987). Similar prevalences of antibody-positive subjects and nasopharyngeal carcinoma cases had been reported in a preliminary analysis of the first 12 932 individuals screened (Zeng et al., 1982). Thirty-five additional cases of nasopharyngeal carcinoma were identified retrospectively in the cohort of antibody-positive individuals during the 10 years after the survey; however, the number of person-years of observation was not available. Four nasopharyngeal carcinomas were seen in subjects who had no IgA antibodies to VCA (Zeng et al., 1993).

In a third survey, conducted in Taiwan (Chen et al., 1989), anti-EBV DNase activity was found in 1250 of 22 596 (5.5%) Government employees and 1176 of 9869 (11.9%) residents of a high-risk area. When those with antibodies were referred for clinical investigation, three and 11 nasopharyngeal carcinoma cases were detected among those who complied with referral in the two groups, corresponding to prevalences of 0.6 and 1.3%, respectively. EBV-negative individuals were not screened for nasopharyngeal carcinoma, and no long-term follow up of the cohort has been performed.

Table 26. Detection of EBV in mass serological surveys and association with nasopharyngeal carcinoma

Reference and region	No. screened	EBV detection	Presence of antibodies		Cases of nasopharyngeal carcinoma in EBV-positive cases		Incidence in EBV-positive cases
			No.	%	No.	%	
Zeng et al. (1979, 1980, 1983a); rural Zangwu County, China	Interim analysis: 56 584 Final cohort: 148 029	IgA VCA	117 3 533	0.2 2.4	18 55	15.4 1.6	Two additional cases in 10-month follow-up
Zeng et al. (1982, 1985); Zeng (1987); Wuzhou City, Zangwu County	Interim analysis: 12 932 Final cohort: 20 726	IgA VCA	680 1 136	5.3 5.5	13 18	5.3 1.6	35 additional cases in 10-year follow-up (Zeng et al., 1993)
Chen et al. (1989); Taiwan Government employees	22 596	Anti-EBV DNAse	1 250	5.5	3/477[a]	0.6	477 who complied with referral
High-risk area	9 869		1 176	11.9	11/829[a]	1.3	829 who complied with referral

[a] Number of people attending a specific clinic at the National Taiwan University Hospital

2.4.3 Cofactors

2.4.3.1 Dietary factors

(a) Cantonese-style salted fish

The higher rates of nasopharyngeal carcinoma among the boat-dwelling Tankas than among the land-dwelling Cantonese in Hong Kong was first noted by Ho (1967), who also observed that salted fish is the principal source of supplementary food in the diet of these people, which consists mainly of rice. He subsequently suggested that Cantonese-style salted fish be investigated as a 'possible etiological factor' for nasopharyngeal carcinoma (Ho, 1971). He made two additional observations: the salting process is inefficient and the product, aged for several days to weeks, becomes partially putrefied, liberating a pungent odour that is offensive to those who were not raised in the southern Chinese culture (Ho, 1972). Later, he recognized that salted fish mixed with soft rice is a popular weaning food in southern China (Ho, 1976).

Eleven case–control studies have been conducted in various Chinese populations with distinct risks for nasopharyngeal carcinoma to investigate the possible association with the consumption of salted fish. Five were conducted in high-risk Cantonese populations (Geser et al., 1978; Henderson & Louie, 1978; Yu et al., 1986, 1989a; Zheng et al., 1994a), three were carried out in populations with about half the incidence rate of Cantonese (Chen et al., 1988b; Yu et al., 1988; Zheng et al., 1994b), one study was conducted in the relatively low-risk population of Tianjin in northern China (Ning et al., 1990), and two studies were conducted in various Chinese populations in Southeast Asia (Armstrong et al., 1983; Lee et al., 1994b). One epidemiological study addressed the relationship between consumption of Cantonese-style salted fish and nasopharyngeal carcinoma risk in a non-Chinese population (Sriamporn et al., 1992).

Henderson and Louie (1978) in the United States interviewed 74 Chinese patients with nasopharyngeal carcinoma and 110 Chinese control subjects. The patients represented incident cases identified from the population-based cancer registries of Los Angeles County and the San Francisco Bay area. The controls were clinic or hospital patients who were frequency matched to the cases on race, age, sex and socioeconomic status; the subjects were all asked about how often they ate salted fish. A statistically significant association with risk for nasopharyngeal carcinoma was seen (p for linear trend = 0.02), and the odds ratio for consumption two or more times per week relative to no consumption was 3.1.

Geser et al. (1978) conducted a hospital-based case–control study in Hong Kong involving 150 patients with nasopharyngeal carcinoma and 150 age- and sex-matched hospital controls. Salted fish intake was not determined for the study subjects, but older women in the households of the patients and controls were interviewed, when possible, about weaning practices. This information was collected from 108 mothers of nasopharyngeal carcinoma patients and 103 mothers of controls. The only food given to babies during and after weaning for which a statistically different prevalence was found between case and control households was salted fish (odds ratio, 2.6; $p < 0.01$).

In Hong Kong, Yu *et al.* (1986) interviewed 250 patients with nasopharyngeal carcinoma under the age of 35 and an equal number of age- and sex-matched friends about their dietary habits three years before diagnosis of the index case and at 10 years of age. Furthermore, 182 mothers of cases and 155 mothers of control subjects were interviewed about the dietary habits of the subjects during weaning, between the ages of one and two years and at the age of 10 years. All of the cases were histologically confirmed at the four hospitals in Hong Kong in which over 90% of new nasopharyngeal carcinoma cases are diagnosed. Salted fish consumption at all periods was significantly related to the risk for this tumour, and increasing frequency of intake was consistently associated with increasing risk. The association with salted fish was stronger when exposure occurred during childhood as compared with adulthood (i.e. three years before interview). Exposure during weaning was associated with an odds ratio of 7.5 (95% CI, 3.9–15); the odds ratio for weekly or more versus less than monthly consumption at age 10 was 38 (95% CI, 14–100). No other dietary or environmental factor studied was significantly associated with nasopharyngeal carcinoma after adjustment for salted fish intake, and the odds ratios for salted fish consumption remained highly significant after all other potential risk factors had been taken into account. It was estimated that over 90% of the cases of nasopharyngeal carcinoma in young people in Hong Kong could be attributed to childhood consumption of Cantonese-style salted fish.

Yu *et al.* (1989a) interviewed 306 patients with histologically confirmed nasopharyngeal carcinoma who lived in Guangzhou, China, and were under the age of 50 years and an equal number of controls matched for age, sex and neighbourhood. Subjects were asked about their dietary pattern three years before diagnosis of the index case and at the age of 10. The mothers of 110 cases under the age of 45 years and of 139 of their matched controls were interviewed about their own consumption of salted fish during the index pregnancy and during lactation and about the subjects' intake of salted fish during weaning, between the ages of one and two years, and at the age of 10. Exposure to salted fish, whether in adulthood or in childhood, was significantly associated with an increased risk for nasopharyngeal carcinoma. The association was strongest for exposure during weaning (odds ratio, 2.1; 95% CI, 1.2–3.6); exposures at all other times were no longer significantly related to the risk for nasopharyngeal carcinoma after adjustment for exposure during weaning. Childhood exposure to salted fish remained a highly significant risk factor after adjustment for other dietary and non-dietary risk factors identified in this study.

Zheng *et al.* (1994a) conducted a hospital-based case–control study in Guangzhou, China, comprising 205 histologically confirmed cases of nasopharyngeal carcinoma under the age of 55 years and an equal number of friends who were individually matched to the cases by age and sex. Subjects were asked about their dietary habits during the past seven years and at the age of 10. Furthermore, the mothers of 151 cases and 195 controls were interviewed about the subjects' dietary habits during the first three years of life and at the age of 10. Salted fish consumption during all of the periods studied was significantly associated with risk for nasopharyngeal carcinoma. The association was strongest for consumption during the first three years of life ($p < 0.001$): the odds ratio for weekly or daily intake relative to less than monthly was 13 (95% CI, 5.2–21). The results

were unchanged after adjustment for the presence of IgA antibodies against EBV VCA. It was estimated that salted fish intake could explain 73% of the cases of nasopharyngeal carcinoma occurring in this Cantonese population.

In a case–control study in Taipei, Taiwan, involving 205 histologically confirmed cases from a single hospital and 205 neighbourhood controls individually matched to the cases by age and sex, Chen *et al.* (1988b) collected information on the frequency of intake of a number of preserved foods before the age of 20, by interview. Individuals who ate salted fish 10 or more times per month had an odds ratio of 1.5 relative to less than monthly intake, which was not statistically significant. Childhood exposure to salted fish was not examined in this study.

Yu *et al.* (1988) interviewed 128 mothers of 231 eligible patients with nasopharyngeal carcinoma under the age of 45 in Yulin Prefecture, Guangxi Autonomous Region, China, in order to examine the relationship between exposures during childhood and subsequent development of nasopharyngeal carcinoma. They also interviewed 174 mothers of 231 individually matched population controls. The mothers were asked about their consumption of salted fish during the index pregnancy and lactation and about the subjects' intake of salted fish during weaning, between the ages of one and two and at the age of 10. Salted fish was not a common weaning food in this population: only 6.3% of controls were exposed. Nonetheless, exposure to salted fish during this early period of life was associated with a 2.6-fold increase in the risk for nasopharyngeal carcinoma (95% CI, 1.2–5.6). The mother's consumption of salted fish during the pregnancy and lactation was also significantly related to risk (p for linear trend = 0.003 and 0.01, respectively). Exposure at the age of 10 was less strongly related, the odds ratios for monthly and weekly intake as compared to less than monthly consumption being 1.5 in both instances and not statistically significant.

Zheng *et al.* (1994b) conducted a case–control study in Zangwu County in eastern Guangxi, China, which involved 88 incident cases of nasopharyngeal carcinoma and twice as many population controls individually matched to the cases by age, sex and neighbourhood of residence. Subjects were asked about their dietary habits during weaning, before the age of two, between the ages of two and 10 and one year before diagnosis of nasopharyngeal carcinoma. Salted fish intake at any of the three times during childhood was significantly related to the risk for nasopharyngeal carcinoma (during weaning, odds ratio, 2.4; p = 0.01). Consumption one year before diagnosis of the tumour was relatively rare (2.3% in cases and 0.6% in controls) and was not significantly related to risk.

Ning *et al.* (1990) studied 100 histologically confirmed cases of nasopharyngeal carcinoma in the Han population in people under the age of 65 identified from a population-based cancer registry covering the Tianjin Metropolitan Area in northern China, and three times as many population controls individually matched to the cases by age, sex and neighbourhood of residence. Questions were asked about the frequency of intake of selected foods, including salted fish, at or before the ages of 10, 20, 30, 40 and 50 years. In this relatively low-risk population, in which salted fish is not a common dietary item, any exposure to salted fish was a significant risk factor for nasopharyngeal

carcinoma (odds ratio, 2.2; 95% CI, 1.3–3.7). Four characteristics of exposure to salted fish contributed independently to the increased risk: (1) low age at first exposure; (2) long duration of consumption; (3) frequent consumption at age 10; and (4) cooking by steaming as opposed to frying, grilling or boiling. The last factor could be determined in this population because, unlike the southern Chinse who almost always steam salted fish, consumers in Tianjin use a variety of methods. It was estimated that 40% of naso-pharyngeal carcinoma cases occurring in this low-risk region could be attributed to the consumption of salted fish.

Armstrong *et al.* (1983) conducted a case–control study among Malaysian Chinese resident in 27 census districts surrounding (and including) metropolitan Kuala Lumpur, in central Selangor. The 100 cases were all histologically confirmed and identified at the only radiotherapy centre in Malaysia. The 100 control subjects were selected from the neighbourhoods in which the cases lived and were individually matched to the index case by sex and age. Subjects were asked about their intake of salted fish during childhood, during adolescence and at the time of interview. Since there was evidence that the diet of many of the patients had changed since manifestation of clinical cancer, the investigators discarded the information on intake at the time of interview which, for a number of the cases, was several years after the diagnosis. Salted fish intake during childhood and ado-lescence were both significantly related to the risk for nasopharyngeal carcinoma, with a stronger association for exposure during childhood. The odds ratio was 3.0 ($p = 0.04$) for any consumption during childhood and 17 (95% CI, 2.7–110) for daily consumption.

In Singapore, Lee *et al.* (1994b) compared 200 Chinese patients in whom naso-pharyngeal carcinoma had been diagnosed consecutively at a major general hospital; the 406 controls were patients at the same hospital who were frequency matched to the cases by ethnicity, age and sex. Information on dietary habits one year previously was collected from all cases and controls, and information on diet during infancy and at the age of 10 was collected from the mothers of cases or controls, if they were available for interview, or from the subjects themselves. A total of 64 mothers of cases and 103 mothers of controls were interviewed, for a participation rate of 28%. The results for diet during infancy were considered by the authors to be inconclusive, and the Working Group considered that the information on diet at the age of 10 is also uninterpretable because information was obtained from both subjects and mothers and is likely to be of variable reliability. No significant relationship was found between salted fish intake one year before diagnosis and the risk for nasopharyngeal carcinoma (p for linear trend = 0.6), although the highest frequency category was associated with an excess fourfold risk (odds ratio, 4.4; 95% CI, 0.7–26).

Two types of salted fish are eaten by the population in northeast Thailand: home-made salted freshwater fish and Cantonese-style salted marine fish available on markets. Sriamporn *et al.* (1992) examined current consumption of both kinds of salted fish in a hospital-based case–control study involving 120 histologically confirmed cases of naso-pharyngeal carcinoma and an equal number of hospital controls individually matched to the cases by age and sex. Intake of Thai-style salted fish was not related to the risk for nasopharyngeal carcinoma, but consumption of Chinese-style salted fish showed a clear,

statistically significant dose–response relationship with risk, weekly consumers having an odds ratio of 2.5 (95% CI, 1.2–5.2) relative to non-consumers.

In summary, the epidemiological data strongly support the hypothesis that Cantonese-style salted fish is a nasopharyngeal carcinogen in humans. The studies suggest that age at exposure is an important co-determinant of risk, earlier age at exposure being associated with a higher risk for disease. Experimental data to support the carcinogenicity of Cantonese-style salted fish is summarized in section 4.5.2.

(b)   Other types of salted fish

There is preliminary evidence that early exposure to other types of salted fish may be responsible for at least some of the increase in risk for nasopharyngeal carcinoma in the native peoples of Southeast Asia and of the Arctic region. Armstrong and Armstrong (1983) studied 13 Malay cases of nasopharyngeal carcinoma and 50 Malay population controls of comparable age and sex distribution in a small case–control study conducted in Selangor, Malaysia. Five cases and four controls had eaten salted fish daily during childhood. The excess was not statistically significant.

In the Philippines, West et al. (1993) studied 104 predominantly Filipino cases of nasopharyngeal carcinoma from a single hospital, 104 hospital controls from the same hospital and 101 community controls, who were matched to the cases by age and sex; the hospital controls were also matched to the cases on type of hospital ward (private versus public), and the community controls were further matched to the cases on neighbourhood of residence. The two sets of controls were combined in the analysis. Individuals with the highest frequency of current intake of salted fish had a nonsignificantly increased risk for nasopharyngeal carcinoma relative to those with the lowest frequency (crude rate, 60%; adjusted rate, 30%). Childhood consumption of salted fish was not investigated in this study.

Lanier et al. (1980b) interviewed 13 nasopharyngeal carcinoma patients and 13 controls in Alaska who were individually matched to the cases by age, sex, race (Inuit, Indian, Aleut) and village of residence. The same questionnaire was administered to 17 patients with other head-and-neck tumours and their similarly matched control subjects. More nasopharyngeal carcinoma patients than controls had eaten salted fish as children, while no such association was observed for the patients with other head-and-neck cancers.

(c)   Other preserved foods

Early exposure to other preserved foods has also been shown to be related to the risk for nasopharyngeal carcinoma in Chinese populations and was addressed in some of the studies described in section 2.4.3.1(a). In Yulin, China, where salted fish is a relatively rare food item, a variety of preserved foods other than salted fish eaten during childhood have significant, independent effects on the risk (Yu et al., 1988). These include salted ducks' eggs (odds ratio, 5.0; $p = 0.03$), salted mustard greens (odds ratio, 5.4; $p = 0.03$) and chung choi (a salted root; odds ratio, 2.0; $p = 0.003$) eaten during weaning, and dried fish (p for linear trend $= 0.002$), fermented black-bean paste (p for linear trend $= 0.009$) and fermented soya-bean paste (p for linear trend $= 0.007$) eaten between the ages of one

and two. In the Cantonese population of Guangzhou, China, childhood exposure to mouldy bean curd (p for linear trend = 0.07), salted shrimp paste (p for linear trend = 0.04), *chan pai mui* (p for linear trend = 0.01) and *gar ink gee* (p for linear trend = 0.03) (the last two are preserved plums) was independently related to the risk for naso-pharyngeal carcinoma after adjustment for intake of salted fish (Yu *et al.*, 1989a). In Taiwan, exposure before the age of 20 to fermented bean products (odds ratio for > 10 times/month versus less than once a month, 1.8; p < 0.05) and smoked meat (odds ratio for > 1/month versus less frequently, 3.3; adjusted for all other risk factors; p < 0.05) were independently associated with the risk for nasopharyngeal carcinoma after adjust-ment for IgA antibody titres against EBV VCA (Chen *et al.*, 1988b). In Tianjin, China, exposure to salted shrimp paste at the age of 10 years was related to risk independently of salted fish intake (odds ratio for weekly/daily versus monthly frequency, 3.2; p = 0.007) (Ning *et al.*, 1990). In Singapore, Lee *et al.* (1994b) noted that consumption of five preserved foods one year before cancer diagnosis was significantly associated with an increased risk. These were *belachan* (salted shrimp paste; p for linear trend = 0.04), salted soya beans (fermented soya-bean paste; p for linear trend = 0.002), tinned pickled vegetables (p for linear trend = 0.004), *szechuan chye* (a salted tuber; p for linear trend = 0.008) and salted mustard greens (*kiam chye*; p for linear trend = 0.007). The effects of salted soya beans, *szechuan chye* and *kiam chye* remained statistically significant in a multivariate model that included other risk factors for nasopharyngeal carcinoma. While the levels of childhood exposure to most of these foods were low in the series studied, *chung choi* was given at weaning in 60% of cases in Yulin (Yu *et al.*, 1988), salted shrimp paste was consumed at the age of 10 by 28% of cases in Tianjin (Ning *et al.*, 1990), and 63% of the cases in Taiwan consumed fermented bean products before the age of 20 (Chen *et al.*, 1988b).

Jeannel *et al.* (1990) conducted a case–control study among Tunisians, who are at intermediate risk for nasopharyngeal carcinoma. Eighty histologically confirmed incident cases identified at the only cancer hospital in Tunisia and 160 population controls indi-vidually matched to the cases by age, sex and neighbourhood of residence were inter-viewed about dietary habits in the year preceding the cancer diagnosis and during child-hood. The intake of several preserved food products during childhood and/or adulthood was found to be significantly associated with the risk for nasopharyngeal carcinoma after adjustment for socioeconomic status. They were *touklia* (a stewing mixture of red and black peppers, paprika, caraway seed and/or coriander seed, salt and olive or soya-bean oil), *quaddid* (dried mutton preserved in olive oil), pickled vegetables, pickled olives and *harissa* (a mixture of red pepper, garlic, caraway seed, salt and olive oil). After adjust-ment for each other and for other potential confounders, only childhood exposure to *touklia, quaddid* and *harissa* were significant risk factors for nasopharyngeal carcinoma in Tunisia. The odds ratio for childhood consumption of *touklia* was 8.6 (95% CI, 1.7–44), that for consumption of *quaddid* more than once a month was 1.9 (95% CI, 1.0–3.7) and that for consumption of *harissa* more than once a month was 4.2 (95% CI, 1.1–17).

Studies of the presence of carcinogens, genotoxins, and EBV-activating substances in samples of foods associated with nasopharyngeal carcinoma are described in section 4.5.2.

(d) Deficits of fresh vegetables and fruit

In an uncontrolled study of 24 patients under the age of 25 with nasopharyngeal carcinoma in Hong Kong, Anderson *et al.* (1978) noted that 'all families felt that vegetables and fruits were bad for babies, and the children had been fed accordingly.' Similar findings were reported in several case–control studies conducted among Chinese. In both Hong Kong (Yu *et al.*, 1986; Ning *et al.*, 1990) and Guangzhou (Yu *et al.*, 1989a), patients with nasopharyngeal carcinoma had a lower intake of fresh vegetables and fruits during weaning, between the ages of one and two and at the age of 10 than controls, and many of the differences were statistically significant. Whereas in Hong Kong the protective effects of fresh vegetables and fruit were no longer significant after adjustment for salted fish intake, in Guangzhou the effects were not due to different consumption of salted fish and other preserved foods by cases and controls (Yu *et al.*, 1989a). In Tianjin (Ning *et al.*, 1990), fewer patients had eaten carrots at the age of 10 than controls. Increased frequency of consumption of garlic, the only other specific vegetable on the questionnaire, also resulted in decreased risks for nasopharyngeal carcinoma, although the effect was not statistically significant. In contrast, Yu *et al.* (1988) did not find a negative association between childhood exposure to fresh vegetables and fruit and the risk for nasopharyngeal carcinoma in Yulin, and Jeannel *et al.* (1990) reported no association between fruit or vegetable intake and nasopharyngeal carcinoma risk in Tunisia.

Clifford (1972) measured serum carotene levels in 17 male African patients with nasopharyngeal carcinoma and 53 male controls and reported a significantly lower level in the cases. In Singapore, Lee *et al.* (1994b) found that the dietary levels of vitamin C, vitamin E and β-carotene one year before diagnosis of nasopharyngeal carcinoma were lower among cases than controls and significantly so for vitamins C and E.

2.4.3.2 Other environmental factors

(a) Fumes, smoke and dust

Dobson (1924) proposed that exposure to smoke from wood fires inside chimneyless houses was the cause of nasopharyngeal carcinoma in southern Chinese, and Clifford (1972) also noted the presence of wood fires in chimneyless houses among tribal groups in Kenya with moderately elevated rates of nasopharyngeal carcinoma.

The relationship between domestic exposure to smoke and risk for nasopharyngeal carcinoma among southern Chinese has been investigated in several case–control studies. Zheng *et al.* (1994b) reported an odds ratio of 5.4 [95% CI, 1.5–20] for use of wood fuel during the year before cancer diagnosis in a case–control study in eastern Guangxi involving 88 cases of nasopharyngeal carcinoma and 176 controls matched for age, sex and neighbourhood. In contrast, Yu *et al.* (1988) observed no association between use of wood fuel and risk in a county adjacent to that studied by Zheng *et al.* (1994b). When lifetime histories of cooking fuels used were collected from each study subject in case–control studies in Hong Kong (Yu *et al.*, 1986) and Guangzhou (Yu *et al.*, 1990), China, no significant association was found with smoke from burning wood.

The role of occupational exposure to smoke, dust and fumes has been examined. A case–control study in Los Angeles and San Francisco (United States) involved 156 histologically confirmed cases of nasopharyngeal carcinoma and 267 hospital or clinic controls matched to the cases by race (whites, blacks, Hispanics, Chinese and other Asians), sex and age (Henderson et al., 1976). Cases were identified through the population-based cancer registries in both metropolitan areas. All interviews were administered in person, and a lifetime occupational history was obtained, including information on usual exposure to fumes, dust, smoke, chemicals or heat in each job. Occupational exposures to fumes (odds ratio, 2.0; $p = 0.006$), dust (odds ratio, 1.5; $p = 0.07$), smoke (odds ratio, 3.0; $p = 0.008$), chemicals (odds ratio, 2.4; $p = 0.006$) and heat (odds ratio, 1.6; $p = 0.05$) were all positively related to the risk for nasopharyngeal carcinoma, but only exposure to smoke showed a clear duration–response relationship (odds ratio for 10 years or less, 1.5; odds ratio for more than 10 years, 7.5; $p < 0.05$).

In the study of Armstrong et al. (1983), described on p. 182, a complete occupational history was obtained from each subject by personal interview. Subjects were specifically asked if they had had regular exposure to a specific dust, smoke or chemical fume. Occupational exposure to smoke (odds ratio, 6.0; $p = 0.006$) and dust (odds ratio, 4.0; $p < 0.001$), irrespective of type, was significantly associated with the risk for nasopharyngeal carcinoma, and a clear duration–response relationship was seen for men and women which was independent of salted fish intake.

In the study of Chen et al. (1988b), described on p. 181, subjects were asked whether they had been exposed occupationally to dust or smoke. Exposure to smoke (odds ratio, 1.7; $p < 0.05$) was significantly associated with risk for nasopharyngeal carcinoma in univariate but not in a multivariate analysis that included other risk factors. Exposure to dust was not related to increased risk (odds ratio, 1.1).

In Guangzhou, China, Yu et al. (1990) interviewed 306 patients under the age of 50 with histologically confirmed nasopharyngeal carcinoma and an equal number of controls matched for age, sex and neighbourhood to obtain a complete occupational history from each respondent. For each job held for six months or longer, the subjects were asked to indicate whether they had been exposed to dust, smoke or chemical fumes and to name the substances involved. To avoid recall bias, a specialist who was unaware of the case or control status of the subjects assessed these exposures on the basis of job title, activity and industry. Occupational exposure to dust was not related to increased risk, on the basis of the subjects' responses (odds ratio, 1.2) or the expert judgement (odds ratio, 0.9); however, exposure to smoke was significantly associated with the risk for nasopharyngeal carcinoma (subjects: odds ratio, 2.1; 95% CI, 1.3–3.5; expert: odds ratio, 1.6; 95% CI, 1.1–2.5). A clear duration–response relationship was seen in both assessments. Exposure to chemical fumes was significantly related to risk, depending on the duration of exposure (odds ratio, 1.7; 95% CI, 1.1–2.4) when based on the subjects' recall but not when based on the expert judgement (odds ratio, 1.0; 95% CI, 0.7–1.4).

In the study of West et al. (1993), described on p. 183, lifetime occupational histories were recorded after interview with each subject. An industrial hygienist who was unaware of the case–control status of the subjects then classified each job as either likely

or unlikely to involve exposure to dust, solvents or exhaust fumes. The investigators found all three exposures to be significantly associated with risk for nasopharyngeal carcinoma. People first exposed to dust 35 or more years before cancer diagnosis had an odds ratio of 4.7 (95% CI, 1.8–13) relative to no exposure; the comparable odds ratios were 2.6 (95% CI, 1.1–6.3) for exposure to solvents and 2.8 (95% CI, 1.1–7.0) for exposure to exhaust fumes. As the three exposures were highly correlated, a combined exposure index was used in the final multivariate model. The index maintained an independent effect on risk after adjustment for other factors.

A number of epidemiological studies have suggested that woodworkers are at increased risk for nasopharyngeal carcinoma. Besides wood dust (see IARC, 1995a), these workers are exposed to various chemicals that are applied to the wood, including pesticides, phenols, chlorophenols and asbestos. Demers et al. (1995) conducted a pooled analysis of five cohort studies of mortality among furniture, plywood and wood-model workers in the United Kingdom and the United States. A total of 28 704 subjects, 7665 of whom died, were studied. A significant excess of nasopharyngeal cancer was noted: nine deaths were observed when 3.8 were expected on the basis of general population rates (standardized mortality ratio (SMR), 2.4; 95% CI, 1.1-4.5). This excess risk was seen among both furniture and plywood workers, and in those with either low or high probability of exposure to wood dust.

Kawachi et al. (1989) used data from the New Zealand Cancer Registry on male patients aged 20 years or more in whom nasopharyngeal carcinoma was diagnosed between 1980 and 1984, to identify possible cancers related to wood-working, since the current or most recent occupation of the patient at the time of cancer diagnosis is recorded in the Registry. Twenty-four cancer sites were examined, compared with all other cancer patients and for each site. Five cases of nasopharyngeal cancer were found among woodworkers (three carpenters and two forestry/logging workers), which represented a nonsignificant excess relative to controls (odds ratio, 2.5; 95% CI, 0.9–6.6).

(b) Formaldehyde

The possible role of formaldehyde (see IARC, 1995b) in development of nasopharyngeal carcinoma has been examined in three large-scale historical cohort studies of exposed workers (Blair et al., 1986, 1987; Stayner et al., 1988; Gardner et al., 1993) and four case–control studies in diverse populations (Olsen et al., 1984; Vaughan et al., 1986a,b; Roush et al., 1987; West et al., 1993).

Blair et al. (1986) evaluated the mortality of 26 561 workers employed in 10 facilities where formaldehyde was produced or used in the United States. About 600 000 person–years of follow-up were available. There were seven deaths from cancer of the nasopharynx in the cohort, when 2.2 were expected on the basis of the mortality rates of the general population ($p < 0.05$). The risk increased with increasing cumulative exposure to formaldehyde among people who were also exposed to particulates (Blair et al., 1987). On the basis of five cases, the SMRs were 190 for cumulative exposure of < 0.5 ppm–years, 400 for 0.5–< 5.5 ppm–years and 750 for ≥ 5.5 ppm–years (this trend was not statistically significant). A closer examination of the data revealed that three of the five deaths from nasopharyngeal carcinoma had occurred in people who had worked at the

plants for less than a year, and that the deaths at one plant out of the 10 studied were responsible for the observed excess (Collins *et al.*, 1988).

Gardner *et al.* (1993) studied the mortality experience of workers in the United Kingdom exposed to formaldehyde in six factories where this chemical was manufactured or used. The cohort consisted of 7660 men who were first employed between 1920 and 1964 and 6357 men first employed between 1965 and 1982. The cohort was followed-up from 1 January 1941 to 31 December 1989. There were no cases of nasopharyngeal cancer, while 1.3 were expected.

In Denmark, Olsen *et al.* (1984) compared the occupational exposures of 266 patients with nasopharyngeal carcinoma with those of 2465 controls with other cancers. Exposure to 12 specific substances or procedures, including formaldehyde, was assessed by industrial hygienists who were unaware of the case or control status of the cancer patients. No significant association was found between exposure to formaldehyde and risk for nasopharyngeal carcinoma, in either men (odds ratio, 0.7; 95% CI, 0.3–1.7) or women (odds ratio, 2.6; 95% CI, 0.3–21.9).

Vaughan *et al.* (1986a,b) conducted a case–control study of 27 cases of nasopharyngeal carcinoma and 552 population controls identified through the random digit dialling method in western Washington State (United States). Occupational exposures and other information were obtained by telephone interviews; however, one-half of the interviews were conducted with next-of-kin. A job–exposure matrix was used to determine if study subjects had had occupational exposure to formaldehyde. A modest, statistically non-significant association was found between duration of exposure to formaldehyde and risk for nasopharyngeal carcinoma. The odds ratios for 1–9 and ≥ 10 years of exposure were 1.2 (95% CI, 0.5–3.1) and 1.6 (95% CI, 0.4–5.8), respectively, relative to no exposure. A history of living in a mobile home (which is believed to be associated with exposure to formaldehyde from the particle-board and plywood commonly used) was positively and significantly related to increased risk, with odds ratios of 2.1 (95% CI, 0.7–6.6) for 1–9 years of living in a mobile home and 5.5 (95% CI, 1.6–19) for ≥ 10 years, relative to no residence in a mobile home. The odds ratio for those with a history of exposure to formaldehyde on the job and living in a mobile home, relative to those with neither exposure, was 6.7 (95% CI, 1.2–39).

Roush *et al.* (1987) compared 173 deaths from nasopharyngeal carcinoma with 605 control subjects selected from the Connecticut (United States) death certificate files. City directories and death certificates were used to reconstruct the occupational status of each study subject 1, 10, 20, 25, 30, 40 and 50 years before death. An industrial hygienist who was unaware of the case or control status then assigned each combination of job, industry and year to give two exposure scores: four degrees of probability of exposure (unexposed, possibly exposed, probably exposed, definitely exposed) and three levels of exposure (zero, low, high). The odds ratio for probable exposure at some level for most of a working life and probable exposure to high levels ≥ 20 years before death was 2.3 (95% CI, 0.9–6.0).

In the study of West *et al.* (1993), described on p. 183, early occupational exposure to formaldehyde was significantly related to the risk for nasopharyngeal carcinoma. First

exposure before the age of 25 was associated with an odds ratio of 2.7 (95% CI, 1.1–6.6) relative to no exposure, and the excess risk persisted after adjustment for other risk factors for nasopharyngeal carcinoma.

(c) Tobacco

The relationship between cigarette smoking (see IARC, 1986) and nasopharyngeal carcinoma has been examined in a number of case–control studies and one cohort study in diverse populations. Although the results of earlier studies, many of which were based on either small sample sizes, very young cases or hospital controls, are equivocal, more recent data from better designed studies are consistent in showing that cigarette smoking is a risk factor for nasopharyngeal carcinoma, regardless of race. Three- to fivefold increased risks were observed among the heaviest smokers in China, the Philippines and the United States (Yu *et al.*, 1990; Nam *et al.*, 1992; Chow *et al.*, 1993; West *et al.*, 1993; Zhu *et al.*, 1995).

In the study of Yu *et al.* (1990), described on p. 186, subjects and their spouses were asked about lifetime use of cigarettes and water pipes. A moderate but statistically significant association was found between tobacco use and nasopharyngeal carcinoma risk. Use of a water pipe and cigarettes were both related to increased risk. Most of the water-pipe users also were cigarette smokers. A lifetime exposure of 30 or more pack–years (assuming that 2.5 g of tobacco, equivalent to 2.5 cigarettes, were consumed each time a subject smoked a water pipe) resulted in a 3.7-fold increased risk for nasopharyngeal carcinoma relative to non-users, after adjustment for all other risk factors (95% CI, 1.2–12).

Nam *et al.* (1992) conducted a case–control study of white cases of nasopharyngeal carcinoma identified from the National Mortality Followback Survey, which provides information from death certificates on people who died at the age of 25 years or more in the United States in 1986. White control subjects were selected from the same database and were matched to the cases by sex and age. Those who had died from causes known to be related to cigarette or alcohol use were excluded from the pool of potential controls. Thus, 204 cases of nasopharyngeal carcinoma and 408 controls were included in the study. Proxy interviews were conducted by using a structured questionnaire, mainly with spouses. Cigarette use was related to the risk for nasopharyngeal carcinoma in both men and women, and the risk increased with increasing duration of use and with increasing number of cigarettes smoked per day. For the heaviest smokers (those with ≥ 60 pack–years of cumulative exposure), the odds ratio was 3.1 (95% CI, 1.6–6.1) in men and 4.9 (95% CI, 1.2–20.9) in women.

Chow *et al.* (1993) used information collected in 1954 and 1957 on 248 046 United States veterans and their mortality through 30 September 1980 to examine the relationship between tobacco use and the development of nasopharyngeal carcinoma. Forty-eight deaths from this cause were studied. Current smokers had a 3.9-fold increased risk for nasopharyngeal carcinoma (95% CI, 1.5–10), and the risk increased with the number of cigarettes smoked per day. There was no excess risk among people who smoked only cigars or pipes.

In the study of West *et al.* (1993), described on p. 183, no association between cigarette smoking and nasopharyngeal carcinoma was found in comparison with hospital controls, but a significant association between heavy use of cigarettes and risk was observed when the cases were compared with community controls. After adjustment for all other risk factors, the odds ratio for smokers of ≥ 31 years was 4.9 (95% CI, 1.6–15).

Zhu *et al.* (1995) carried out a case–control study in eight areas of the United States covered by population-based cancer registries. The cases were in men aged 15–39 in 1968 in whom nasopharyngeal cancer had been diagnosed in 1984–88. The controls were men selected from the general populations of the eight study areas by random-digit dialling and matched to the cases by age. A total of 113 cases and 1910 controls were included; 62% of the cases were white (including Hispanics), 11% were black and 27% were Asian. Most of the subjects were interviewed directly by telephone. Cigarette smoking was found to be related to the risk for nasopharyngeal carcinoma, and the risk increased monotonically with increasing number of cigarettes smoked per day and with increasing number of pack–years of cumulative exposure. The odds ratio for individuals with ≥ 45 pack–years of exposure was 3.9 (95% CI, 2.0–7.8) relative to nonsmokers.

Vaughan *et al.* (1996) conducted a case–control study in five locations in the United States covered by population-based cancer registries. Telephone interviews were completed with 231 cases and with 246 controls matched to the cases on age, sex and race. Most of the cases occurred in non-Hispanic whites (77%) or African Americans (10%). A statistically significant dose–response relationship was observed between cigarette smoking and risk for nasopharyngeal carcinoma, which was confined to differentiated squamous-cell carcinomas and those classified as epithelial tumours not otherwise specified; no association was observed between cigarette smoking and the 54 cases of undifferentiated non-keratinizing carcinoma.

The possible role of passive smoking in the development of nasopharyngeal carcinoma has been examined in two case–control studies, with inconclusive results. In the study of Yu *et al.* (1988), described on p. 181, mothers were asked about the smoking habits of all household members around the time of birth of the index subject and when he or she was 10 years old. The fathers of more cases than controls had smoked when the subjects were born (odds ratio, 1.5; $p = 0.05$), and the risk increased with increasing number of cigarettes smoked by the father (p for linear trend = 0.04). Very few mothers had smoked (5.5% of case mothers and 5.2% of control mothers), and the odds ratio was 1.0. More cases than controls had been exposed to tobacco smoke from other household members, but the difference was not statistically significant. Overall, the presence of a smoker in the household around the time of birth was associated with an increased risk for nasopharyngeal carcinoma (odds ratio, 2.0; $p = 0.004$). Exposure at birth and at the age of 10 was highly correlated, with a slightly stronger association for exposure at birth; there was no significant residual effect for exposure at the age of 10 after adjustment for exposure at birth. Adjustment for personal cigarette smoking did not affect these findings.

In the study of Yu *et al.* (1990), described on p. 186, in which a significantly positive association with active smoking was observed, the same set of questions as used in the

study described above was given to the mothers of cases and controls. No associations were observed. Having a spouse who smoked was also not related to the risk for naso-pharyngeal carcinoma (odds ratio, 1.2; 95% CI, 0.6–2.4). The odds ratio for any domestic exposure to passive smoking was 0.9 (95% CI, 0.6–1.4). The results were unchanged when the analysis was restricted to non-tobacco users.

(d) Alcohol

The possible association between alcohol intake and the development of nasopharyn-geal carcinoma has been investigated in a number of case–control studies among Chinese in and outside of China and among whites in the United States (Lin *et al.*, 1973; Henderson *et al.*, 1976; Geser *et al.*, 1978; Shanmugaratnam *et al.*, 1978; Armstrong *et al.*, 1983; Mabuchi *et al.*, 1985; Chen *et al.*, 1988b; Ning *et al.*, 1990; Nam *et al.*, 1992; Vaughan *et al.*, 1996). Only the studies of Nam *et al.* (1992) and Vaughan *et al.* (1996) found an association. Nam *et al.* (1992) relied on surrogate interviews for infor-mation on exposure, as all of the cases and controls were identified from death certi-ficates. The 80% excess risk noted in that study after control for level of cigarette smoking could have been the result of residual confounding. Vaughan *et al.* (1996) reported a significant association between heavy alcohol use (21 or more drinks per week) and risk for nasopharyngeal carcinoma after adjustment for cigarette use. The association was confined to differentiated squamous-cell carcinoma and epithelial tumours not otherwise specified.

(e) Herbal drugs

A number of Chinese herbs have been shown to induce EBV antigens in human lymphoblastoid cell lines carrying the EBV genome (Hirayama & Ito, 1981; Zeng *et al.*, 1983b; Zeng, 1987), raising the possibility that exposure to these products may affect the risk for nasopharyngeal carcinoma. The geographical distribution of *Croton tiglium*, the seeds of which are used in Chinese herbs, has been noted to loosely parallel that of naso-pharyngeal carcinoma within China (Hirayama & Ito, 1981). In fact, croton seeds are rarely used in herbal mixtures due to their extreme potency. In the studies of Yu *et al.* (1986, 1988, 1990), questions on lifetime use of croton seeds were asked, but none of the subjects reported exposure to this herb. Yu *et al.* (1990) also examined lifetime use of *Phyllanthus emblica* and *Croton crassifolius*, two EBV-inducing herbs that are commonly prescribed in Guangzhou (according to Government sales figures); no rela-tionship with nasopharyngeal carcinoma was observed.

Use of herbal drugs in general was reported to be associated with nasopharyngeal carcinoma in three case–control studies (Lin *et al.*, 1973; Hildesheim *et al.*, 1992; Zheng *et al.*, 1994b). Lin *et al.* (1973) interviewed 343 patients with nasopharyngeal carcinoma identified in four counties in Taiwan and 1017 population controls matched to the cases by age, sex and neighbourhood of residence. Use of herbal drugs was associated with the risk for nasopharyngeal carcinoma, occasional users having an odds ratio of 1.7 and frequent users an odds ratio of 3.5 (*p* < 0.001), relative to non-users. The authors acknowledged that usage may have been related to the clinical symptoms of the tumour

and/or recall bias. [Use of herbal drugs is a part of the traditional life style that is an established risk factor for nasopharyngeal carcinoma.]

In the Philippines, Hildesheim *et al.* (1992) studied 104 predominantly Filipino cases of nasopharyngeal carcinoma (see West *et al.*, 1993, p. 183). Information on the use of herbal medicines was collected from study subjects at personal interviews. Since the prevalence of use was similar in the two groups of control subjects, they were combined for the analysis. Patients with nasopharyngeal carcinoma were significantly more likely than controls to have used herbal medicines (odds ratio, 2.5; 95% CI, 1.4–4.5), and the result was unchanged after adjustment for potential confounders. [The same methodological concerns outlined at the end of the last paragraph apply to this study.]

In the study of Zheng *et al.* (1994b), described on p. 181, subjects were asked about their use of herbal drugs during the year before cancer diagnosis and during childhood. Recent use was strongly associated with the risk for nasopharyngeal carcinoma (odds ratio, 4.5; $p = 0.006$), and the risk was reduced when childhood use was considered (odds ratio, 1.8; $p = 0.07$).

In contrast, three case–control studies conducted in southern China of the frequency of use of the most popular herbal tea in the region, 'cooling soup', during adulthood (three years before cancer diagnosis) or childhood (at the age of 10) showed no association between exposure and risk (Yu *et al.*, 1986, 1988, 1989a). Herbal medicines are almost never given to Chinese infants.

(f)   Incense and anti-mosquito coils

Domestic exposure to burning incense and anti-mosquito coils has been postulated as a risk factor for nasopharyngeal carcinoma and has been investigated in a number of case–control studies conducted in various southern Chinese populations. In Taiwan (Lin *et al.*, 1973; Chen *et al.*, 1988b), Hong Kong (Yu *et al.*, 1986), Guangxi, Yulin prefecture (Yu *et al.*, 1988) and Guangzhou (Yu *et al.*, 1990), such exposures were not associated with the risk for nasopharyngeal carcinoma. Yu *et al.* (1986, 1988, 1990) examined the frequencies of the two exposures at birth, at the age of 10 and three years before cancer diagnosis, while Lin *et al.* (1973a) and Chen *et al.* (1988b) compared any with no exposure to the two types of smoke. In Singapore, two case–control studies of incense burning and risk for nasopharyngeal carcinoma showed no association (Shanmugaratnam *et al.*, 1978; Lee *et al.*, 1994b).

The study of Shanmugaratnam *et al.* (1978) was a hospital-based study with two sets of hospital controls: non-nasopharyngeal carcinoma patients from the same clinics and wards where the cases were recruited and medical, surgical and orthopaedic patients from a different Government hospital. The two sets of controls were not comparable to the cases with regard to socioeconomic status; the cases were less educated than the controls with ear-nose-and-throat conditions and were more educated than the 'other hospital' controls. Anti-mosquito coil burning was associated with increased odds ratios of 1.3–1.4 ($p < 0.05$) in comparison with both control groups when users were compared with non-users and those of unknown status. The frequencies of the groups with no use, any use and unknown status were not given.

In the study of West *et al.* (1993), described on p. 183, use of mosquito coils in the year before cancer diagnosis was investigated. Daily users of anti-mosquito coils had a sixfold increased risk for nasopharyngeal carcinoma relative to non-users (odds ratio, 5.9; 95% CI, 1.7–20).

(g) Chinese nasal oil

The use of Chinese nasal oil, the main ingredients of which are camphor and menthol, has been postulated as a risk factor for nasopharyngeal carcinoma. Yu *et al.* (1986, 1990) noted greater recent (three years before) use among cases than controls, which was related to the clinical symptoms of nasopharyngeal carcinoma. The more relevant exposure during childhood was examined in three case–control studies (Yu *et al.*, 1986, 1988, 1990). Although in two studies (Yu *et al.*, 1988, 1990) the mothers of more cases than controls reported use during the subjects' childhood, most of the exposures were infrequent and/or unsubstantiated by a medical condition that would suggest intense, sustained use. Several other case–control studies of Chinese have shown greater recent use in cases than controls, but none could rule out the possibility that the oil was used to treat symptoms of nasopharyngeal carcinoma (Lin *et al.*, 1973; Shanmugaratnam *et al.*, 1978; Lee *et al.*, 1994b).

2.4.3.3 Host factors

Case–control studies have established several associations between HLA locus A and B antigens and risk for nasopharyngeal carcinoma in southern Chinese. The presence of both A2 and BW46 antigens was associated with a twofold increased risk for naso-pharyngeal carcinoma among Chinese in Singapore, Malaysia, Hong Kong and Guangzhou (Simons *et al.*, 1976, 1977, 1978, 1980; Chan *et al.*, 1983a). Interestingly, the frequency of the A2-BW46 phenotype is twice as common among Cantonese than in the Chiu Chau/Fujianese dialect group, parallelling the twofold difference in naso-pharyngeal carcinoma incidence between these two ethnic groups (Simons *et al.*, 1976). Other HLA antigens that show an association with nasopharyngeal carcinoma in southern Chinese are B16 (RR, 6.0), B17 (RR, 2.1–2.3), especially in combination with B17 (RR, 2.4–2.5), A11 (RR, 0.5) and B13 (RR, 0.5) (Simons *et al.*, 1978; Chan *et al.*, 1981, 1983a; Wu *et al.*, 1989). Preliminary HLA locus DR typing in this high-risk population has shown significant differences in antigen frequencies between cases and controls (Chan *et al.*, 1981, 1983b; Wu *et al.*, 1989). A linkage study of affected pairs of siblings in southern Chinese in China, Hong Kong and Singapore suggests that a gene (or genes) closely linked to the HLA locus is associated with a 20-fold increased risk for naso-pharyngeal carcinoma (Lu *et al.*, 1990). A recent study in Singapore Chinese showed that allele DRB10803 is associated with a 2.8-fold increase (95% CI, 1.0–7.5). In the same study, the investigators found a 3.5-fold increased risk (95% CI, 1.6–7.6) among individuals with allele 4 of the D6S1624 microsatellite locus located on chromosome 6 (Ooi *et al.*, 1997).

An association between HLA profile and nasopharyngeal carcinoma risk has also been reported in non-Chinese populations. B46 has been associated with the occurrence of this tumour in Thais (Chan *et al.*, 1986), and antigen B17 has been found to be posi-

tively associated with the risk of Malays (Chan *et al.*, 1985) and Australian whites (Simons & Shanmugaratnam, 1982). Other antigens shown to be associated with naso-pharyngeal carcinoma in selected populations are: A29 in Kenyans and Tanzanians, B18 in Malays, A3 in Australian whites, B5 in Germans and A2 in whites in the United States (Hall *et al.*, 1982; Simons & Shanmugaratnam, 1982; Chan *et al.*, 1985; Burt *et al.*, 1994). In contrast to southern Chinese, white Americans, Europeans, Tunisians and Malays rarely have BW46, and no association with nasopharyngeal carcinoma was seen in these populations (Betuel *et al.*, 1975; Chan *et al.*, 1979b, 1985; Beigel *et al.*, 1983; Moore *et al.*, 1983).

2.4.3.4 Familial aggregation

Multiple cases of nasopharyngeal carcinoma occurring in first-degree relatives have been documented in diverse populations, ranging from high-risk southern Chinese and Alaskan and Greenland natives, low- to intermediate-risk Africans, to low-risk Caucasians (Stinson, 1940; Bell & Maguda, 1970; Nevo *et al.*, 1971; Ho, 1972; Williams & de Thé, 1974; Brown *et al.*, 1976; Jonas *et al.*, 1976; Lanier *et al.*, 1979; Gajwani *et al.*, 1980; Fischer *et al.*, 1984; Yu *et al.*, 1986; Schimke *et al.*, 1987; Yu *et al.*, 1990; Albeck *et al.*, 1993). Familial aggregation can be the result of shared genes, shared environments or both. Among the high-risk southern Chinese, a potent environmental factor that is strongly correlated within families — dietary exposures at weaning — has been identified, and consistent associations with certain HLA antigens imply the presence of disease susceptibility genes. Genetic studies in non-Chinese populations also suggest the involvement of hereditary factors in the development of nasopharyngeal carcinoma, and analytical studies have implicated environmental factors. Familial clustering of naso-pharyngeal carcinoma is therefore likely to be a product of genetic constitution and environmental exposures.

2.5   Comparison of characteristics of Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma

Table 27 gives a comparison of the epidemiology, virological markers and sites of African Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma in areas of high risk for those tumours.

2.6   Other malignancies

2.6.1   *Lymphoepithelial carcinomas outside the nasopharynx*

The detection of EBV in virtually all undifferentiated nasopharyngeal carcinomas prompted studies into the possible association of the virus with other lymphoepithelial carcinomas. These studies concentrated initially on carcinomas morphologically similar to type-3 nasopharyngeal carcinoma, which can be distinguished from gastric carcinoma with lymphoid stroma, which may account for up to 50% of gastric carcinomas (Matsunou *et al.*, 1996). Lymphoepithelial carcinomas of the stomach are a relatively rare subtype of gastric neoplasm (Rowlands *et al.*, 1993).

Table 27. Characteristics of Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma in high-risk regions

Characteristic	African Burkitt's lymphoma	Hodgkin's disease	Nasopharyngeal carcinoma
Epidemiology			
Age	< 15 years (mode, 8–10)	Bimodal (1st mode, 15–50 years; 2nd mode, > 50 years)	> 15 years (mode, 45–54)
Sex	Males > females	Males > females	Males > females
High-risk regions	Equatorial Africa	Developed countries	Southeast Asia and south China
Period of exposure to probable carcinogens	Early	Early or post-adolescence	Early and continuous
Co-factors	Holoendemic malaria	None identified	Selected preserved foods
Virological markers			
Nucleic acids, proteins			
EBV DNA	+	+ (30–50%)	+
EBER	+	+ (30–50%)	+
EBNA	+	–	+
Serology			
VCA IgA	–	–	+
IgG	+	–	–
IgM	+	+	–
Anti-EBNA	–	++	+
Reactive stroma			
T Cells	–	++	+
B Cells	–	–	–
NK and other cells	–	+	–

EBER, EBV-encoded RNA; EBNA, EBV nuclear antigen; VCA, viral capsid antigen; Ig, immunoglobulin; NK, natural killer

An association of EBV with lymphoepithelial carcinomas arising outside the naso-pharynx was initially proposed on the basis of the detection of EBV genomes in DNA extracts (Table 28). Saemundsen *et al.* (1982) found EBV genomes in DNA extracted from a lymphoepithelial salivary gland carcinoma in a Greenland Inuit. This observation was subsequently confirmed by Hamilton-Dutoit *et al.* (1991a), who also localized the viral genomes to the tumour cells by DNA in-situ hybridization in 11/11 of these carcinomas. Further studies have shown the consistent association of salivary gland lympho-epithelial carcinomas with EBV (Chan *et al.*, 1994; Leung *et al.*, 1995a; Kotsianti *et al.*, 1996; Tsai *et al.*, 1996a). Moreover, the viral episomes in these tumours have been shown to be of monoclonal origin (Leung *et al.*, 1995a). An EBV-associated lympho-epithelial carcinoma in the lachrymal sac was reported in a Chinese patient (Leung *et al.*, 1996).

Using DNA in-situ hybridization, Weiss *et al.* (1989b) detected EBV DNA in all of six nasopharyngeal lymphoepitheliomas but not in lymphoepithelial carcinomas occurring in the skin, cervix uteri, tonsil, pharynx or larynx. Of 14 lymphoepithelial carcinomas outside the nasopharynx, the only one that contained EBV was a carcinoma of the lung in a Chinese woman. This observation was confirmed by subsequent reports (Table 29; Butler *et al.*, 1989; Gal *et al.*, 1991; Pittaluga *et al.*, 1993; Wöckel *et al.*, 1995). Pittaluga *et al.* (1993) were also able to show monoclonal viral episomes in some of their cases. [The Working Group noted that the cases in these studies that contained EBV were generally in Asians.]

On the basis of PCR investigations, Burke *et al.* (1990) and Min *et al.* (1991) first suggested that lymphoepithelial gastric carcinomas are associated with EBV infection. Clonal viral episomes were subsequently detected in such cases by Southern blot hybridization (Pittaluga *et al.*, 1992). Studies with in-situ hybridization have confirmed those reports by localizing the virus to the malignant epithelial cells in such cases (Shibata *et al.*, 1991b; Niedobitek *et al.*, 1992b; Oda *et al.*, 1993; Matsunou *et al.*, 1996). Several studies have indicated that about 80% of lymphoepithelial gastric carcinomas are associated with EBV (Rowlands *et al.*, 1993; Osato & Imai, 1996; see Table 30).

The association of EBV with lymphoepithelial carcinomas arising at other anatomical sites is tenuous. Nicholls *et al.* (1994) detected EBV in one of five non-keratinizing tonsillar carcinomas among Chinese patients, but Niedobitek *et al.* (1991b) found no evidence for an association with EBV in Chinese or Malays. Mori *et al.* (1994) detected EBV in a single lymphoepithelial carcinoma of the oesophagus by PCR and EBER in-situ hybridization. Morphologically similar carcinomas of the breast, so-called medullary carcinomas, have consistently been found to be EBV-negative (Niedobitek *et al.*, 1991b; Kumar & Kumar, 1994; Lespagnard *et al.*, 1995), as were lymphoepithelial carcinomas of the skin, thyroid gland, larynx, urinary bladder, uterine cervix and vulva (Weiss *et al.*, 1989b; Carr *et al.*, 1992; Weinberg *et al.*, 1993; Martinez-Leandro *et al.*, 1994; Requena *et al.*, 1994; Axelsen & Stamp, 1995; Gulley *et al.*, 1995; MacMillan *et al.*, 1996; Shek *et al.*, 1996).

Table 28. Presence of EBV in lymphoepithelial and other carcinomas

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Salivary gland				
Saemundsen *et al.* (1982)	Inuit	Lymphoepithelial	ISH	1/1
		Adenocarcinoma		0/1
Hamilton-Dutoit *et al.* (1991a)	Inuit Non-Inuit	Lymphoepithelial-like	ISH	11/11 0/2
Chan *et al.* (1994b)	Chinese	Lymphoepithelial Other	ISH	5/5 0/55
Kotsianti *et al.* (1996)	NR (Greek)	Lymphoepithelial-like	ISH	1/1
Tsai *et al.* (1996a)	Chinese	Lymphoepithelial Adenocarcinomas	ISH	7/7 0/49
Thymus				
Dimery *et al.* (1988)	NR (USA)	Thymoma (lymphoepithelioma)	Southern blot	1/1
Teoh *et al.* (1989)	Chinese	Thymomas (lymphoepithelial-like)	NR	2/13
McGuire *et al.* (1988)	Chinese	Thymomas	Southern blot	3/3
Leyvraz *et al.* (1985)	Hispanic	Lymphoepithelial-like	*Bam*HI W	1/1
Borisch *et al.* (1990)	NR (Switzerland)	Epithelial tumours	Southern blot	0/32
Niedobitek *et al.* (1991b)	NR (Germany)	3 lymphoepithelial-like + 11 other	ISH	0/14
Mann *et al.* (1992)	NR (USA)	Carcinoma Thymoma	ISH	1/7 0/14
Head and neck (other than nasopharyngeal carcinoma)				
Tyan *et al.* (1993)	Chinese	Various	PCR	30/44
Weiss *et al.* (1989b)	Caucasian and black	Lymphoepithelial Squamous-cell	ISH	0/2 0/4

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Tonsil				
Bricháček et al. (1984)	NR (Czechoslovakia)	Squamous-cell carcinoma	ISH	6/7
Niedobitek et al. (1991b)	NR (Germany)	Squamous-cell carcinoma	ISH	0/26
		Lymphoepithelial-like		0/2
Nicholls et al. (1994)	Chinese	Undifferentiated carcinoma	ISH	1/5
		Squamous-cell carcinoma		0/5
Weiss et al. (1989b)	Caucasian	Lymphoepithelial	ISH	0/3
		Squamous-cell carcinoma		0/1
Cervix				
Martínez-Leandro et al. (1994)	Caucasian	Lymphoepithelial-like	ISH	0/1
Niedobitek et al. (1991b)	NR (Germany)	Carcinoma	ISH	0/14
Leung et al. (1995a)	Chinese	Lymphoepithelial-like	ISH	0/1
Payne et al. (1995)	NR (United Kingdom)	Pre-invasive squamous lesions	ISH	0/3
Weinberg et al. (1993)	Black	Lymphoepithelial	PCR and ISH	0/1
Hilton et al. (1993)	Caucasian	Squamous-cell and adenocarcinomas	ISH	0/24
		Intraepithelial		0/10
Landers et al. (1993)	Caucasian	Squamous-cell carcinoma	PCR	8/18
		CIN-III	ISH	2/25
		CIN-II		2/25
		CIN-I + normal	PCR and ISH	0/50
Se Thoe et al. (1993)	NR (Malaysia)	Carcinoma	ISH	5/8
Weiss et al. (1989b)	Caucasian	Lymphoepithelial	ISH	0/1
Vagina				
Dietl et al. (1994)	NR (Germany)	Lymphoepithelial	ISH	0/1

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Vulva				
Axelsen & Stamp (1995)	NR (Denmark)	Lymphoepithelial	ISH	0/1
Testis				
Rajpert-de Meyts et al. (1994)	NR (Denmark)	Germ-cell	PCR EBER ISH	0/19
Breast				
Niedobitek et al. (1991b)	NR (Germany)	Medullary carcinoma	ISH	0/9
Kumar & Kumar (1994)	NR (USA)	Lymphoepithelial-like	ISH	0/1
Labrecque et al. (1995)	NR (United Kingdom)	Various	PCR	19/91
			EBER ISH	6/19
			DNA ISH	12/19
Lespagnard et al. (1995)	NR (Belgium)	Medullary carcinoma	PCR and ISH	0/10
Thyroid gland				
Shek et al. (1996)	Chinese	Lymphoepithelial	ISH	0/1
Parotid gland				
Huang et al. (1988)	Chinese	Undifferentiated	ISH	0/1
Gallo et al. (1995)	Caucasian	Undifferentiated	ISH	3/7
Urinary bladder				
Gulley et al. (1995)	NR (USA)	Lymphoepithelial	ISH	0/11
Skin				
Weiss et al. (1989b)	Caucasian	Lymphoepithelial	ISH	0/4
Carr et al. (1992)	White	Lymphoepithelial	ISH	0/1
Requena et al. (1994)	NR (Spain)	Lymphoepithelial	ISH	0/1

Table 28 (contd)

Cancer site and reference	Ethnicity	Morphology	Method of detection of EBV	EBV-positive/ total tested
Oesophagus				
Mori *et al.* (1994)	NR (Japan)	Lymphoepithelial-like	ISH and PCR	1/1
		Other		0/29
Colon and rectum				
Yuen *et al.* (1994)	Chinese	Adenocarcinoma	ISH	0/36
Larynx				
Bricháček *et al.* (1983)	NR (Czechoslovakia)	Poorly differentiated carcinoma	ISH	3/5
MacMillan *et al.* (1996)	NR (USA)	Lymphoepithelial-like	ISH	0/8
Paranasal sinus				
Leung *et al.* (1995b)	Chinese	29 carcinomas	ISH	7/29
		8 keratinizing squamous-cell carcinomas		4/8
				1/11
		11 transitional-cell carcinomas		1/4
		4 adenocarcinomas		0/2
		2 mucoepithelial carcinomas		0/2
		2 adenoid cystic carcinomas		1/2
		2 undifferentiated carcinomas		
Lopategui *et al.* (1994)	Asian	NR	ISH	7/11
	NR (western)	NR		0/11
Lachrymal sac				
Leung *et al.* (1996)	Chinese	Undifferentiated lymphoepithelial carcinoma	ISH	1/1

NR, not reported; ISH, in-situ hybridization; PCR, polymerase chain reaction; CIN, cervical intraepithelial neoplasia; EBER, EBV-encoded RNA

Table 29. Presence of EBV in lung carcinomas

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Weiss *et al.* (1989b)	Asian Caucasian	Lymphoepithelial-like carcinoma	ISH	1/1 0/3
Pittaluga *et al.* (1993)	Chinese	Lymphoepithelial-like carcinoma Other carcinomas	ISH	5/5 0/132
Conway *et al.* (1996)	Caucasian, Hispanic	Adenocarcinoma Pleural mesothelioma	ISH	0/80 0/50
Ferrara & Nappi (1995)	Caucasian	Lymphoepithelial-like carcinoma	ISH	0/2
Wöckel *et al.* (1995)	Caucasian	Lymphoepithelial-like carcinoma	ISH and PCR	0/1
Butler *et al.* (1989)	Chinese Caucasian	Lymphoepithelial-like carcinoma	ISH	1/1 1/3
Gal *et al.* (1991)	Chinese	Lymnphoepithelial-like carcinoma	ISH and PCR	1/1
Wong *et al.* (1995)	Chinese	167 carcinomas	ISH	9/167 (all 9 lympho-epithelial-like)

ISH, in-situ hybridization

2.6.2   Other carcinomas

2.6.2.1   Stomach

EBV was first detected in gastric adenocarcinomas by Shibata and Weiss (1992), in 22 of 138 cases (16%) in the United States by PCR and DNA and EBER in-situ hybridization. This observation prompted several groups to study gastric adenocarcinomas in other areas. Studies in Europe showed a generally lower proportion of EBV-associated gastric adenocarcinomas, ranging between about 2 and 8% (Rowlands *et al.*, 1993; Ott *et al.*, 1994; Selves *et al.*, 1996b). Interestingly, in one study, no difference in the prevalence of EBV was found between tumours from the United Kingdom and from Japan (Rowlands *et al.*, 1993). A number of studies were carried out in Japan where the incidence of gastric carcinoma is very high. In several large studies comprising well over 2000 cases, EBV was detected in about 7% of cases (Tokunaga *et al.*, 1993; Fukayama *et al.*, 1994; Imai *et al.*, 1994a; Osato & Imai, 1996). Shin *et al.* (1996) found that 12 of 89 consecutive gastric tumours in Korean patients were EBV-positive; nine of these EBV-infected carcinomas were of the lymphoepithelial lymphoma type. These studies are summarized in Table 30.

Table 30. Presence in EBV in gastric carcinomas

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Burke et al. (1990)	Filipino NR (USA)	Lymphoepithelial-like Adenocarcinoma	PCR	1/1 0/1
Min et al. (1991)	White	Lymphoepithelial-like	ISH and PCR	3/3
Shibata et al. (1991b)	Japanese Caucasian (USA)	Lymphoepithelial-like	PCR	6/7 1/1
Pittaluga et al. (1992)	Chinese	Lymphoepithelial-like	ISH	1/1
Shibata & Weiss (1992)	NR (USA)	Adenocarcinoma	ISH and PCR	22/138
Oda et al. (1993)	Japanese	Lymphoepithelial-like Other	ISH and PCR ISH	13/14 2/8
Rowlands et al. (1993)	NR (UK) and Japanese	Intestinal Diffuse Mixed Unclassified Selected lympho- epithelial-like	ISH	1/81 0/44 2/28 0/21 6/6
Fukayama et al. (1994)	Japanese	Lymphoepithelial-like Other	ISH and PCR	6/6 2/66
Mori et al. (1994)	Japanese	Lymphoepithelial-like Other	ISH and PCR	2/2 0/29
Ott et al. (1994)	NR (Germany)	Lymphoepithelial-like Other	ISH	4/4 3/35
Tokunaga et al. (1993)	Japanese	Carcinoma Lymphoepithelial-like	ISH	69/999 8/9
Yuen et al. (1994)	Chinese	Intestinal Lymphoepithelial-like Diffuse Mixed	ISH	6/52 1/3 0/14 0/5
Harn et al. (1995)	Chinese	Lymphoepithelial-like Other	ISH and PCR ISH	1/1 5/54
Blasco et al. (1996)	NR (Argentina)	Lymphoepithelial-like	ISH	1/1
Gulley et al. (1996)	NR (USA)	Carcinoma Other	ISH	11/95 3/4
Matsunou et al. (1996)	Japanese	Lymphoepithelial-like Other (synchronous)	ISH	22/26 4/4
Selves et al. (1996b)	NR (France)	Intestinal Lymphoepithelial-like Diffuse Mixed	ISH	1/22 4/6 0/21 0/10
Shin et al. (1996)	Korean	Adenocarcinoma Lymphoepithelial-like Normal gastric	ISH	12/89 9/10 0/37

Table 30 (contd)

Reference	Ethnicity	Morphology	EBV detection method	EBV-positive/ total tested
Vasef *et al.* (1996)	NR (USA) (1 patient with 2 tumours)	Lymphoepithelial-like N-Cell lymphoma in MALT	ISH and PCR	1/1 1/1

NR, not reported; ISH, in-situ hybridization; PCR, polymerase chain reaction; MALT, mucosa-associated lymphoid tissue

EBV has been detected in both intestinal and diffuse gastric adenocarcinomas. No difference in EBV positivity was found in carcinomas in different locations in the stomach (Rowlands *et al.*, 1993; Tokunaga *et al.*, 1993; Fukayama *et al.*, 1994). The frequency of EBV positivity in gastric adenocarcinomas was 9.2% in men and 3.1% in women in a study of 999 cases in Japan (Tokunaga *et al.*, 1993) (see also Table 30).

Viral genomes in EBV-associated gastric carcinomas have consistently been found to be monoclonal (Pittaluga *et al.*, 1992; Imai *et al.*, 1994a; Gulley *et al.*, 1996). While Fukayama *et al.* (1994) reported the detection of EBV in shed gastric epithelial cells by in-situ hybridization, evidence of viral infection of normal gastric mucosa was not found in other studies with similar methods (Rowlands *et al.*, 1993; Tokunaga *et al.*, 1993; Gulley *et al.*, 1996). Gulley *et al.* (1996) reported the detection of EBV in dysplastic gastric mucosa adjacent to areas of EBV-positive adenocarcinomas.

2.6.2.2 Other sites

Examination of adenocarcinomas and squamous-cell carcinomas arising at other anatomical sites for the presence of EBV has produced mostly negative results (see Table 28).

Squamous-cell carcinomas of the oesophagus and colorectal adenocarcinomas were shown not to contain EBER in two studies (Mori *et al.*, 1994; Yuen *et al.*, 1994). The number of cases investigated, however, was small and an association of the virus with a small proportion of such carcinomas cannot be excluded. In addition, since EBER transcription may be suppressed in well-differentiated squamous-cell tumours, this technique is inappropriate for detecting EBV in these tumours.

Bricháček *et al.* (1984) found that six of seven squamous-cell carcinomas of the tonsils contained EBV, but this finding was not confirmed in subsequent studies with EBV DNA and EBER in-situ hybridization (Niedobitek *et al.*, 1991b; Nicholls *et al.*, 1994). Bricháček *et al.* (1983) also reported the detection of EBV in a small group of poorly differentiated laryngeal carcinomas by DNA in-situ hybridization, in contrast to the absence of the virus in lymphoepithelial carcinomas at this site (MacMillan *et al.*, 1996).

In studies of carcinomas of the salivary glands (Chan *et al.*, 1994b) and lungs (Wong *et al.*, 1995; Conway *et al.*, 1996), no association with EBV was demonstrated.

Carcinomas of the paranasal sinus constitute a wide morphological spectrum of tumours including undifferentiated carcinomas, adenocarcinomas and squamous-cell carcinomas (Leung *et al.*, 1995b). The group of undifferentiated sinonasal carcinomas includes some cases with morphological features similar to those of nasopharyngeal carcinoma. In two studies, EBV was detected in at least some sinonasal carcinomas arising in Asian patients (Lopategui *et al.*, 1994; Leung *et al.*, 1995b). The EBV-positive cases included undifferentiated carcinomas, squamous-cell carcinomas and adenocarcinomas. None of the undifferentiated sinonasal carcinomas arising in western patients was EBV-positive (Lopategui *et al.*, 1994).

EBV was detected by DNA PCR in 19 of 91 cases of breast carcinoma. Of the 18 cases found to contain EBV by PCR, six were also shown to be EBV-positive by EBER in-situ hybridization, and 12 of 18 tested were EBV-positive by *Bam* HI W in-situ hybridization (Labrecque *et al.*, 1995). [The Working Group noted that it was not clear whether some cases were positive by both methods.]

Fuelled by a report suggesting that the cervix may be a site of EBV shedding (Sixbey *et al.*, 1986), several groups examined the possible association of EBV infection with cervical carcinoma. Using in-situ hybridization with biotinylated probes, Landers *et al.* (1993) found EBV DNA in 8% of grade-II cervical intraepithelial neoplasia (CIN), in 8% of CIN III and in 43% of cervical carcinomas. A similar finding was reported by Se Thoe *et al.* (1993). Other studies in which EBV DNA or EBER in-situ hybridization was used consistently failed to detect the virus in CIN lesions or in invasive cervical carcinomas (Niedobitek *et al.*, 1991b; Hilton *et al.*, 1993; Payne *et al.*, 1995).

EBV DNA was detected by Southern blot hybridization in some thymomas, i.e. benign epithelial tumours of the thymus, and in thymic carcinomas, both often showing a prominent reactive lymphoid cell infiltrate (Leyvraz *et al.*, 1985; Dimery *et al.*, 1988; Katzin *et al.*, 1988; McGuire *et al.*, 1988; Teoh *et al.*, 1989). These tumours were mainly obtained from Asian patients. Subsequent studies by PCR and in-situ hybridization of thymic epithelial tumours in western patients provided no convincing evidence of an association with EBV (Borisch *et al.*, 1990; Niedobitek *et al.*, 1991b; Mann *et al.*, 1992).

2.6.3   Smooth-muscle tumours

Immunosuppressed transplant recipients and HIV-infected patients are at increased risk for smooth-muscle tumours, both benign leiomyomas and malignant leiomyosarcomas (Mueller *et al.*, 1992; Lee *et al.*, 1995b). The increase is observed primarily in immunosuppressed children; cases in adults are observed rarely. The tumours that occur in this situation have frequently been found to contain EBV.

McClain *et al.* (1995) tested five leiomyosarcomas and two leiomyomas from five HIV-infected children aged less than nine years and one HIV-infected adult, aged 24, by EBER-1 in-situ hybridization. Essentially all cells in all seven tumours showed positive signals. In contrast, none of seven smooth-muscle tumours (three leiomyosarcomas and four leiomyomas) from seven HIV-negative children (under 15) were EBV-positive. EBV clonality was assessed in two of the HIV-associated cases of leiomyosarcoma: one

showed biclonality; the other consisted of two tumours at different sites, each of which contained a different clone of EBV.

Lee *et al.* (1995b) tested six smooth-muscle tumours from three children under six years of age who had undergone immunosuppression for liver allotransplantation. Most cells in all six tumours were shown to contain EBV by EBER-1 in-situ hybridization; all three tumours contained monoclonal EBV. Southern blot analysis of one tumour suggested that the EBV DNA was associated with host sequences, indicating viral integration. When tested by immunohistochemistry, all six tumours were LMP-negative but three of three tumours tested were EBNA-2-positive.

These results were corroborated by additional case reports of EBV-positive smooth muscle tumours in AIDS patients (van Hoeven *et al.*, 1993; Prévot *et al.*, 1994) and transplant recipients (Timmons *et al.*, 1995; Kingma *et al.*, 1996; Morel *et al.*, 1996), although an EBV-negative case was described in one transplant recipient (van Gelder *et al.*, 1996).

2.6.4 *Other tumours*

Conway *et al.* (1996) reported the absence of the virus in a series of mesotheliomas. When PCR and EBER in-situ hybridization were used, no evidence of EBV was found in testicular germ-cell tumours (Rajpert-de Meyts *et al.*, 1994).

3. Studies of Cancer in Animals

3.1 EBV in non-human species

Considerable efforts have been made to develop suitable animal models to study EBV infection and pathogenesis; however, such attempts have had little success, because Old World primates carry their own EBV-like virus and cross-reacting antibody (Kieff, 1996). New World primates do not carry EBV homologues, however, and at least five species appear to be infectable by EBV, i.e. the cotton-topped tamarin (*Saguinus oedipus oedipus*), white-lipped marmoset (*Saguinus fuscicollis*) and common marmoset (*Callithrix jacchus*), owl monkey (*Aotus trivirgatus*) and squirrel monkey (*Saimiri sciureus*). The most susceptible non-human primate for the carcinogenicity of EBV is the cotton-topped tamarin. This is, however, an endangered species and not widely available for use in laboratories. When common marmosets are infected with EBV, only some animals develop an infectious mononucleosis-like syndrome (Wedderburn *et al.*, 1984).

3.1.1 *Infection of non-human primates with EBV*

3.1.1.1 *New World primates*

The special susceptibility of cotton-topped tamarins to EBV is not well understood. The response to EBV infection varies from occult infection to benign lymphoproliferation to malignant lymphoma (Shope *et al.*, 1973; Deinhardt *et al.*, 1975; Werner *et al.*, 1975; Miller *et al.*, 1977; Rabin *et al.*, 1977; Johnson *et al.*, 1983; Cleary *et al.*, 1985;

Niedobitek *et al.*, 1994). The lymphomas induced in cotton-topped tamarins are morphologically similar to EBV-associated lymphomas in humans (Rabin *et al.*, 1977; Cleary *et al.*, 1985) and are frequently observed in the mesenteric nodes and small intestine. Each tumour nodule in an animal has a dominant clonotype, but many different clones are found in different locations.

Shope *et al.* (1973) induced malignant lymphomas in four of eight cotton-topped tamarins inoculated with cell-free EBV extracted from a lymphoblastoid cell line (B95-8) originally transformed *in vitro*. The first tumour developed after 222 days.

Epstein *et al.* (1973) reported that one of three owl monkeys inoculated with EB3 cells from a Burkitt's lymphoma (frozen and thawed) developed lymphoproliferative disease without evidence of true lymphoma. Antibodies to VCA were detected in the serum obtained from this animal.

Leibold *et al.* (1976) inoculated EBV-transformed squirrel monkey lymphocytes (6–12 × 10^8 cells) by intraperitoneal or subcutaneous injection into three squirrel monkeys. All three animals developed multiple undifferentiated malignant lymphomas and died 8–10 days after inoculation. Necropsy specimens showed the presence of EBV DNA and contained 7–21 genomes per cell. Lymphoblastoid cell lines were established from the tumour tissue and were found to express EBNA and EA.

Werner *et al.* (1975) showed that inoculation of concentrated EBV from a transforming (Kaplan) mononucleosis cell line resulted in malignant lymphoproliferation in one of three cotton-topped tamarins six weeks after inoculation, whereas inoculation of non-transforming EBV (P3HR-1) did not result in lymphoproliferation. The tumour induced by the Kaplan cell line was a lymphosarcoma and contained EBV DNA. The tumour-bearing animals produced anti-VCA antibodies.

Sundar *et al.* (1981) reported that one of six white-lipped marmosets inoculated with a transforming EBV (B95-8) developed diffuse malignant lymphoma within 18 weeks. EBV-DNA was detected in the pathologically enlarged mandibular lymph nodes, and the animal had an anti-VCA antibody titre of 1:320.

In a study in which eight cotton-topped tamarins were inoculated with sufficient quantities of transforming EBV (B95-8), multiple tumours were induced in each animal within 14–21 days which were identified histologically as large-cell lymphomas. The tumour cells contained multiple copies of the EBV genome. Cell lines derived from the tumours showed no chromosomal abnormalities. Hybridization of tumour DNA with immunoglobulin gene probes revealed that the tumours were oligo- or monoclonal in origin; in each animal, individual tumours arose from different B-cell clones (Cleary *et al.*, 1985).

Horizontal transmission of EBV in common marmosets was demonstrated when two animals experimentally infected with EBV (from M81-transformed cells) were paired with two animals seronegative to EBV. The seronegative animals seroconverted after four to six weeks, and the presence of EBV DNA was demonstrated in their peripheral blood lymphocytes (Cox *et al.*, 1996).

3.1.1.2 Old World primates

Levine *et al.* (1980a) inoculated 42 rhesus monkeys (*Macaca mulatta*), four chimpanzees (*Pan troglodytes*) and one cynomolgus monkey (*Macaca fascicularis*) with non-transforming EBV (P3HR-1). None of the animals developed any clinical manifestation or tumour within three to eight years. [The Working Group noted that the lack of response in this study could be due either to the use of a non-transforming EBV strain or the presence in these primates of their own EBV-like herpesvirus (see section 3.2).]

3.1.2 Transformation of monkey cells by EBV in vitro

Desgranges *et al.* (1976) transformed peripheral blood mononuclear cells of common marmosets with concentrated EBV (HKLY-28 virus) obtained from a nasopharyngeal carcinoma lymphoblastoid cell line. The monkey cells produced more EBV than the original human cell line, and the transforming activity of the EBV obtained from monkey cells was similar to that of EBV from B95-8 cell lines.

Ablashi *et al.* (1977) showed that squirrel monkey peripheral blood mononuclear cells infected with transforming EBV (B95-8) were immortalized and that these cells contained EBV DNA.

Griffin and Karran (1984) showed that specific EBV DNA fragments could immortalize a subpopulation of epithelial cells of the African green monkey (*Cercopithecus aethiops*). EBV-related sequences were observed in all the established cell lines.

3.1.3 Rodent models for EBV infection and pathogenesis

3.1.3.1 Severe combined immunodeficiency (SCID) mouse model

The SCID mouse model has been widely used to study lymphomas induced by EBV *in vivo*, EBV protein expression and specific therapies against EBV-induced lymphoproliferation (e.g. anti-CD40 and anti-CD23 monoclonal antibodies) (Garnier *et al.*, 1993; Funakoshi *et al.*, 1995; Katano *et al.*, 1996). SCID mice lack mature B and T cells but can be immunologically reconstituted to a certain extent by xenografts of human leukocytes. If the lymphocytes are derived from human EBV-seropositive donors, the animals frequently develop multiple foci of proliferating EBV-positive human B cells within 10–16 weeks. The SCID mouse lesions are composed of immunoblasts and plasmacytoid cells.

In SCID mice grafted with EBV-positive human peripheral blood mononuclear cells, Okano *et al.* (1990) detected EBV-positive lymphoproliferative lesions that expressed LMP and B-lymphocyte activation antigen (CD23) and adhesion molecules (ICAM-1 and CD18). The authors suggested that these lymphoid lesions in SCID mice were comparable to EBV-immortalized lymphoblastoid cells and not to malignant lymphomas like Burkitt's lymphoma.

SCID mice were grafted with peripheral blood lymphocytes from immunodeficient patients with X-linked lymphoproliferative syndrome. Mice receiving lymphocytes from EBV-seronegative patients were then infected with B95-8 EBV. The mice developed EBV-induced oligoclonal or polyclonal disease comparable to that occurring in both

immunodeficient patients and normal immunocompetent donors. The lesions were diffuse, monomorphic and human B-cell-related, and activated associated antigens were present. Analysis of the sera revealed that the B-cell populations were oligoclonal or polyclonal; however, phenotypic and cytogenetic analysis did not reveal the mono-clonality generally observed in Burkitt's lymphoma (Nakamine et al., 1991; Purtilo et al., 1991).

Walter et al. (1992) compared the local growth of a Burkitt's lymphoma with the disseminated, invasive growth of EBV-immortalized lymphoblastoid cells in SCID mice. The lymphoma grew progressively but was locally restricted, whereas the lympho-blastoid cells were both locally invasive and disseminated into lymphoid tissue.

Katano et al. (1996) studied the lymphoid tumours induced in SCID mice after grafting of human EBV-infected B cells. Molecular analysis of the tumours revealed expression of EBV LMP-1 molecules at quantitatively different levels in the individual tumours.

[The Working Group noted that the SCID mouse model is limited to the study of lymphoproliferative disorders.]

3.1.3.2 Nude mouse model

Ablashi et al. (1978) inoculated EBV-positive P3HR1 cells (1.0×10^6 cells in 0.2 mL medium) into 30 athymic nude mice. All animals developed lymphoid tumours after an average latent period of 36 days. The tumours were poorly differentiated lymphomas of varied cell size and shape. The nuclei were variably hyperchromatic and were reminiscent of those of Burkitt's lymphoma. EBNA was detected in 80% of these cells and in 90% of the cells used for the inoculum, but VCA was detected in only 2% of the cell lines obtained from the tumour and in 13% of cells in the inoculum.

Yang et al. (1982) inoculated cells from 36 biopsy samples of nasopharyngeal carcinoma into BALB/c nude mice. In three mice, the inoculum grew to detectable tumours with metastases within 20–43 days. The tumours were then passaged 6–15 times over a period of 12–22 months into other groups of nude mice, with an average take of 67–73%. Touch smears from the tumours showed the presence of EBNA. Only two of four of the cell cultures derived from tumours expressed VCA after induction with 5-iodo-2'-deoxy-uridine. Co-cultivation of epithelial cells with human cord blood mononuclear cells provoked transformation of the latter, and the transformed cells produced IgM.

3.2 EBV-like viruses isolated from non-human primates

The Gammaherpesvirinae subfamily of Herpesviridae is subdivided into two genera: Lymphocryptovirus (e.g. HHV4 or EBV) and Rhadinovirus (e.g. ateline herpesvirus-2) (Roizman et al., 1992). For further details of the classification of herpesviruses that infect animals, see the Introduction.

Lymphocryptoviruses (so-called gamma-1) include EBV-like viruses isolated from Old World primates such as chimpanzees (Pan troglodytes), baboons (Papio hamadryas) and orangutans (Pongo pygmaeus). The gamma-1 lymphocryptoviruses are B lympho-

tropic, transforming, generally restricted in their host range and similar to each other in genomic organization (Kalter, 1994). Their genomes are collinearly homologous to that of EBVand are more closely related to that of the saimiri herpesvirus-1, a member of the *Rhadinovirus* genus, than to genomes of the beta- or alphaherpesviruses.

Non-human primate rhadinoviruses (gamma-2 herpesviruses) are discussed in the monograph on KSHV/HHV8.

Lymphocryptoviruses cross-react antigenically with EBV and can transform or immortalize cells. These viruses are transmitted horizontally and infect their host at the juvenile stage, inducing the production of specific antibodies (Deinhardt & Deinhardt, 1979). The first isolation of a lymphocryptovirus in non-human primates was reported by Landon *et al.* (1968), who established a permanent lymphoblastoid cell line from a healthy chimpanzee in which they detected a herpesvirus. A similar virus was reported in chimpanzees (Gerber *et al.*, 1976b), which was B-lymphotropic and transformed human and other primate cells. Since the first isolation of *Herpesvirus pan* (pongine herpesvirus 1) from chimpanzees (Gerber *et al.*, 1977), the following primate lymphocryptoviruses have been reported: *Herpesvirus gorilla* (pongine herpesvirus 3; Neubauer *et al.*, 1979a), *Herpesvirus pongo* (pongine herpesvirus 2) from orangutans (Rasheed *et al.*, 1997), *Herpesvirus papio* (cercopithecine herpesvirus 12) from baboons (Falk *et al.*, 1976), *Herpesvirus cercopithecus* (cercopithecine herpesvirus 14) from African green monkeys (Böcker *et al.*, 1980) and herpesviruses from rhesus monkeys (*Macaca mulata*; Rangan *et al.*, 1986), cynomolgus monkeys (*M. fascicularis*; Feichtinger *et al.*, 1992; Li *et al.*, 1994) and *M. arctoides* (Lapin *et al.*, 1985).

The double-stranded DNA of these viruses has a molecular mass of about 110×10^6, and the genome of cercopithecine herpesvirus 12 (baboons) and pongine herpesvirus 1 (chimpanzees) is about 170 kb. The virion has a diameter of 118–220 nm. The viral morphogenesis is similar to that of EBV. The natural host range is limited to the family or order of its host. DNA sequence homology among primate gamma-1 herpesviruses is approximately 40%. The DNAs of cercopithecine herpesvirus 12 and pongine herpesvirus 1 show extensive collinear homology (Lee *et al.*, 1980, 1981; Kieff, 1996).

3.2.1 Herpesvirus papio *(cercopithecine herpesvirus 12)*

Agrba *et al.* (1975) reported the establishment of two lymphoblastoid cell lines (SPG-1, KMPG-1) from bone marrow and spleen of hamadryas baboons with malignant lymphomas in the Sukhumi colony. Herpesvirus particles were detected in these cell lines by electron microscopy. Upon further investigation, other herpesvirus types (HSV-1, HSV-2, cytomegalovirus) were ruled out. The virus was later named *Herpesvirus papio* (HVP). Increased antibody titres to this virus were demonstrated in baboons which developed lymphoma (Voevodin *et al.*, 1983).

A similar virus was isolated in the United States from several baboon colonies (*Papio hamadryas, P. ambis, P. cynocephalus*) by Falk *et al.* (1976). Antibodies that reacted with viral antigens in both HVP and EBV cell lines (P3HR1, B95-8) were demonstrated. All of the established cell lines were B cell-derived, and 5–15% contained antigens (see also Falk, 1979).

HVP is indigenous to Africa and is restricted to that continent; however, animals bearing the virus have been transported throughout the world. Although no specific seasonal variation in infection is known in the wild, peak infection in the Sukhumi colony occurred in the autumn and spring (Kalter, 1994).

3.2.1.1   Cell lines, persistence and transformation

To date, the cell lines established from peripheral blood mononuclear cells or other tissues of HVP-infected tumour-bearing animals have been B lymphocytes (Falk, 1979), and infected monkey and human cord blood mononuclear cells exhibit B-cell markers; however, the markers are not uniform (Deinhardt & Deinhardt, 1979). HVP transforms lymphocytes of several species of non-human primates (*Macaca mulatta*, *M. arctoides*, *Hylobates lar*, *Saguinus* sp., *Callithrix jacchus*, *Saimiri sciureus*) as well as human cord blood mononuclear cells *in vitro*. The presence of the virus can be demonstrated by the presence of antigens (Ohno *et al.*, 1978; Falk, 1979). The nuclear antigen of HVP is similar to EBNA. In a comparative study, all EBNA-positive sera reacted with HVP nuclear antigen, but none of the sera containing this antigen reacted with EBNA.

3.2.1.2   Prevalence of infection with Herpesvirus papio

Voevodin *et al.* (1985), using an assay to detect anti-VCA to HVP, showed that the prevalence of antibody in two groups of baboons, one with a high prevalence of lymphoma and the other lymphoma-free, was 80–90% in both groups, in contrast to the results of previous studies in which complement fixation was used (Voevodin *et al.*, 1979). Newly imported baboons had a significantly lower prevalence (35%). Moreover, the prevalence of infection was age-dependent, increasing during the first years of life, reaching the highest rate (100%) at five years of age, staying stable up to 18 years of age and decreasing subsequently. Infection in newly imported animals increased with age up to 71%. Nevertheless, when antibodies to early, capsid and nuclear antigens were compared in baboons in the Sukhumi colony, animals with lymphoid disease had higher frequencies and higher titres than did control animals (Neubauer *et al.*, 1979b).

3.2.1.3   Molecular biology

The organization of the genome of HVP shows similar unique features to that of EBV DNA (see section 1.1) and shows 40% homology with EBV DNA (Lee *et al.*, 1980). Replication of HVP involves both *cis*- and *trans*-acting functions, analogous to those found in EBV (Pesano & Pagano, 1986). For more information, see Lee *et al.* (1980, 1981) and Kieff (1996).

It has been shown that non-producer HVP cell lines have two or three viral genome equivalents per cell in an apparently integrated form, whereas in HVP producer cell lines the HVP-free DNA fraction is composed of both linear and circular DNA molecules that are similar in length to the circular DNA of EBV in human cell lines, as shown by electron microscopy (Falk *et al.*, 1979).

Howe and Shu (1988) showed that two small RNAs of HVP hybridize to EBERs, and two genes of HVP RNAs, *HVP-1* and *HVP-2*, are located together in a small unique

region at the left end of the viral genome and are transcribed by RNA polymerase III in a rightward direction, like the *EBERs*. There is considerable similarity between *EBER-1* and *HVP-1* RNA, except for an insert of 22 nucleotides which increases the length of *HPV-1* RNA to 190 nucleotides. *HVP-2* RNA and *EBER-2* are less similar, but both are about 170 nucleotides long. Like EBERs, *HVP* RNAs are abundant in HVP-transformed cells and are efficiently bound to cellular proteins. EBNA-1 and EBNA-2 of EBV do not cross-react well with HPV nuclear antigen, and cloning of *EBNA-2* of the HVP homologue showed significant nucleotide and amino acid divergence, as predicted by serological studies. The HVP *EBNA-2* homologue does not retain the acidic transactivating domain of EBV *EBNA-2*. This gene, as in EBV, is important in B-cell immortalization and the resultant alterations in B-cell growth pattern which regulate expression from specific viral and cellular promoters. Ling *et al.* (1993b) showed that, in comparison with the *EBNA-2* of EBV, all of which are either type 1 or type 2, the HVP *EBNA-2* gene falls into neither type 1 or type 2. The divergence of *EBNA-2* of HVP from that of EBV is thus ideally suited for comparative studies. Similarities between the EBV and the HVP *LMP-2* gene indicate that the latter is important in infection *in vivo* (Franken *et al.*, 1995).

These molecular biological studies with HVP provide not only an understanding of the action and function of various viral and cellular genes but also a unique opportunity to study their role in pathogenesis, because they can infect, replicate and cause B-cell lymphoproliferation in various non-human primates.

3.2.1.4 *Pathogenesis and immune response to* Herpesvirus papio

HVP is a potent transforming agent, since it can efficiently transform human and non-human primate peripheral blood mononuclear cells. Falk *et al.* (1976) established three lymphoblastoid cell lines from the splenic lymphocytes of a lymphomatous baboon (*Papio hamadryas*) in which they could identify the presence of HVP. Adult or newborn marmosets of three species (*Saguinus oedipus oedipus, S. fascicollis, Callithrix jacchus*) were inoculated with lymphoblastoid cells derived from this baboon (Deinhardt *et al.*, 1978). All six adult animals developed moderate leukocytosis, relative lymphocytosis beginning 7–16 days after inoculation. Three of the animals, two of which also had developed large tumours at the site of inoculation, developed marked generalized lymphadenopathy and died 13–22 days after inoculation. None of the eight newborn marmosets died after inoculation; they developed only minimal lymph-node swelling during the first two to three weeks after inoculation, without haematological abnormalities.

Gerber *et al.* (1977) inoculated two HVP-seronegative baboons (*Papio cynocephalus*), two rhesus monkeys (*Macaca mulatta*) and two cotton-topped tamarins (*Saguinus oedipus oedipus*) either intraperitoneally, intravenously or subcutaneously, with a total of 3.0 ml of HVP with a transforming titre of 10^{3-5} units derived from a spontaneously transformed baboon lymphoid cell line. The animals were kept for six months. Both baboons seroconverted, and HVP was excreted by the oropharyngeal route. Seroconversion was seen in the rhesus monkeys but not the tamarins, and neither species excreted detectable

levels of HVP from the oropharynx. None of the inoculated animals had detectable disease or palpable tumours.

3.2.2   Gamma-1 herpesvirus from cynomolgus monkey (Macaca fascicularis)

Fujimoto *et al.* (1990) established a B-lymphoblastoid cell line from lymph nodes of an apparently healthy cynomolgus monkey (*Macaca fascicularis*) in which EBV-like herpesvirus was demonstrated. The cell-free supernatant from the cell line transformed the lymphocytes of another cynomolgus monkey.

Feichtinger *et al.* (1992) inoculated cynomolgus monkeys with simian immuno-deficiency virus type 1, and about 40% of the animals developed B-cell lymphoma. A herpesvirus termed HVMF1 was detected in all tumours tested, and lymphoma tissue and two established cell lines reacted with EBV-specific DNA probes (Li *et al.*, 1993, 1994). Homology to EBV was found in *EBNA-2*, *EBNA-5*, *EBERs* and *oriP*, and proteins that cross-reacted with EBNA-1 and EBNA-2 were identified by western blotting. On the basis of the close homology with EBV, the investigators proposed that malignant B-cell lymphoma in cynomolgus monkeys experimentally infected with simian immuno-deficiency virus could be used as a model for EBV-associated lymphomagenes is in immunodepressed persons.

3.2.3   Rabbit model of malignant lymphoma induced by EBV-like virus from Macaca arctoïdes

An EBV-like herpesvirus isolated from *Macaca arctoïdes* was successfully used to induce malignant lymphoma in rabbits (*Oryctolagus cuniculus*). Sixteen out of 32 infected rabbits became ill between days 21 and 143 after inoculation, and in 15/16 animals with clinical signs, lymphoproliferative disease was shown by histological examination. The disease consisted either of malignant lymphoma or lymphoid hyper-plasia of the spleen and parenchymal organs (Wutzler *et al.*, 1995).

3.3   Other models of relevance to EBV

3.3.1   Murid herpesvirus 4

Murid herpesvirus 4, or MHV-68, is a B-lymphotropic gammaherpesvirus which on phylogenetic grounds was classified as a gamma-2 herpesvirus. It induces B-lympho-proliferative disease in mice and has therefore been proposed as a model for EBV infection. As it is a gamma-2 herpesvirus, it is discussed in the monograph on KSHV/-HHV8.

3.3.2   Marek's disease

Marek's disease virus (MDV), first described by Josef Marek in 1907, has clearly played a major role in comparative herpesvirology and oncology (Calnek, 1986). MDV is a lymphotropic herpesvirus which, after an early cytolytic infection, induces T-cell lymphomas in chickens, its natural host. The lymphoma cells are latently infected with MDV, but the viral contribution to the transformed phenotype is not fully understood.

The integration pattern of MDV DNA suggests the clonal nature of tumour formation, and MDV-transformed cell lines established *in vitro* maintain the integration pattern of primary lymphomas (Delecluse *et al.*, 1993b).

Malignant lymphomas occur in a variety of organs and tissues, including the spleen, gonads, liver, lungs, heart, mesentery, bursa of Fabricius, thymus, adrenal gland, pancreas, proventriculus, intestine, skeletal muscle and skin. The various strains of MDV have different organ distributions. Marek's disease is also characterized by the formation of lesions in peripheral nerves, the spinal roots and/or the root ganglia. The affected peripheral nerves show loss of cross-striations, enlargement, discolouration and sometimes oedema. Affected spinal root ganglia become enlarged and appear translucent and somewhat discoloured (Calnek & Witter, 1991).

MDV is transmitted through direct or indirect contact between chickens. The fully infectious virus becomes airborne when it is shed from the feather follicle epithelium during skin keratinization (Calnek *et al.*, 1970). Both diseased and apparently unaffected chickens can continually shed MDV continuously for many weeks (Kenzy & Cho, 1969).

Propagation of the causative agent in cell culture (Churchill & Biggs, 1967) was followed by transmission of the disease with the cell culture-propagated virus (Churchill & Biggs, 1968). Eventual transmission of the disease with cell-free infectious herpesvirus particles isolated from the feather follicles of infected chickens was the definitive study that dispelled doubt that MDV is the causative agent of Marek's disease (Calnek *et al.*, 1970).

Marek's disease is the first, most important system in which a herpetic affliction and/or a neoplastic condition has been successfully controlled by vaccination. Vaccination against Marek's disease was first demonstrated in 1969 with a serotype-1 strain attenuated by passage in cell culture (Churchill *et al.*, 1969). Herpesvirus of turkeys, isolated in 1969 (Kawamura *et al.*, 1969), provides good protection against Marek's disease (Okazaki *et al.*, 1970).

Three avian herpesviruses share common antigen determinants: Marek's disease herpesvirus 1 (gallid herpesvirus 2), Marek's disease herpesvirus 2 (gallid herpesvirus 3) and turkey herpesvirus 1 (maleagrid herpesvirus 1) (Witter *et al.*, 1970).

A major step in MDV virology was made by Buckmaster *et al.* (1988), who showed that MDV and turkey herpesvirus sequences bear greater similarity to varicella-zoster virus (human herpesvirus 3), an alphaherpesvirus, than to EBV sequences, and that the MDV and turkey herpesvirus genomes are collinear with that of varicella-zoster virus. Therefore, even if some biological aspects of Marek's disease resemble EBV infection in humans, it cannot be considered virologically to be a true model for human EBV infection because MDV is clearly an alphaherpesvirus.

4. Other Data Relevant to an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Growth transformation

4.1.1 *Role of EBV*

In view of the role of viral gene products in the development of non-Hodgkin's lymphoma, it is clear that an important factor is the viral gene functions that have been shown to be involved in growth transformation of normal resting human B lymphocytes (Henle *et al.*, 1967; Pope *et al.*, 1968). Growth transformation by EBV is regarded as an in-vitro equivalent of the lymphoproliferation occurring in EBV-infected individuals in the absence of a functional immune system. Growth transformation by EBV *in vitro* is defined as the induction of immortalization. EBV-infected, proliferating B lymphocytes are similar to lymphocytes activated in response to antigens, mitogens or treatment with IL-4 and anti-CD40 (Banchereau & Rousset, 1991), resulting in expression of a similar repertoire of activation markers and adhesion molecules (Åman *et al.*, 1986; Hurley & Thorley-Lawson, 1988; Alfieri *et al.*, 1991). EBV-infected tumour cells in non-Hodgkin's lymphomas *in vivo*, however, probably also have genetic and epigenetic changes, in addition to the phenotype of the immortalized B lymphocyte studied *in vitro*.

In EBV-transformed B cells, only a small number of viral genes is expressed (see section 1.1.5 for nomenclature), including those for six nuclear antigens, EBNA-1, -2, -3A, -3B, -3C and EBNA-LP, the genes for three membrane proteins, LMP-1, LMP-2A and LMP-2B (two splice variants of LMP-2), *BHRF1*, *BARF0* and two small nuclear non-polyadenylated RNAs (EBERs). *BHRF1* encodes a viral homologue of Bcl-2 (Cleary *et al.*, 1986) which is highly expressed during the lytic cycle. *BHRF1* has also been reported to be transcribed during latency in EBV-immortalized cells (Austin *et al.*, 1988; Oudejans *et al.*, 1995a), while the BHRF1 protein has not been detected in latently infected cells. *BARF0*, a reading frame contained in a set of transcripts first described in nasopharyngeal carcinomas passaged in nude mice (Hitt *et al.*, 1989; Gilligan *et al.*, 1990b, 1991), has recently been shown to encode a 30-kDa protein in lymphoblastoid cell lines and nasopharyngeal carcinoma samples (Fries *et al.*, 1997).

4.1.2 *Minimal set of transforming genes*

The transforming potential of EBV is maintained within about one-third of the viral genome (Mark & Sugden, 1982; Kempkes *et al.*, 1995a; Robertson & Kieff, 1995). Genetic analyses of recombinant viruses have also shown that not all of the genes expressed in growth-transformed cells are required for initiation and maintenance of transformation. Neither the *EBERs* (Swaminathan *et al.*, 1991) nor *EBNA-3B* (Tomkinson & Kieff, 1992a), *BARF0* (Robertson *et al.*, 1994), *EBNA-LP* (Hammerschmidt & Sugden, 1989) or *LMP-2* is required for transformation (Longnecker *et al.*, 1992; Kim & Yates, 1993). Viral *IL-10*, a gene of the lytic cycle with suspected B-cell growth factor activity, is also not required (Swaminathan *et al.*, 1993); however, *EBNA-LP* and *LMP-2* were

shown to significantly improve the outgrowth of transformed cells (Mannick *et al.*, 1991; Allan *et al.*, 1992; Brielmeier *et al.*, 1996). *EBNA-1*, *EBNA-2*, *EBNA-3A*, *-3C* and *LMP-1* are essential for initiation of transformation (Cohen *et al.*, 1989; Kaye *et al.*, 1993; Tomkinson *et al.*, 1993). *EBNA-1* (Yates *et al.*, 1984, 1985), *EBNA-2* (Kempkes et al., 1995b) and *LMP-1* (Sandberg et al., 1997) are also definitely required for maintenance of transformation, whereas *EBNA-3A* is not (Kempkes *et al.*, 1995a). Information on the role of *EBNA-3C* in the maintenance of transformation is not available. The minimal set of viral genes required for the initiation and maintenance of growth transformation is still unknown, however, and the question of whether the combination of *EBNA-1*, *EBNA-2*, *EBNA-3A*, *EBNA-3C* and *LMP-1* is also sufficient for transformation has not yet been addressed experimentally. Functional redundancy among the non-essential genes might permit the deletion of one but not several simultaneously.

4.1.3 *Growth transformation* in vitro *and induction of lymphoproliferation* in vivo

It is not known whether induction of B-cell proliferation by EBV in an immunocompromised host *in vivo* requires the same set of viral genes as EBV-induced B-cell transformation *in vitro*, or a larger or smaller set of viral genes. The genes required for growth transformation *in vitro* are obviously most likely to be involved in the induction of lymphoproliferation *in vivo* and therefore represent the main focus here. It should be kept in mind, however, that the genes required *in vitro* are not necessarily those required *in vivo*. Proliferating cells may receive signals from their surroundings that may bypass the requirement for an active viral oncogene. Alternatively, genes that are unnecessary for growth transformation *in vitro* may be absolutely essential for the induction of lymphoproliferation *in vivo*. Given the complexity of the virus–host interaction, which has evolved over millions of years, this is the more likely possibility. The complete life cycle of the virus is influenced by its interaction with the immune system of the host *in vivo* and therefore plays an important role in the development of EBV-associated malignancies. Control of latency *in vivo*, reactivation, re-entry into the lytic cycle, reinfection of new target cells and interference by the immune system at any of these levels are therefore of key importance for EBV-induced lymphoproliferation *in vivo*. These events not only implicate the viral genes involved in growth transformation and their cellular targets but also reflect the complexity of the virus–host interaction *in vivo*, which can be studied only in a model system of a related primate virus and its natural host.

4.1.4 *Viral transcription pattern after infection of human primary B lymphocytes by EBV*

Infection of B cells with EBV is mediated through CD21, the receptor for complement component C3d (Fingeroth *et al.*, 1984). Binding of the virus triggers a signalling cascade initiated from the CD21 molecule, which activates the cell and gives it the competence to respond to the growth-promoting action of *EBNA-2* and *EBNA-LP* (Hutt-Fletcher, 1987; Sinclair *et al.*, 1994). The viral genome is circularized within about 16–20 h after infection (Hurley & Thorley-Lawson, 1988) and gives rise to large transcripts initiated in the W promoter of the large internal repeats (Woisetschlaeger *et al.*, 1990).

After expression of *EBNA-1* and *EBNA-2*, transcription switches to the C promoter and gives rise to multiply spliced transcripts that code for EBNA-LP, EBNA-2, EBNA-3A, -3B, -3C and EBNA-1 (Schlager *et al.*, 1996; Woisetschlaeger *et al.*, 1990). *OriP*, to which EBNA-1 binds, acts not only as an origin of replication (Yates *et al.*, 1984) but also as an EBNA-1-dependent enhancer of the C promoter (Sugden & Warren, 1989) and the *LMP-1* promoter (Gahn & Sugden, 1995). C promoter transcription occludes the W promoter and brings the viral genome under its own transcriptional control (Puglielli *et al.*, 1996). Expression of the *LMPs* is under transcriptional control of *EBNA-2*. *LMP-1* and *LMP-2B* are transcribed from a bi-directional promoter that responds to *EBNA-2* (Laux *et al.*, 1994a; Johannsen *et al.*, 1995), whereas *LMP-2A* is transcribed from a separate promoter controlled by *EBNA-2* (Zimber-Strobl *et al.*, 1993).

4.1.5   Viral proteins involved in growth transformation

4.1.5.1   EBNA-1

EBNA-1 is the only viral protein expressed in growth-transformed cells that binds directly to DNA in a sequence-specific manner through its DNA binding and dimerization domain in the C terminus (Ambinder *et al.*, 1991; Bochkarev *et al.*, 1995). EBNA-1 binds to the plasmid origin of replication (*oriP*) which consists of multiple tandem repeats of EBNA-1 binding sites followed by two symmetrical binding sites (Rawlins *et al.*, 1985; Reisman *et al.*, 1985). Cooperative binding of EBNA-1 to the multiple binding sites on *oriP* is required, and sufficient, for episomal replication of the viral genome. Furthermore, *oriP* acts as an EBNA-1-dependent enhancer and plays a crucial role in the regulation of viral transcription from both the C and the *LMP-1* promoter in growth-transformed cells (Sugden & Warren, 1989; Gahn & Sugden, 1995). Because of their role in episomal replication and transcription regulation, EBNA-1 and *oriP* are of prime importance for EBV-induced proliferation of lymphocytes *in vitro* and *in vivo*.

Apart from its role in regulation of viral replication and transcription, EBNA-1 might also affect cellular replication and transcription if binding sites for this protein exist in the cellular genome. Comparison of EBV-negative Burkitt's lymphoma cell lines with EBV-positive group I Burkitt's lymphoma cell lines expressing EBNA-1, however, indicates that the growth pattern and cellular phenotypes are identical (Rowe *et al.*, 1987a; Falk *et al.*, 1993).

EBNA-1 can bind RNA *in vitro* through arginine/glycine (RGG) motifs (Snudden *et al.*, 1994). EBNA-1 also activates expression of the lymphoid recombinase genes (RAGs) through an as yet unidentified mechanism (Srinivas & Sixbey, 1995). Activation of the RAGs could promote chromosomal rearrangement and translocations and possibly also facilitate viral integration. This may indicate that EBNA-1 can activate expression of critical cellular genes and affect cellular growth control. EBNA-1 is also expressed in the permissive EBV infection, hairy leukoplakia, indicating that it contributes to replicative EBV infection (Murray *et al.*, 1996).

Evidence that EBNA-1 may itself have oncogenic potential is provided by the finding that lymphomas developed in two transgenic mouse lines expressing EBNA-1 under the

control of the immunoglobulin heavy-chain intron enhancer (Wilson *et al.*, 1996a). Moreover, EBV-positive EBNA-1-expressing Akata cell clones were reported to be able to clone in soft agar and to induce tumours in nude mice (Shimizu *et al.*, 1994).

Another hallmark of EBNA-1 is an array of glycine–alanine repeats located in the N-terminal part of the protein. These repeats were shown to prevent presentation of MHC class I-restricted CTL epitopes (Levitskaya *et al.*, 1995), which represents a novel, unique mechanism for bypassing recognition by cytotoxic T cells and maintaining viral latency.

4.1.5.2   *EBNA-LP*

EBNA-2 and *EBNA-LP* are the first viral genes expressed after infection of primary human B cells with EBV (Rooney *et al.*, 1989; Allday *et al.*, 1989). *EBNA-LP* appears to be important at least for initiation of B-cell transformation, since mutant virus lacking the C-terminal exons has a severely reduced transformation potential, and cells infected with mutant virus require a feeder layer for outgrowth (Mannick *et al.*, 1991). Ectopic expression of *EBNA-LP* in these cells restored the growth behaviour (Allan *et al.*, 1992).

EBNA-LP was reported to bind to *p53* and *Rb* (Szekely *et al.*, 1993), but there is no evidence that the function of *p53* and *Rb* is modified by such binding (Allday *et al.*, 1995; Inman & Farrell, 1995). *EBNA-LP* was recently described as localizing to the nuclear compartment defined by accumulation of the promyelocytic leukaemia gene product in normal cells (Dyck *et al.*, 1994; Weis *et al.*, 1994; Szekely *et al.*, 1996), the main function of which is again unknown. A role for *EBNA-LP* in cell cycle regulation is suggested by the finding that, with *EBNA-2*, it can induce cyclin D2 and cell cycle activation in primary human B cells pretreated with gp340 (Sinclair *et al.*, 1994) and that *EBNA-LP* phosphorylation is dependent on the cell-cycle stage (Kitay & Rowe, 1996a). Recent studies suggest that *EBNA-LP* greatly enhances *EBNA-2*-induced *trans*-activation of *LMP-1* expression through interaction with the *EBNA-2* acidic *trans*-activation domain (Harada & Kieff, 1997). Expression of *EBNA-LP* in transgenic mice has no effect on development or tumour incidence; the animals die of heart failure, probably due to a toxic effect of EBNA-LP itself (Huen *et al.*, 1993).

4.1.5.3   *EBNA-2*

EBNA-2 has long been suspected to play a crucial role in growth transformation, because a 6.6-kb deletion in the P3HR1 viral strain, encompassing the boundary of the large internal repeats to the long unique region (Ragona *et al.*, 1980; Bornkamm *et al.*, 1980, 1982; Jeang & Hayward, 1983), is associated with loss of transforming capacity, while infectivity is maintained (Miller *et al.*, 1974). A recombinant transforming virus lacking the deletion was observed after either superinfection of Raji cells with P3HR1 virus (Fresen *et al.*, 1980; Skare *et al.*, 1985) or by reconstituting the deletion in the P3HR1 virus with cloned fragments spanning the deletion and harbouring the *EBNA-2* gene (Cohen *et al.*, 1989; Hammerschmidt & Sugden, 1989). Analysis of the virus harboured by the parental Jijoye line from which P3HR1 had been obtained by single cell cloning (Hinuma *et al.*, 1967) revealed the surprising finding that the *EBNA-2* genes of

Jijoye and B95-8 share only about 50% sequence homology (Dambaugh *et al.*, 1984; Adldinger *et al.*, 1985). Although strain differences between EBV types 1 (B95-8 prototype) and 2 (Jijoye and AG876 prototypes; Dambaugh *et al.*, 1984) were later also found in other genes (Sample *et al.*, 1990), the difference in the transforming ability of the two strains (Rickinson *et al.*, 1987) could be pinpointed to the differences in the *EBNA-2* genes (Cohen *et al.*, 1992).

EBNA-2 expressed from the SV40 promoter in transgenic mice induced tubular kidney hyperplasia which advanced to adenocarcinoma (Törnell *et al.*, 1996).

EBNA-2 is a *trans*-activator of many cellular (*CD23*, *CD21*, *c-fgr*) and viral genes (*LMP-1*, *LMP-2*, *Cp*) but cannot bind to DNA directly. A cellular protein that mediates binding of *EBNA-2* to its response element was first described in the *LMP-2A* promoter (Zimber-Strobl *et al.*, 1993) and later shown also to have binding sites in the C and *LMP-1* promoters (Ling *et al.*, 1993a; Grossman *et al.*, 1994; Laux *et al.*, 1994b; Yalamanchili *et al.*, 1994). This protein turned out to be recombination signal binding protein Jκ (RBP-Jκ) (Grossman *et al.*, 1994; Henkel *et al.*, 1994; Waltzer *et al.*, 1994; Zimber-Strobl *et al.*, 1994), a ubiquitously expressed protein which is highly conserved in evolution, with homologues in species as distant as *Drosophila* (suppressor of hairless, *Su(H)*) (Furukawa *et al.*, 1992; Schweisguth & Posakony, 1992) and *Caenorhabditis elegans* (Christensen *et al.*, 1996). Its name is misleading since it proved not to bind to the immunoglobulin Jκ sequence (Tun *et al.*, 1994). The genetics of *Drosophila* provided a clue to its physiological function. RBP-Jκ/Su(H) is a DNA-binding protein and a downstream target of a cellular receptor called Notch (Fortini & Artavanis-Tsakonas, 1994), which in mammals is part of a family of related proteins. Ligand binding activates Notch, and subsequent processing and transport of Notch to the nucleus converts the repressor RBP-Jκ (Hsieh & Hayward, 1995; Waltzer *et al.*, 1995) into an activator and turns on target genes (Jarriault *et al.*, 1995; Hsieh *et al.*, 1996). EBNA-2 and activated Notch interact with similar regions in the RBP-Jκ molecule and fulfil similar functions (Hsieh *et al.*, 1996; Sakai & Honjo, 1997). EBNA-2 may therefore be regarded as a functional homologue of a constitutively active Notch receptor. By analogy with other viral oncoproteins, it seems likely that *EBNA-2* has functions additional to activation of RBP-Jκ. Binding of EBNA-2 to RBP-Jκ, although necessary, is not sufficient for *trans*-activation of EBNA-2-regulated promoters. In the *LMP-1* promoter, binding of PU.1 is additionally involved in *trans*-activation by EBNA-2 (Laux *et al.*, 1994b; Johannsen *et al.*, 1995), and other proteins involved are still to be identified. The region in EBNA-2 that interacts with RBP-Jκ has been mapped to amino acids 318–327 (Yalamanchili *et al.*, 1994; Ling & Hayward, 1995), a region highly conserved among the *EBNA-2* genes of EBV types 1 and 2 and HVP (Ling *et al.*, 1993b). It is one of the three regions of *EBNA-2*, including the C-terminal *trans*-activation domain and an N-terminal domain with unknown function, that are critically required for transformation (Cohen *et al.*, 1991). Additional details on the biochemistry and interaction with basal transcriptional machinery are given in Section 1.

In view of the functional equivalence between EBNA-2 and an activated Notch receptor, Notch signalling may partially substitute for EBNA-2 function in activation of viral and cellular genes in the EBNA-2-negative forms of latency.

4.1.5.4   EBNA-3A, -3B and -3C

EBNA-3A, -3B and -3C differ in type 1 and type 2 strains, but these differences do not affect the transforming ability of the viruses (Sample et al., 1990; Tomkinson & Kieff, 1992b). EBNA-3A and -C are required for transformation, whereas EBNA-3B is not (Tomkinson & Kieff, 1992a; Tomkinson et al., 1993). This is quite surprising, since EBNA-3B is a major target for recognition of latently infected cells by cytotoxic T cells (Moss et al., 1996) and might be counterselected if it were not an essential gene for the viral life cycle in vivo. The EBNA-3C and -3B proteins have been reported to participate in transcriptional regulation of the LMP-1, CD21 and CD23, and CD40, CD77 and vimentin genes, respectively (Wang et al., 1990a; Allday et al., 1993; Silins & Sculley, 1994). EBNA-3A, -3B and -3C proteins were recently shown to inhibit transcriptional activation of EBNA-2-responsive promoters (Le Roux et al., 1994) by preventing RBP-Jκ and EBNA-2/RBP-Jκ complexes from binding to their cognate RBP-Jκ binding sites (Waltzer et al., 1996; Robertson et al., 1996; Zhao et al., 1996). EBNA-3 proteins are thus believed to counterbalance and finely tune the action of EBNA-2, and it is likely that they have other functions. EBNA-3C has been described as countering the action of the cyclin-dependent kinase inhibitor p16 INKLA and inactivating the retinoblastoma protein (Rb) functionally, like the viral proteins HPV E7 and adenovirus E1A (Parker et al., 1996).

4.1.5.5   LMP-1

LMP-1 is the only known EBV gene which, acting alone, can transform rodent fibro-blasts (Wang et al., 1985). LMP-1-expressing rodent fibroblasts grow in low concentrations of serum, acquire anchorage-independent growth in soft agar and become tumorigenic. In primary B cells, LMP-1 induces DNA synthesis and up-regulation of CD23, CD21 and CD54 (Peng & Lundgren, 1992).

LMP-1 is highly toxic if expressed at high levels (Hammerschmidt et al., 1989; Floettmann et al., 1996). When expressed at lower levels, it induces a number of phenotypic and functional changes such as up-regulation of adhesion molecules and activation markers (e.g. CD23, CD39, CD40, CD54 and CD58; Wang et al., 1988b, 1990b), restoration of immunological function, i.e. coordinated up-regulation of peptide transporters and HLA class-I and class-II molecules (Cuomo et al., 1990; de Campos-Lima et al., 1993b; Zhang et al., 1994a; Rowe et al., 1995) and induction of cyclin D2 (Arvanitakis et al., 1995) and stress-response genes that prevent apoptosis, such as bcl-2 and A20 (Henderson et al., 1991; Laherty et al., 1992; Rowe et al., 1994). Most of these functions are usually attributed to the ability of LMP-1 to induce NFκB (Hammarskjold & Simurda, 1992), but this is not the only signalling pathway to which LMP-1 contributes (Miller et al., 1997). The molecular link between LMP-1 and induction of the expression of genes such as bcl-2 is in fact still missing (Martin et al., 1993; Rowe et al., 1994). When expressed in epithelial cells, LMP-1 inhibits cell differentiation in keratinocyte raft cultures in vitro (Dawson et al., 1990) and induces morphological transformation and aberrant keratin expression (Fåhraeus et al., 1990b) and severe epidermal hyperplasia in transgenic mice (Wilson et al., 1990).

LMP-1 is absolutely required for both the initiation and maintenance of B-cell transformation by EBV (Kaye *et al.*, 1993). Genetic experiments have shown that the first transmembrane domain and the neighbouring amino acids of the cytoplasmic N-terminal domain are required for B-cell transformation (Kaye *et al.*, 1993), while the function of the 155 C-terminal amino acids can be complemented by co-cultivation of the cells on a fibroblast feeder layer (Kaye *et al.*, 1995). Constitutive *LMP-1* expression in EBV-transformed cells carrying a conditional *EBNA-2* gene has provided evidence that *LMP-1* in the absence of functional *EBNA-2* promotes survival of the cells without maintaining proliferation, similar to the stimulation of the endogenous CD40 receptor by CD40 ligand, which is even more effective in promoting cell survival than LMP-1 (Zimber-Strobl *et al.*, 1996). The functional similarity between LMP-1 and CD40 is corroborated by the fact that the two proteins recruit the same types of signalling molecules on the cytoplasmic side of the membrane. These molecules, which are known as tumour necrosis factor (TRAF) receptor-associated factors mediate activation of NFκB (Mosialos *et al.*, 1995). Deletion of the TRAF interaction domain within the LMP-1 molecule abolishes B-cell transformation by EBV (Izumi *et al.*, 1997).

4.1.5.6   *LMP-2A and -2B*

A clue to the function of LMP-2A has been provided by the observations that (i) it has eight tyrosine motifs that can be phosphorylated and interact with other kinases and adapter proteins; (ii) it is a substrate for members of the src family of kinases expressed in B cells (fyn, lyn) and is stably phosphorylated on tyrosine (Longnecker *et al.*, 1991; Burkhardt *et al.*, 1992); (iii) it is associated with another stably phosphorylated tyrosine kinase (syk) (Miller *et al.*, 1995a); (iv) it shares the sequence motif $(YXXL/I)_2$ with signal transducing subunits of antigen receptors in B and T cells (Reth, 1989; Beaufils *et al.*, 1993); and (v) it inhibits anti-immunoglobulin-mediated Ca^{2+} mobilization, PLCγ2 activation and anti-immunoglobulin-induced reactivation of the lytic cycle, which can be bypassed by TPA with Ca^{2+} ionophore (Miller *et al.*, 1994b, 1995a). These data are consistent with a model in which LMP-2A sequesters the receptor-associated tyrosine kinase, blocking its autophosphorylation and downstream signalling events (Miller *et al.*, 1995a).

Consistent with this model, *LMP-2A* and -*2B* proved not to be required for B-cell transformation by EBV *in vitro* (Longnecker *et al.*, 1992, 1993a,b), even though they contribute to the efficiency of transformation (Brielmeier *et al.*, 1996). Maintenance of latency and prevention of entry into the lytic cycle due to inhibition of the B-cell receptor may, however, be a more important issue for the virus *in vivo* than *in vitro*.

4.1.6   *Cellular genes induced during growth transformation by EBV*

Normal cells are induced to proliferate by signalling molecules (soluble molecules such as hormones and growth factors or matrix or cell-borne molecules) that interact with specific receptors, deliver a signal to the cell, transmit it into the nucleus and convert the cell to a new transcriptional programme and a proliferative response. As seen with oncogenes, malignant growth is a consequence of genetic changes which, on the one hand,

abrogate negative control mechanisms and, on the other, render the signalling process constitutive. This is achieved by shortcuts introduced into the signal transduction pathway at some level between the membrane and the nucleus.

Since different proliferative signals have to converge into a common programme leading to entry into S phase, the proteins that control these checkpoints, such as Rb and p53, are frequent targets for transforming viruses like papillomaviruses, SV40 and adenoviruses, which abrogate the cellular control mechanisms. In contrast, there is no evidence for a direct functional interaction between EBV latency proteins and either Rb or p53; however, it is likely that EBV indirectly affects these pathways. Recent data suggest that EBNA-3C overcomes the action of cyclin-dependent kinase inhibitor p16 and in this way affects cellular cycle control mechanisms (Parker *et al.*, 1996). In addition, *LMP-1* expression inhibits p53-mediated apoptosis (Okan *et al.*, 1995; Fries *et al.*, 1996).

In comparison with resting B cells, EBV-transformed lymphoblastoid cell lines show dramatic changes in their gene expression programme. In fact, EBV appears to mimic physiological B-cell activation brought about by antigen and helper T cells, and up-regulates many kinds of cell-surface molecules that might be important in signalling and growth regulation. Among the cellular genes induced by EBV are those that code for growth factors such as IL-5 (Paul *et al.*, 1990), IL-6 (Tanner & Tosato, 1992; Tanner *et al.*, 1996), IL-10 (Burdin *et al.*, 1993; Nakagomi *et al.*, 1994), TNFα and lymphotoxin (Estrov *et al.*, 1993; Gibbons *et al.*, 1994), thioredoxin (Wakasugi *et al.*, 1990), receptors such as the transferrin receptor, CD21, CD23, the TNFα receptor (Gibbons *et al.*, 1994; Wei *et al.*, 1994), TRAF-1 (Mosialos *et al.*, 1995), a protein related to the p40 subunit of the IL-12 receptor (Devergne *et al.*, 1996), G-protein-coupled receptors (Dobner *et al.*, 1992; Birkenbach *et al.*, 1993), activation markers (CD39), adhesion molecules (CD44, CD48, CD54, CD58) and molecules involved in cytoskeleton formation such as vimentin and actin-bundling protein (Birkenbach *et al.*, 1989; Mosialos *et al.*, 1994; Wang *et al.*, 1990b; Yokoyama *et al.*, 1991). It seems likely that these molecules participate in the induction and maintenance of growth transformation by EBV by establishing autocrine loops (only convincingly shown for lymphotoxin) or cell–cell-mediated cross-stimulatory pathways.

It should be borne in mind, however, that there is currently no way of testing such hypotheses. A conditional knock-out system for cellular genes (e.g. conditional induction of antisense RNA or ribozymes) is urgently required to elucidate the role of individual cellular genes for the maintenance of growth transformation.

4.2 Burkitt's lymphoma

4.2.1 *Molecular abnormalities in relation to the tumour-cell precursor*

4.2.1.1 *Translocation of the c-myc oncogene*

The discovery of non-random chromosomal translocations associated with Burkitt's lymphoma (Manolov & Manolova, 1972; Zech *et al.*, 1976; Bernheim *et al.*, 1981) paved the way to an understanding of the genetic derangements that are a central component of its pathogenesis.

From the observations that a chromosomal breakpoint on chromosome 8, band q24 is common to all three of the observed translocations in Burkitt's lymphoma and that the breakpoints are located on chromosomes 14, 2 and 22, at the heavy- and light-chain immunoglobulin loci (Croce *et al.*, 1979; Lenoir *et al.*, 1982; Malcolm *et al.*, 1982; McBride *et al.*, 1982), a strategy for identifying a potential oncogene on chromosome 8q24 was devised. Probes from the immunoglobulin regions were used to clone adjacent DNA fragments, which were soon shown to contain *c-myc* (Dalla-Favera *et al.*, 1982; Taub *et al.*, 1982; Adams *et al.*, 1983). Each of the chromosomal translocations results in the juxtaposition of *c-myc* to immunoglobulin sequences — either *c-myc* is translocated distal to heavy-chain sequences on chromosome 14q32, or light chain sequences from chromosomes 2p11 (kappa) or 22q12 (lambda) are translocated distal to *c-myc* on chromosome 8 (reviewed by Magrath, 1990; Lüscher & Eisenman, 1990). The *c-myc* gene is known to be important in the control of cell proliferation and cell death (Askew *et al.*, 1991; Evan *et al.*, 1992).

The juxtaposition of *c-myc* to immunoglobulin genes in a B-cell lymphoma suggests that the latter genes themselves have an important role to play in pathogenesis. Firstly, they are probably relevant to the genesis of the translocations, because recombinational events occur in immunoglobulin genes during B-cell ontogeny. The physiological breakage and relegation of the DNA strand to the regions in which the chromosomal breakpoints occur is unlikely to be due to chance; either the chromosomes are more readily broken in areas of the DNA strand that are exposed to recombinases, or translocations are actually created by the same recombinases that are responsible for VDJ rearrangements. Secondly, the transcriptional deregulation of the *c-myc* gene, which results from the translocation, is due to its juxtaposition to transcriptional control elements within the immunoglobulin locus (Madisen & Groudine, 1994; Polack *et al.*, 1993; Hörtnagel *et al.*, 1995). The net consequence of the translocation appears to be that *c-myc* is regulated as if it were an immunoglobulin gene, i.e. it is constitutively expressed in these immunoglobulin-synthesizing tumour cells. Thirdly, structural changes within the regulatory and coding regions of *c-myc*, reminiscent of somatic mutations in the hypervariable regions of the immunoglobulin genes, invariably accompany the translocation (Rabbitts *et al.*, 1984; Pelicci *et al.*, 1986; Magrath, 1990; Zajac-Kaye *et al.*, 1990; Bhatia *et al.*, 1995; Raffeld *et al.*, 1995).

Because the *c-myc*–immunoglobulin translocation is the central element in the pathogenesis of Burkitt's lymphoma, one possible role for cofactors is to increase the likelihood that a translocation will occur. On the basis of evidence derived by Southern blot that the J region of the immunoglobulin heavy chain locus involved in the translocation is not rearranged in a large fraction of Burkitt's lymphomas, it has been surmised that the translocation occurs early in B-cell differentiation (Bhatia *et al.*, 1995). This assumption is consistent with the notion that precursor B cells in the process of, or just about to, rearrange their immunoglobulin genes are particularly susceptible to translocation. By extrapolating from mouse models, it can be calculated that 10^{10} new B cells are generated daily in humans (Opstelten & Osmond, 1983). An environmental agent that increases the rate of B-cell ontogeny could simultaneously increase the frequency of occurrence of translocations, even if the latter is a purely stochastic event. Both EBV and malaria cause

B-cell hyperplasia and thus could influence the development of Burkitt's lymphoma in this way (Lenoir & Bornkamm, 1987). When both agents are present, as in African infants, the risk of developing a translocation is likely to be increased. Other agents, such as phorbol esters present in medicinal plants, have been proposed to increase the risk for chromosomal translocations additionally (Osato *et al.*, 1990; van den Bosch *et al.*, 1993).

EBV cannot only induce cell proliferation and thus increase on a statistical basis the likelihood that a *c-myc*–immunoglobulin translocation will arise, but it may also directly promote immunoglobulin gene recombination. Such gene recombination in lymphoid cells is dependent upon expression of the recombination activating genes *RAG-1* and *RAG-2*. To minimize the risk of aberrant recombination, the expression of these genes is limited to a narrow window in the development of B cells. EBV infection of sporadic Burkitt's lymphoma-negative cells and transfection of *EBNA-1* have been shown to up-regulate *RAG* expression (Kuhn-Hallek *et al.*, 1995; Srinivas & Sixbey, 1995). Expression of *LMP-1* and of *RAG-1* were, however, found to be mutually exclusive. The possibility that inappropriate *RAG* expression increases the likelihood of aberrant immunoglobulin gene recombination is supported by the observation that EBV-transformed, fetal liver-derived precursor B cells frequently develop translocations involving the immunoglobulin heavy-chain locus (Altiok *et al.*, 1989). B Lymphocytes that have undergone aberrant recombination are normally eliminated by apoptosis. Precursors to Burkitt's lymphoma cells with aberrant recombinations may be illegitimately rescued from apoptosis by EBV-encoded *LMP-1* if the malignant stage involves type II or type III latency phenotypes.

If the role of EBV in the pathogenesis of Burkitt's lymphoma were confined to the likelihood that a *myc* translocation occurs, it would follow that once such a translocation has developed the viral genome is no longer necessary.

4.2.1.2   The Burkitt's lymphoma-cell phenotype resembles that of a germinal-centre cell

While there is evidence that the chromosomal translocations occur in precursor B cells, the immunophenotype of Burkitt's lymphoma is that of a germinal-centre cell, indicating that differentiation must have occurred after the translocation. This may be an explanation for the essentially invariable expression of surface immunoglobulin, since differentiating B cells that fail to express a functional immunoglobulin molecule are destined to undergo apoptosis. The need for genetic changes, or possibly the expression of EBV genes that permit a translocation-bearing cell to avoid apoptosis, is likely to be an essential component of lymphomagenesis.

Consistent with the hypothesis that Burkitt's lymphoma cells have a germinal-centre phenotype is the presence of mutations in the hypervariable region of the heavy immunoglobulin chain in these cells (Chapman *et al.*, 1995; Klein *et al.*, 1995; Tamaru *et al.*, 1995), raising the possibility that exposure to antigens may be relevant to the pathogenesis of Burkitt's lymphoma. Burkitt's lymphoma cells do not, however, express activation antigens (Gregory *et al.*, 1987b) and in this respect, differ markedly from EBV-

transformed lymphoblastoid cell lines. This phenotype seems to be a consequence of the c-*myc* translocation (Polack *et al.*, 1996).

4.2.1.3 *Mutations in* p53 *in Burkitt's lymphoma*

Mutations in *p53* have been detected in up to 37% of biopsy samples from primary Burkitt's lymphomas (Gaidano *et al.*, 1991; Bhatia *et al.*, 1992). The incidence of *p53* mutation is independent of the geographic origin of the tumour, the chromosomal translocation or the association with EBV. The specific mutations in *p53* in Burkitt's lymphoma are distinct from those found in non-lymphoid tumours, as most are clustered in codons 213–248. The frequency of *p53* mutation is higher in cell lines, most of which are derived from recurrent tumours, more than 70% of which harbour mutations (Gaidano *et al.*, 1991; Farrell *et al.*, 1991; Bhatia *et al.*, 1992). This finding may indicate that *p53* mutation contributes to tumour progression. One consequence of the absence of *LMP-1* expression in Burkitt's lymphoma (Rowe *et al.*, 1987a) is the lack of its protective effects on *p53*-mediated apoptosis (Okan *et al.*, 1995; Fries *et al.*, 1996). Mutations in *p53* are rare in EBV-associated malignancies in which *LMP-1* is expressed (Spruck *et al.*, 1992; Sun *et al.*, 1992; Fåhraeus *et al.*, 1988).

4.2.2 *EBV infection in Burkitt's lymphoma*

Although chromosomal translocations affecting c-*myc* and the immunoglobulin loci are always present in Burkitt's lymphoma, EBV is not invariably found. About 95% of the cases in endemic areas of Africa and Papua–New Guinea contain EBV, but the prevalence in Burkitt's lymphoma outside the endemic areas varies with underlying disease and geographic region from 15 to more than 90% (see section 2.1). Both EBV type 1 and type 2 may be associated with Burkitt's lymphoma (Zimber *et al.*, 1986). A definitive conclusion about the pathogenetic role of EBV in Burkitt's lymphoma cannot be reached solely on the basis of epidemiological observations; molecular studies provide a number of additional insights and are the most likely source of unequivocal evidence for a pathogenetic role of the virus.

4.2.2.1 *EBV is monoclonal in Burkitt's lymphoma*

One requirement that must be fulfilled if EBV is to subserve a pathogenetic role in Burkitt's lymphoma is that the virus must not only be present in the tumour cells but must also have entered the cell before its final conversion to a malignant lymphoma. Since malignant tumours originate from a single cell which contains all of the necessary genetic abnormalities, EBV should be present in all tumour cells and should also be monoclonal, i.e. all tumour cells should be derived from the same EBV-infected cell. The detection of a homogeneous number of terminal repeats in EBV episomes in Burkitt's lymphoma tumour cells showed that the virus is indeed clonal and indicated that the tumour developed from a single EBV progenitor cell (Raab-Traub & Flynn, 1986; Neri *et al.*, 1991).

4.2.2.2 Integration of viral DNA in Burkitt's lymphoma cells

Many oncogenic viruses contribute to tumorigenesis by insertional mutagenesis, e.g. by activation of proto-oncogenes and/or inactivation of tumour suppressor genes. Integration of the EBV genome has been observed in several Burkitt's lymphoma-derived cell lines (Henderson *et al.*, 1983; Delecluse *et al.*, 1993a); however, no preferential integration site has, so far, been identified. Moreover, integration is not a *sine qua non*, since EBV-positive Burkitt's lymphoma cells may (rarely) lose the viral genomes when grown *in vitro* (Takada *et al.*, 1995), and EBV-positive and EBV-negative cells co-exist in a Burkitt lymphoma line (Trivedi *et al.*, 1995).

4.2.2.3 Expression of EBV genes in EBV-associated Burkitt's lymphoma

If EBV exerts a direct oncogenic effect, one or more viral genes should be expressed in tumour cells. This criterion is also fulfilled, although the pattern of expression of EBV proteins is markedly different in Burkitt's lymphoma and in lymphoblastoid cell lines. This suggests that there are basic differences in the mechanisms whereby EBV transforms B cells on the one hand and contributes to the development, or maintenance, of Burkitt's lymphoma on the other. Although the full range of latency genes is expressed in EBV-transformed cell lines, only *EBNA-1* and the *EBERs* are invariably expressed in Burkitt's lymphoma cells (Rowe *et al.*, 1986, 1987a; Niedobitek *et al.*, 1995). Rare cells within a tumour may express other EBV genes, such as *EBNA-2*, *LMP* and *BZLF1* (Niedobitek *et al.*, 1995), and may even enter the lytic cycle (Gutiérrez *et al.*, 1993).

The differences in latent gene expression in lymphoblastoid cell lines and Burkitt's lymphoma are regulated at the level of promoters in these cell types. In lymphoblastoid cell lines, all of the EBNA proteins are encoded from spliced transcripts derived from a common long precursor initiated from promoters in the W and/or C *Bam*HI fragments of the EBV genome, whereas in Burkitt's lymphoma *EBNA-1* is transcribed from the Q promoter (Schaefer *et al.*, 1995). Q Promoter-initiated transcripts that encode *EBNA-1* are negatively regulated by *EBNA-1* (Sample *et al.*, 1992).

A recent study revealed that transgenic mice that express *EBNA-1* under the control of immunoglobulin enhancer develop B-cell lymphomas (Wilson *et al.*, 1996c). This suggests that *EBNA-1* may have additional properties beyond maintenance and regulation of the viral genome and that it contributes to the development of malignancy.

4.2.2.4 Expression only of EBNA-1 is associated with reduced immunogenicity

The limited expression of EBV gene products in Burkitt's lymphoma cells provides significant protection from immune recognition, as most of the CTL response is directed against EBNA-3 proteins. In addition, EBNA-1 is not processed or presented in class I MHC molecules (Levitskaya *et al.*, 1995). The EBNA-1 protein contains a simple repeat of glycine and alanine. Transfer of this repeat element to a heterologous protein sequesters that protein from processing and expression within MHC class I. This unique property enables an EBV-infected cell that expresses only *EBNA-1* to escape immune recognition.

Burkitt's lymphoma cells not only lack EBV-derived immunogenic proteins, they also express low levels of HLA class-I molecules (Masucci *et al.*, 1987; Andersson *et al.*, 1991; Gavioli *et al.*, 1992; Imreh *et al.*, 1995), adhesion molecules, including LFA-1 (CD11a), ICAM-1 (CD54) and LFA-3 (CD58), and co-stimulatory molecules, B7-1 and B7-2 (CD80 and CD86), that participate in antigen presentation, while CD10, CD38 and CD77 are un-regulated (Gregory *et al.*, 1988, 1990; Billaud *et al.*, 1990; Ling *et al.*, 1989; Magrath, 1990; Martel-Renoir *et al.*, 1995). As a consequence, Burkitt's lymphoma cells are virtually invisible to the immune system, as evidenced by the fact that the cells are unable to elicit an allostimulatory T-cell response (Cuomo *et al.*, 1990). The phenotype of Burkitt's lymphoma cells is a consequence of high *c-myc* expression and non-expression of the viral proteins involved in growth promotion, except for EBNA-1 (Polack *et al.*, 1996).

4.2.2.5 *The proliferation programme driven by c-myc–immunoglobulin is incompatible with expression of EBNA-2 and LMP-1 in the type-III latency programme*

After the discovery of the chromosomal translocations in Burkitt's lymphoma cells, it was suspected that, in analogy to the well-documented cooperation of oncogenes (Hunter, 1991), EBV viral gene products would cooperate with an activated *c-myc* gene to promote a fully malignant phenotype. It was therefore not anticipated that *EBNA-2* and *LMP-1* would have adverse effects on the proliferation of Burkitt's lymphoma cells and even abrogate their tumorigenic potential (Torsteinsdottir *et al.*, 1989; Cuomo *et al.*, 1992; Floettmann *et al.*, 1996). This can be explained, at least in part, by the fact that *EBNA-2* inhibits the transcriptional regulatory elements of the immunoglobulin heavy chain locus. *EBNA-2* down-regulates IgM expression, and, in cells in which *c-myc* transcription is driven by translocation into the immunoglobulin heavy-chain locus, it down-regulates *c-myc* expression concomitantly (Jochner *et al.*, 1996).

Thus, while *EBNA-2* is absolutely required for proliferation of EBV-infected normal cells, switching on the function of *EBNA-2* inhibits proliferation of Burkitt's lymphoma cells with the t(8;14) translocation (Kempkes *et al.*, 1996). In cells with the variant t(2;8) translocation, *EBNA-2* does not affect *c-myc* expression directly but slows proliferation in an indirect manner (Polack *et al.*, 1996). *EBNA-2* expression and proliferation mediated by constitutive activation of *c-myc* are thus mutually exclusive.

EBV-positive Burkitt's lymphoma cells tend to change their phenotype and switch to the type III latency programme, expressing *EBNA-2* and *LMP-1*, when explanted into tissue cultures, which at first sight seems contradictory to what is stated above. Burkitt's lymphoma cells in type I latency are highly susceptible to apoptosis and evolve variants resistant to apoptosis *in vitro* which have switched to the type III latency programme (Gregory *et al.*, 1991). Under these conditions, however, the cells proliferate significantly more slowly (Falk *et al.*, 1993) and express lower levels of *c-myc* RNA and protein than Burkitt's lymphoma cells in type-I latency (Jochner *et al.*, 1996), due to the effects of *EBNA-2* on heavy-chain-regulated *c-myc* (Bornkamm *et al.*, 1988; Spencer &

Groudine, 1991), while the expression of *EBNA-2* and *LMP-1* seems sufficiently high to protect the cells from apoptosis.

4.2.2.6 Contribution of the viral strategy for latent persistence to lymphomagenesis

The different type I latency programmes together represent a strategy necessary for the virus to maintain a permanent reservoir of viral genomes in B cells in normal EBV-infected individuals. This strategy also contributes to lymphomagenesis (see section 4.2.1 above; Rowe *et al*, 1992; Chen *et al.*, 1995a; Miyashita *et al.*, 1995). Under physiological conditions, the type-I latency programme allows maintenance of EBV DNA without risk of elimination by CTL, as discussed elsewhere. This programme, which under physiological conditions appears to operate only in non-proliferating B cells, is maintained in Burkitt's lymphoma cells.

4.2.2.7 EBNA-1 subtypes

Recently, subtypes of EBNA-1 that differ in their amino-acid sequence have been identified (Bhatia *et al.*, 1996). These differences do not seem to reflect geographical regions. Interestingly, a subtype marked by alanine at position 487 is the commonest form in normal individuals but is rarely found in tumours. In contrast, the subtype marked by leucine at 487 but containing several additional amino-acid substitutions is frequently found in Burkitt's lymphomas and not in normal individuals (Gutiérrez *et al.*, 1997). This subtype, *V-leu*, differs only at position 487 from another subtype, *V-pro*, which is present in normal lymphocytes but only in association with other variants. These data suggest that the subtypes arise *in vivo* and that mutations in *EBNA-1* may be relevant to the pathogenesis of Burkitt's lymphoma.

4.2.3 Effects of malaria on B-cell activation and EBV infection

While epidemiological evidence for a role of malaria as a predisposing factor (see section 2.1.4.1) is strong, it will be important to identify the specific mechanism whereby malaria influences the pathogenesis of Burkitt's lymphoma. To date, the mechanism has not been identified, although experimental evidence derived from studies in mice provide paradigms that may be applicable to Burkitt's lymphoma.

In a study conducted by Jerusalem (1968), 64% of mice infected with *Plasmodium berghei* eventually developed malignant lymphoma, while Osmond *et al.* (1990) showed that infection of mice with *P. bergei yeollii* increased the proportion of immature B cells (pro-B cells) in the bone marrow and decreased the pool of mature B cells. It is this expansion of precursor B cells that is likely to be responsible for the increased susceptibility of *Plasmodium*-infected mice to the development of lymphoma.

In a study of concurrent infection, 10 of 12 adult mice infected with *P. berghei yeollii* and Moloney leukaemia virus developed lymphoma, in comparison with one of 11 infected only with Moloney virus and none of 10 infected with malaria alone (Wedderburn, 1970). Similar findings were reported by Salaman *et al.* (1969). Moloney virus does not contain an oncogene and activates oncogene expression by a process of promoter insertion. The combination of precursor B-cell hyperplasia and genetic lesions

induced by Moloney virus is likely to account for the increased frequency of lymphomas in the doubly infected mice.

A similar situation may apply to African children. If malarial infection in humans also expands pro-B- and pre-B-cell populations, the risk for developing Burkitt's lymphoma may be increased, as the pool of cells that could develop a *c-myc*–immunoglobulin translocation is larger. EBV infection may well add an additional potentially tumorigenic lesion, although the molecular pathways involved are unlikely to include promoter insertion.

While expansion of precursor B-cell populations in children in regions where malaria is hyperendemic has not been examined, there is evidence that the B-cell system is hyperactive during the course of infection. Children with malaria have very high serum levels of IgG and IgM, most of which are not due to anti-plasmodial antibodies and plateau after the age of five to six years (McGregor, 1970). Turnover of IgG is as much as seven times greater in adults from holoendemic malarious regions than among those from control regions (Cohen et al., 1961; Cohen & McGregor, 1963).

Chronic malaria, like HIV infection, leads to a shift in the helper T-cell response towards T_h2 cells (von der Weid & Langhorne, 1993). T_h2 cytokines, such as IL-10, suppress the humoral arm of the immune response, support the early steps of B-cell immortalization by EBV (Burdin et al., 1993) and suppress CTL function. As a consequence, the number of B lymphocytes latently infected with EBV increases, while the ability of T cells to suppress the outgrowth of EBV-infected lymphoblastoid cells is impaired (Gunapala et al., 1990; Moss et al., 1983a; Whittle et al., 1984; Lam et al., 1991).

In addition, EBV seroconversion is known to occur much earlier in populations of low socioeconomic status or those who live in crowded conditions than in populations in which families are smaller and have more spacious accommodation (Niederman et al., 1968; Hinuma et al., 1969; Henle & Henle, 1970, 1979). In developing countries, seroconversion occurs in the majority of children by the age of three years (Diehl et al., 1969; Kafuko et al., 1972), occurring earlier in rural areas than in urban zones (Biggar et al., 1981), and is usually not associated with a clinical syndrome (Diehl et al., 1969). It is possible, as originally suggested by de Thé (1977), that early infection with EBV predisposes to the development of EBV-associated Burkitt's lymphoma because of differences in the immune system of infants and older individuals and, in the context of African children, because of the presence of coincident B-cell hyperplasia and immunosuppression caused by malaria.

4.2.4   Plant products

Phorbol esters interact with protein kinase C and are potent inducers of cell differentiation. They can induce the differentiation of Burkitt's lymphoma-derived cell lines (Benjamin et al., 1984; Ho et al., 1987; Osato et al., 1990). Several authors have demonstrated that extracts of *Euphorbia tirucalli* and related plants, or even extracts of soil or reservoir water near places where *Euphorbia* plants are growing, can induce the expression of EA and VCA in Raji cells (Ito et al., 1981). Lin et al. (1982) showed that

TPA, a phorbol ester, increases viral replication, as measured by an increase in genome copies. This potential activation of EBV replication could result in an increased number of lymphocytes that become infected with EBV.

Similar extracts have been reported to increase the outgrowth of EBV-transformed B cell lines and to decrease EBV-specific T-cell killing (Osato *et al.*, 1990; Imai *et al.*, 1994b). In addition, of potentially considerable interest, Osato *et al.* (1990) observed that dual exposure of B lymphocytes to EBV and deoxyphorbol ester, the active principle of *Euphorbia*, increased the numbers of chromosomal translocations and deletions involving chromosome 8. Thus, the presence of these tumour-promoting compounds in the environment could influence both EBV infection and the genesis of chromosomal abnormality.

4.2.5 Genetic disposition

Although familial Burkitt's lymphoma has been reported (see section 2.1.2), genes that could account for an increased risk for the development of Burkitt's lymphoma have not been identified, although it is probable that genetic determinants of the response to relevant cofactors such as malaria, e.g. the presence of sickle-cell trait (Williams, 1966; Pike *et al.*, 1970; Nkrumah & Perkins, 1976), may influence the risk for development of this tumour in Africa. Similarly, inherited immunodeficiency syndromes, such as X-linked lymphoproliferative disorder (Provisor *et al.*, 1975; Purtilo, 1976), are associated with a markedly increased risk for the development of non-Hodgkin's lymphomas (Harrington *et al.*, 1987), which sometimes contain *c-myc*–immunoglobulin gene translocations and have the histological appearance of Burkitt's lymphoma (Egeler *et al.*, 1992).

4.2.6 Burkitt's lymphoma in AIDS patients

Burkitt's lymphoma arising in the context of AIDS is associated with EBV infection in only 30–40% of cases, which is greater than that observed in sporadic Burkitt's lymphoma (Ernberg & Altiok, 1989; Gaidano & Dalla-Favera, 1995). This percentage is, however, much lower than that in other non-Hodgkin's lymphomas (see section 4.3.1.3).

In comparison with other non-Hodgkin's lymphomas, AIDS-associated Burkitt's lymphoma develops earlier in the progress of the disease and in less severely immuno-compromised individuals. While the characteristics of endemic Burkitt's lymphoma may also apply to AIDS-related disease, there are some important differences: (i) EBV is found in only a proportion of AIDS-related Burkitt's lymphomas, (ii) the patients are much older than those with endemic Burkitt's lymphoma, and (iii) the pattern of *c-myc* translocation is different, involving the switch region rather than the VDJ region.

The clinical, molecular and viral features of AIDS-related Burkitt's lymphoma indicate that its pathogenesis is similar to that of sporadic Burkitt's lymphoma in immunocompetent individuals; however, in the context of AIDS, these lymphomas occur at increased incidence. This suggests the contribution of an AIDS-related factor. That this factor is unlikely to be simply immunosuppression is indicated by the fact that Burkitt's lymphomas occur only rarely in transplant patients and then only in association with rela-

tively low immunosuppression (Niedobitek *et al.*, 1997b). Chronic immune stimulation by malaria has been suggested to play a role in the pathogenesis of endemic Burkitt's lymphoma, and stimulation by e.g. schistosomiasis may be involved in the development of cases in other regions (Araujo *et al.*, 1996). Indeed, it has been proposed that HIV-induced polyclonal B-cell proliferation may contribute to the development of AIDS-related Burkitt's lymphoma (Kalter *et al.*, 1985; Seigneurin *et al.*, 1987; Gaidano & Dalla-Favera, 1995).

For a discussion of lymphomas in AIDS patients, see section 4.3.1.3.

4.3   Other Non-Hodgkin's lymphomas and lymphoproliferative conditions

4.3.1   *Immunosuppressed patients*

The most conspicuous features of human infections with EBV are their ubiquitous distribution in the adult population and that they are mostly asymptomatic. Equally impressive is the high frequency of EBV-positive lymphomas occurring in patients with severe immunosuppression caused by e.g. inherited immune defects and iatrogenic or HIV-associated immunosuppression. These facts together serve as the strongest evidence for a direct oncogenic effect of EBV infection in B cells, when physiological immune regulation and natural defence barriers have broken down.

4.3.1.1   *Primary immune defects due to genetic abnormalities with EBV-positive lymphoproliferation as one consequence*

Patients with X-linked lymphoproliferative syndrome suffer from a number of serious complications after primary infection with EBV. The genetic basis of this syndrome is described in Section 1 (Skare *et al.*, 1993). Other relevant primary immune defects that may involve EBV-related complications are Wiskott-Aldrich syndrome, common variable immunodeficiency and ataxia telangectasia. These genetic disorders result in different types of defects affecting the immune system, in particular T-cell or NK-cell maturation, but also B-cell maturation in common variable immunodeficiency (Purtilo, 1991; Sander *et al.*, 1992; Nakanishi *et al.*, 1993). Lymphoproliferative disorders in these patients encompass a disease spectrum ranging from polyclonal lymphoid hyperplasia at one end to overtly malignant monoclonal high-grade non-Hodgkin's lymphoma at the other (for a review, see Niedobitek *et al.*, 1997c). In particular, in the Wiskott-Aldrich and X-linked lymphoproliferative syndromes, many of the lymphoproliferations are polyclonal (Falk *et al.*, 1990; Nakanishi *et al.*, 1993). Most cases of non-Hodgkin's lymphoma arising in association with primary immune defects are of the B-cell phenotype and are EBV-positive (Falk *et al.*, 1990; Purtilo, 1991; Sander *et al.*, 1992; Nakanishi *et al.*, 1993). Whereas the pattern of EBV gene expression in most polyclonal lymphoproliferations is similar to that in lymphoblastoid cell lines (type-III latency), monoclonal lesions often display a type-I latency. In some cases, EBNAs have been expressed in the absence of detectable LMP-1. Whether this represents a new form of EBV latency or merely reflects technical problems is currently uncertain. The recent detection of a similar phenotype in EBV-associated smooth-muscle tumours (Lee *et al.*,

1995b) suggests that the EBNA-positive/LMP-1 phenotype indeed represents a novel form of stable EBV latency.

Efforts are being made to clone the *XLP* gene and to characterize its function (see Section 1). This will eventually afford a better understanding of the role of EBV in the development of lymphoproliferation in these patients. In general terms, the frequent occurrence of virus-associated lymphoproliferation in patients with genetic immune defects illustrates the significance of intact immune regulation in maintaining the EBV–host balance. Notably, however, immunosuppression does not appear to be associated with nasopharyngeal carcinoma, an epithelial tumour. The range of lymphoproliferations observed may reflect progression from polyclonal lymphoblastoid cell line-like lesions to monoclonal lymphomas as one of the pathogenetic mechanisms of EBV-positive non-Hodgkin's lymphomas (see discussion on post-transplant lymphoproliferative disorders below). In situations with type II or type III latency in the lymphoproliferative tissue, it is conceivable that EBV contributes to driving cell proliferation or, at least, contributes to extended cell survival.

4.3.1.2 Post-transplant lymphoproliferative disorders

Post-transplantation lymphoproliferative disorders are a major complication in allograft recipients, occurring in 1–20% of patients (Nalesnik & Starzl, 1994). The incidence tends to be lowest for renal transplant recipients and highest for lung transplant patients (Nalesnik & Startzl, 1994; Montone *et al.*, 1996a; Swinnen, 1996), which may reflect more intensive use of immunosuppressive therapy in the latter. The incidence of post-transplant lymphoproliferative disorders in bone-marrow transplant patients is generally low, although it may be up to 24% in recipients of mismatched T cell-depleted marrow (Shapiro *et al.*, 1988). More recently, similar lesions have been reported in patients being treated for rheumatoid disease with methotrexate (Kamel *et al.*, 1993, 1994). The lymphoproliferations in these patients appear to be morphologically and biologically similar to post-transplant lymphoproliferative disorders and are thus not discussed separately in this context. The vast majority of such cases have been shown to be of B-cell origin and to be associated with EBV infection (Locker & Nalesnik, 1989; Swerdlow, 1992; Craig *et al.*, 1993); however, EBV-negative non-Hodgkin's lymphomas of B- and T-cell phenotypes occur occasionally in transplant recipients (Nalesnik & Starzl, 1994). These may represent sporadic lymphomas developing independently of iatrogenic immunosuppression, and are not discussed here.

The general consensus is that post-transplant lymphoproliferative disorders developing in recipients of solid-organ transplants are of host origin, while those occurring in bone-marrow transplant patients are of donor origin (Zutter *et al.*, 1988; Chadburn *et al.*, 1995a; Weissmann *et al.*, 1995); however, exceptions in both directions have been reported. Thus, Zutter *et al.* (1988) demonstrated that two of 12 cases of post-transplant lymphoproliferative disorder arising in bone-marrow recipients were of host origin, in keeping with the reported persistence of EBV-positive B cells of host origin in bone-marrow transplant patients (Gerhartz *et al.*, 1988). More surprisingly perhaps, Larson

et al. (1996) and Mentzer *et al.* (1996) showed recently that post-transplant lymphoproliferative disorders of donor origin may develop in recipients of solid organs.

For reasons as yet unknown, post-transplant lymphoproliferative disorder frequently develops in the gastrointestinal tract and in the transplanted organ (Guettier *et al.*, 1992; Opelz & Henderson, 1993; Palazzo *et al.*, 1993; Mentzer *et al.*, 1996; Randhawa *et al.*, 1996). Histologically, these conditions represent a spectrum ranging from benign poly-clonal, polymorphic lymphoproliferations at one end to frankly malignant monoclonal, monomorphic lymphomas at the other (Swerdlow, 1992; Craig *et al.*, 1993). Typically, B cells in these lymphoproliferations express a broad spectrum of virus-encoded latent proteins, including EBNA-1, EBNA-2 and LMP-1 (Young *et al.*, 1989a; Thomas *et al.*, 1990). This form of EBV latency (type III) is similar to that seen in lymphoblastoid cell lines *in vitro* and, accordingly, these cells usually display a lymphoblastoid-like pattern of cellular gene expression, including lymphocyte activation and adhesion molecules (Thomas *et al.*, 1990). A large proportion of cases of post-transplant lymphoproliferative disorder also show lytic infection, usually in a small subset of cells (Patton *et al.*, 1990; Rea *et al.*, 1994; Montone *et al.*, 1996b). Whether this is sufficient justification for the inclusion of acyclovir in the treatment of these conditions, as often advocated, is un-certain (Zutter *et al.*, 1988; Patton *et al.*, 1990; Davis *et al.*, 1995). It seems likely that entry into the lytic cycle is simply a reflection of the underlying immune defect.

It is generally held that the above-mentioned morphological spectrum of post-trans-plant lymphoproliferative disorder represents an evolutionary process (Craig *et al.*, 1993). Thus, it is believed that the pathogenesis of the condition starts with EBV-driven polyclonal B-cell proliferation; the acquisition of additional genetic changes then leads to the emergence of dominant B-cell clones and eventually to fully developed malignant lymphomas which are morphologically indistinguishable from lymphomas arising in immunocompetent individuals. This scheme is based on the demonstration in studies of immunoglobulin gene rearrangement and analysis of the structure of the viral terminal repeat sequences that some polymorphic cases are polyclonal. Histologically mono-morphic large-cell lymphomas, however, have been shown to be monoclonal using both approaches. In addition, in one group of polymorphic lesions minor clonal components are detectable on the background of polyclonal B-cell proliferations (Locker & Nalesnik, 1989). Furthermore, only monomorphic, monoclonal cases of post-transplant lympho-proliferative disorder contain alterations involving oncogenes or tumour suppressor genes, e.g. *c-myc* translocations or mutations of the N-*ras* and *p53* genes (Delecluse *et al.*,1995b; Knowles *et al.*, 1995). In a recent study, 24 separate lesions in one patient were all clonally distinct (Chadburn *et al.*, 1995b). In a series of 11 patients with recur-rent post-transplant lymphoproliferative disorders, the recurrent lesions were either relapses of the original disease or clonally distinct. In some cases, progression from poly-morphic disease to frankly malignant lymphoma, including Hodgkin's disease and T-cell lymphomas, was observed. Significantly, no primarily monomorphic case recurred as polymorphic disease, further supporting the notion that the disease progresses from early polymorphic polyclonal lesions to monomorphic monoclonal disease (Wu *et al.*, 1996).

This concept of the pathogenesis of post-transplant lymphoproliferative disorder is also supported by the frequent regression of lesions in response to a reduction in immu-

nosuppressive therapy (Starzl *et al.*, 1984). The evidence described above would imply that early polyclonal lesions are more susceptible to this approach than late monoclonal lesions. Indeed, it has been observed that cases that develop less than one year after transplantation have a much higher response rate than those occurring more than one year later (Armitage *et al.*, 1991). Similarly, polyclonal cases appear to respond better than monoclonal lesions; however, this distinction is not absolute, and a trial of reduced immunosuppression has been recommended even in patients with monoclonal disease (Nalesnik *et al.*, 1992). Lucas *et al.* (1996) demonstrated recently that the frequency of EBV-specific CTL precursors in the peripheral blood of bone-marrow recipients returned to normal in nine of 13 patients within six months after transplantation. In this series, one patient with low precursor CTL levels four months after transplantation developed post-transplant lymphoproliferative disorder, further underlining the significance of suppressed T-cell immunity in its pathogenesis. Regression of lymphoproliferative lesions has also been reported in patients with rheumatoid disease after withdrawal of methotrexate therapy, further reflecting the similarity of some of these lesions to post-transplant lymphoproliferative disorder (Kamel *et al.*, 1993; Salloum *et al.*, 1996; Thomason *et al.*, 1996).

Studies to identify transplant patients who are at risk of developing post-transplant lymphoproliferative disorder have defined certain risk factors. Individuals who are EBV-negative before transplantation and undergo seroconversion after transplantation appear to be particularly prone to development of the condition (Ho *et al.*, 1985; Walker *et al.*, 1995). Further risk factors are the duration and severity of immunosuppression, including use of OKT3, CDw52 and anti-LFA1 antibodies (Opelz & Henderson, 1993; Nalesnik & Starzl, 1994; Gerritsen *et al.*, 1996; Walker *et al.*, 1995). More direct approaches to monitoring the risks of individual patients include quantitative PCR assays to measure the viral DNA load in peripheral blood and techniques for counting the number of virus-carrying B cells in blood or allograft biopsy samples (Randhawa *et al.*, 1992; Crompton *et al.*, 1994; Riddler *et al.*, 1994; Savoie *et al.*, 1994; Kenagy *et al.*, 1995).

Several recent studies have indicated a certain heterogeneity of post-transplant lymphoproliferative disorders. Yoshizawa *et al.* (1993) isolated a cell line from a case which resolved in response to reduced immunosuppression. This cell line, SUBL, displayed a pre-B-cell phenotype and genotype and carried a t(2;3)(p11;q27) translocation, indicating that factors other than proliferation of mature B cells can also give rise to the condition. Furthermore, some EBV-positive T-cell lymphomas have been reported (Borisch *et al.*, 1992b; Kumar *et al.*, 1993; Waller *et al.*, 1993) in which the virus was found by in-situ hybridization to be present in only rare neoplastic cells; thus, the role of EBV in the pathogenesis of these tumours remains uncertain (Borisch *et al.*, 1992b). The virus is consistently detected in the rare cases of Hodgkin's disease that occur in the context of transplantation (Garnier *et al.*, 1996), reflecting the detection of EBV in most cases of HIV-associated Hodgkin's disease (Uccini *et al.*, 1990; Boiocchi *et al.*, 1993b; Herndier *et al.*, 1993).

The analysis of EBV gene expression patterns in post-transplant lymphoproliferative disorders shows considerable variability both between and within lesions. Thus, not all cases display type-III latency (Rea *et al.*, 1994; Delecluse *et al.*, 1995a; Oudejans *et al.*,

1995a). Particularly in monomorphic lymphomas, EBV latent protein expression may be restricted to type-I or type-II latency (Rea *et al.*, 1994; Delecluse *et al.*, 1995a). Moreover, even in those cases in which both EBNA-2 and LMP-1 (indicative of type-III latency) can be identified, expression of these proteins is often seen in variable subsets of cells (Thomas *et al.*, 1990; Rea *et al.*, 1994; Delecluse *et al.*, 1995a; Oudejans *et al.*, 1995b). Thus, a relatively small proportion of cells may display type-III latency and others show type-I or type-II latency. In addition, cells in which EBNA-2 is expressed in the absence of LMP-1 have been observed, as also seen in infectious mononucleosis (Oudejans *et al.*, 1995b; Niedobitek *et al.*, 1997b).

4.3.1.3   AIDS

In the general population, the incidence of non-Hodgkin's lymphomas is increasing rapidly, but they still account for only about 4% of all cancers (Parkin *et al.*, 1992). In contrast, non-Hodgkin's lymphomas are very common in HIV-infected individuals, primarily at extranodal sites. Particularly common are primary central nervous system lymphomas, which account for 1.6% of non-Hodgkin's lymphomas in the HIV-negative population (Krogh-Jensen *et al.*, 1994). In a recent Norwegian study, the results of 153 autopsies of AIDS patients were evaluated, representing 73% of all AIDS patients who died. The overall accumulated cancer incidence was 35%. In this series, more cases of primary central nervous system lymphoma (19 cases) than systemic non-Hodgkin's lymphoma (12 cases) were identified (Goplen *et al.*, 1997). In a study in the United States, central nervous system lymphomas constituted only some 20% of all AIDS-related non-Hodgkin's lymphomas (Beral *et al.*, 1991). In larger surveys, 1–4% of AIDS patients had lymphomas (for review, see IARC, 1996). These figures are likely to be underestimates because of the usually low autopsy rates for AIDS patients. Morphologically, AIDS-related non-Hodgkin's lymphomas fall into two broad groups: diffuse large B-cell non-Hodgkin's lymphomas, which often show a prominent immunoblastic component, and Burkitt's lymphoma and Burkitt's-like lymphoma. As lymphomas in AIDS patients have been reviewed recently (IARC, 1996), we focus here only on the EBV-related lymphomas and mechanistic aspects.

(a)   Viral factors

The two histopathological types of AIDS-related non-Hodgkin's lymphoma show striking differences in their relationship to EBV, suggesting different pathogenetic mechanisms. Most diffuse large B-cell non-Hodgkin's lymphomas, particularly those with immunoblastic differentiation, and all AIDS-related central nervous system lymphomas are EBV-positive (MacMahon *et al.*, 1991; for review, see IARC, 1996). Diffuse large B-cell lymphomas have been reported to occur relatively late in AIDS patients (Gaidano & Dalla-Favera, 1995), and more advanced depression of the immune system is a risk factor for their development (Pedersen *et al.*, 1991). The exceedingly rare primary effusion lymphomas fall into the group of diffuse large B-cell non-Hodgkin's lymphomas on morphological grounds; however, they appear to be a special case because of the unusual site of presentation and because the tumour cells often harbour KSHV/HHV8 in addition to EBV (Cesarman *et al.*, 1995). This suggests that the two

viruses cooperate in the pathogenesis of this disease, but the nature of the co-infection is at present uncertain. More recently, primary effusion lymphomas harbouring KSHV/-HHV8 only have also been described (Chang *et al.*, 1994), and thus the role of EBV in the development of this special type of non-Hodgkin's lymphoma remains uncertain.

Regardless of histological type, most AIDS-related non-Hodgkin's lymphomas appear to be monoclonal, both with respect to their antigen receptor genes and to the EBV episomes (Ballerini *et al.*, 1993; Delecluse *et al.*, 1993c; Shibata *et al.*, 1993). This situation differs from that in lymphoproliferative processes occurring in transplant patients (see section 4.3.1.2), which are frequently polyclonal; however, Delecluse *et al.* (1993c) demonstrated that rare cases may be polyclonal and thus resemble post-transplant lymphoproliferative disorder.

(b) Disturbances of immunity as cofactors

As EBV-positive, AIDS-related B-cell lymphomas consistently lack the HIV genome, a direct contribution of HIV to tumorigenesis beyond suppression of the immune system is unlikely (Knowles, 1993; IARC, 1996). Shiramizu *et al.* (1994) reported rare cases of HIV-containing tumours, but this work requires confirmation.

As discussed below, HIV may contribute by inducing severe immunosuppression, leading to a loss of EBV-specific T-cell immunity, or by chronic stimulation of the B-cell system. The relative risk for AIDS-related non-Hodgkin's lymphoma increases with duration of HIV infection (Rabkin *et al.*, 1992) and to a certain extent with immune suppression (Muñoz *et al.*, 1993). T Cell-mediated immunosurveillance is known to play an important part in controlling the proliferation of EBV-infected B lymphocytes in humans (for review, see Rickinson *et al.*, 1992; Masucci & Ernberg, 1994). The spontaneous outgrowth of EBV-positive cells in peripheral blood from AIDS patients is greater than that in immunocompetent individuals (Birx *et al.*, 1986; Ragona *et al.*, 1986; Rinaldo *et al.*, 1986). Similarly, the number of EBV-carrying lymphocytes detected in peripheral lymphoid tissues is greater in HIV-infected individuals than in non-immunocompromised controls (Niedobitek *et al.*, 1992a). This evidence indicates that disturbances in EBV-specific immunity are a pathogenetic factor in the development of some EBV-associated, AIDS-related non-Hodgkin's lymphomas. The location to the central nervous system of one predominant group of AIDS-associated lymphomas may reflect the fact that this site is less accessible for immunosurveillance (MacMahon *et al.*, 1991).

The absence of those viral latent genes that are commonly recognized by cytotoxic T cells from AIDS-associated Burkitt's lymphomas is in keeping with the generally better immune system of these patients in comparison with those with EBV-associated diffuse large-cell non-Hodgkin's lymphomas. It also suggests that the pathogenetic mechanisms leading to the development of these two lymphoma types are different (Knowles, 1996); however, a few AIDS-related cases of non-Hodgkin's lymphoma fall between the two morphological categories, diffuse large B-cell non-Hodgkin's lymphoma and Burkitt's lymphoma, and these cases carry *c-myc* translocations and may thus represent evolution of Burkitt's lymphoma *in vivo* (Delecluse *et al.*, 1993c). This process may be similar to the shift of Burkitt's lymphoma cells to the lymphoblastoid cell line-like phenotype observed *in vitro*. That this may also occur *in vivo* is suggested by the detection of iso-

lated cells expressing LMP-1 and/or EBNA-2 in cases of endemic and AIDS-related Burkitt's lymphoma (Hamilton-Dutoit *et al.*, 1993a; Niedobitek *et al.*, 1995). Such a shift could be expected to coincide with progressive deterioration of the immune system.

(c)   Oncogenes and genetic abnormalities as possible cofactors

There is some evidence that disruption of the cytokine network contributes to the pathogenesis of AIDS-related non-Hodgkin's lymphoma. Thus, it has been shown that B cells from HIV-infected patients, unlike those of normal controls, constitutively express IL-6 and TNFα (for review see Gaidano & Dalla-Favera, 1995). This is of relevance, as IL-6 is known to promote the growth of EBV-positive B cells (Tanner & Tosato, 1992). IL-6 expression has been shown in endothelial cells and macrophages admixed with the neoplastic cells in biopsy samples of AIDS-related non-Hodgkin's lymphomas (Emilie *et al.*, 1992a). Moreover, EBV-positive AIDS-related Burkitt's lymphomas have been shown to express IL-10 (Emilie *et al.*, 1992b; Masood *et al.*, 1995), another cytokine which may directly or indirectly promote the growth of EBV-positive B cells (Vieira *et al.*, 1991; Herbst *et al.*, 1996b). It appears therefore, that IL-6 and IL-10 (and possibly other cytokines) can contribute to the development of AIDS-related non-Hodgkin's lymphoma through paracrine or autocrine regulatory loops.

As discussed previously, AIDS-related Burkitt's lymphomas, both EBV-positive and EBV-negative, have been consistently shown to harbour the characteristic *c-myc* trans-location. These translocations have been detected in a minority of diffuse large B-cell lymphomas and cases with morphological features between large B-cell lymphoma and Burkitt's lymphoma (Ballerini *et al.*, 1993; Delecluse *et al.*, 1993c; Bhatia *et al.*, 1994). Although *p53* point mutations have been detected in 60% of Burkitt's lymphomas in AIDS patients, they were not seen in any other type of non-Hodgkin's lymphoma (for review, see IARC, 1996). Chromosomal translocations in AIDS-related non-Hodgkin's lymphomas frequently involve *bcl-6*, a proto-oncogene that affects B-cell maturation, which maps to 3q27 (Ye *et al.*, 1993). This is the region mainly affected in large-cell lymphomas, rearrangements being seen in 20% of cases. Although the rearrangements of *bcl-6* and *c-myc* occur in approximately 20% of cases of diffuse large B-cell non-Hodgkin's lymphoma, they have been reported to be mutually exclusive (Gaidano *et al.*, 1994).

Point mutations were detected in N-*ras* and K-*ras* in four of 27 AIDS-related non-Hodgkin's lymphomas investigated, but not in non-Hodgkin's lymphomas in immuno-competent hosts (Ballerini *et al.*, 1993).

Deletion of the long arm of chromosome 6 at band q27 was described in five of 13 large-cell lymphomas, some of which were EBV-positive (Pastore *et al.*, 1996). This rearrangement is seen in a wide range of non-Hodgkin's lymphomas in immuno-competent hosts (Gaidano *et al.*, 1992). Genetic abnormalities in chromosome 1q have been reported in AIDS-related cases of Burkitt's lymphoma by several groups, similar to those seen in endemic cases (Bernheim & Berger, 1988; Polito *et al.*, 1995), site 1q 21-25 being frequently involved.

Thus, AIDS-related non-Hodgkin's lymphomas, whether EBV-associated or not, frequently show genetic changes implicated in the pathogenesis of malignant lymphomas, including c-myc translocations, p53, N-ras and K-ras point mutations and deletions in the long arm of chromosome 6 (Gaidano & Dalla-Favera, 1995).

(d) Pathogenesis of EBV-associated, AIDS-related non-Hodgkin's lymphoma: A scenario

AIDS-related non-Hodgkin's lymphomas thus appear to fall into two broad categories with distinct pathogenetic pathways. Diffuse large B-cell lymphomas occur in severely immunocompromised individuals, are mostly EBV-positive and express type-II or -III latency patterns. While most of these cases are monoclonal and morphologically malignant, rare EBV-positive polyclonal lymphoproliferations have been described. It appears, therefore, that the pathogenesis of these lesions is similar to that of post-transplant lymphoproliferative disorder (Gaidano & Dalla-Favera, 1995), with an initial EBV-driven proliferation of B cells leading eventually to the outgrowth of fully malignant lymphomas. This process would depend on the acquisition of additional genetic change, e.g. c-myc or bcl-6 rearrangements (Gaidano & Dalla-Favera, 1995).

The clinical, molecular and viral features of AIDS-related Burkitt's lymphoma suggest a pathogenesis similar to that of sporadic Burkitt's lymphoma. The comparatively high incidence of these lymphomas in AIDS patients points to the contribution of an AIDS-related factor independent of HIV-mediated immunosuppression. As these lymphomas have been shown to be HIV-negative (Knowles, 1993), a direct role of HIV is unlikely. The primary event in AIDS-related Burkitt's lymphoma appears to be chronic stimulation of the immune system, leading to polyclonal B-cell hyperplasia; this process may well be driven initially by HIV infection. The relatively low rate of EBV infection in these lymphomas suggests that the function of the virus can be substituted by other, as yet unidentified, factors.

4.3.2   T-Cell lymphomas

The relatively frequent detection of EBV in T-cell lymphomas does not fit easily into the well-established B-cell lymphotropism of the virus in vitro. As noted earlier (see Section 1), a subset of T cells has been shown to express CD21 or a related molecule that could act as the receptor for EBV. Interpretation of the detection of EBV in T-cell non-Hodgkin's lymphomas and an assessment of the role of the virus in the pathogenesis of T-cell lymphomas are complicated by two factors. Firstly, if EBV infection of certain T cells in vitro leads to a predominantly lytic infection, EBV infection of T cells may be accidental rather than part of the viral strategy to establish persistent infection. Such accidental infection of a cell type not adapted to latent EBV infection may contribute to the development of EBV-associated T-cell lymphomas. Secondly, in many cases, the virus is detected in only a small proportion of tumour cells (Anagnostopoulos et al., 1996). Particularly in T-cell lymphomas, this problem is compounded by the polymorphism of the tumour cells. Thus, it may be difficult to decide if an individual cell is a tumour cell or a reactive lymphoid cell. Furthermore, the identification of EBV in neoplastic cells usually rests on the detection of the EBERs, which may not be expressed

even in the presence of EBV. Nevertheless, there seems to be good evidence to suggest that the reported detection of EBV in a fraction of cells in T-cell lymphomas reflects the actual situation (Asada *et al.*, 1994; Imai *et al.*, 1996).

The question of the significance of the virus in the pathogenesis of these lymphomas thus arises. Although the virus is present at the onset of the neoplastic process, it may subsequently be lost from the tumour cells. While there is now some evidence to suggest that this may happen *in vitro* (Takada *et al.*, 1995), it has not yet been shown to occur *in vivo*. The alternative scenario would be secondary infection of established neoplastic T cells with the virus. While this would exclude a contribution of the virus to the initial neoplastic process, it would be compatible with a contribution to the disease process, similar to the role of late *p53* gene mutations in the progression of follicular lymphomas. The frequent expression of the LMP-1 protein of EBV in T-cell lymphomas (d'Amore *et al.*, 1996) would seem to argue in favour of such a role.

EBV is often monoclonal in T-cell lymphomas containing a subset of virus-carrying tumour cells, which argues against simple superinfection of an established tumour (Ott *et al.*, 1992). Studies of EBV-associated T-cell lymphomas at primary presentation and at relapse could be informative.

In addition to the peripheral T-cell lymphomas occurring in western populations, in which subpopulations of the tumour cells often carry the virus, there are clearly some T-cell non-Hodgkin's lymphomas in which the presence of monoclonal EBV genomes is a defining characteristic of the neoplastic population. This group is exemplified by angio-centric sinonasal T-cell lymphomas, which occur predominantly in Asian populations. The role of the virus in the pathogenesis of these tumours is uncertain; however, the clonal nature of the EBV genome and its detection in virtually all tumour cells suggests a role of EBV early in tumorigenesis. While mechanisms have not been identified in lymphomatous granulomatosis, it has been shown that the EBV-infected B cells express LMP-1 (Takeshita *et al.*, 1996).

4.4 Hodgkin's disease

Over the last decade, evidence has accumulated implicating EBV in the pathogenesis of Hodgkin's disease. Up to 50% of cases in western countries (Weiss *et al.*, 1989a; Herbst *et al.*, 1991a; Armstrong *et al.*, 1992) and up to 100% in some other populations (Weinreb *et al.*, 1996b) carry the virus in the presumably neoplastic HRS cells. Sero-epidemiological studies have shown elevated antibody titres to EBV antigens in Hodgkin's disease patients both at the time of presentation and some years before the onset of the disease (Mueller *et al.*, 1989; Mueller, 1997). Furthermore, patients with infectious mononucleosis have a fourfold increase in risk for developing Hodgkin's disease (Muñoz *et al.*, 1978). More direct evidence linking EBV with Hodgkin's disease has accumulated (see also section 1.2.2). Thus, EBV DNA has been detected in tumour biopsies and has been localized to malignant HRS cells by in-situ hybridization (Weiss *et al.*, 1987; Anagnostopoulos *et al.*, 1989; Weiss *et al.*, 1989a). Significantly, it has been shown by EBER in-situ hybridization that the virus is present in virtually all tumour cells in EBV-associated Hodgkin's disease (Wu *et al.*, 1990; Herbst *et al.*, 1992). This finding

is consistent with the detection of monoclonal EBV episomes in cases of Hodgkin's disease by Southern blot hybridization and indicates that the EBV infection took place before initiation of clonal cellular proliferation (Weiss *et al.*, 1987; Anagnostopoulos *et al.*, 1989). In agreement with this idea, it has been shown that patients with multifocal Hodgkin's disease have the virus in all affected tissues and that the virus persists in recurrent disease (Coates *et al.*, 1991a; Boiocchi *et al.*, 1993a; Brousset *et al.*, 1994; Vasef *et al.*, 1995).

Further evidence suggesting a role for EBV in the pathogenesis of Hodgkin's disease comes from the analysis of EBV gene expression in HRS cells. Transcriptional studies by RT-PCR have shown that *EBNA-1* expression in Hodgkin's disease tissues is driven exclusively by the F promoter; *LMP-1* and usually *LMP-2A* and *LMP-2B* are also expressed, in addition to *EBER*s (Deacon *et al.*, 1993). Immunohistochemical studies have now confirmed that EBNA-1, LMP-1 and LMP-2A are indeed expressed by the HRS cells in EBV-associated Hodgkin's disease, while EBNA-2 is consistently not detectable (Herbst *et al.*, 1991a; Pallesen *et al.*, 1991a; Delsol *et al.*, 1992; Grässer *et al.*, 1994; Niedobitek *et al.*, 1997a). This pattern of EBV latent gene expression is consistent with type-II latency. These studies indicate that the viral genome is actively expressed in HRS cells and thus reveal that EBV is not merely a silent passenger in these cells.

The main function of *EBNA-1* is maintenance of the viral episome (Yates *et al.*, 1984); however, a recent study of *EBNA-1*-transgenic mice suggests that this gene may also contribute to the development of lymphoid tumours (Wilson *et al.*, 1996a). Whether *EBNA-1* expression in HRS cells contributes to the pathogenesis of Hodgkin's disease remains uncertain.

LMP-2A expression is thought to block the induction of the lytic viral cycle after cross-linking of the B-cell receptor complex, by acting as a dominant negative inhibitor of Src and Syk kinases (Longnecker & Miller, 1996). In line with this scheme and with the frequent detection of LMP-2A in HRS cells (Niedobitek *et al.*, 1997c), it has been shown that EBV lytic cycle proteins are expressed only infrequently in HRS cells (Pallesen *et al.*, 1991c; Brousset *et al.*, 1993). Several studies suggest that *LMP-2A* and *LMP-2B* are not required for EBV-induced B-cell transformation (Longnecker *et al.*, 1992, 1993a,b); however, recent work by Brielmeier *et al.* (1996) indicates that *LMP-2A* enhances the efficiency of transformation and that this effect is not dependent on prevention of entry into the lytic cycle. Thus, *LMP-2A* expression may contribute to the malignant phenotype of HRS cells in EBV-associated Hodgkin's disease.

Of particular relevance is the detection of LMP-1 in HRS cells in virtually all cases of virus-associated Hodgkin's disease (Herbst *et al.*, 1991a; Pallesen *et al.*, 1991a; Delsol *et al.*, 1992) and the ability of LMP-1 to induce expression of activation markers such as CD23, CD30 and CD70 (Wang *et al.*, 1988b, 1990a; Isaacson *et al.*, 1992; Hamilton-Dutoit *et al.*, 1993a); however, these antigens are strongly expressed in HRS cells of both EBV-positive and EBV-negative cases.

There is growing evidence to suggest that EBV infection modulates the phenotype of Hodgkin's disease. Thus, EBV is preferentially associated with the development of mixed cellularity Hodgkin's disease. Moreover, the presence of EBV in HRS cells has

been shown to correlate with increased expression of lymphocyte activation antigens and decreased expression of the CD20 B-cell antigen (Bai et al., 1994). Of particular relevance is the association of EBV infection with the expression of T_h2 cytokines such as IL-10 and IL-6 in HRS cells (Herbst et al., 1996b; Klein et al., 1996). Thus, although definite proof of an etiological role of EBV in the pathogenesis of Hodgkin's disease has yet to be attained, the available evidence strongly implicates the virus as a cofactor in the pathogenesis and morphogenesis of a significant proportion of cases.

It has been observed that cases of Hodgkin's disease arising in the setting of iatrogenic or HIV-induced immunosuppression are almost invariably EBV-associated. Moreover, regression of EBV-positive cases of Hodgkin's disease has been reported after restoration of the immune system (Berger & Delecluse, 1993), providing further evidence for an etiological role of EBV in the pathogenesis of Hodgkin's disease.

4.5 Nasopharyngeal carcinoma

4.5.1 EBV infection

4.5.1.1 Molecular and biochemical studies

EBV-positive nasopharyngeal carcinoma cells cannot yet be cultivated in vitro, and only a small proportion of tumour samples can be propagated in nude mice. Therefore, EBV infection in nasopharyngeal carcinoma has been characterized primarily by analysis of viral nucleic acids and proteins in samples obtained at biopsy and tumours passaged in nude mice (Raab-Traub et al., 1983; Busson et al., 1988). EBV episomes have been detected in nasopharyngeal carcinoma samples by electron microscopy (Kaschka-Dierich et al., 1976) and by restriction enzyme analyses based on identification of the ends of the EBV genome (Raab-Traub & Flynn, 1986). Clonal EBV episomes are detected in such samples (Raab-Traub & Flynn, 1986), indicating that every copy of the viral genome within each cell is identical and the EBV episomes in every cell within the tumour are identical. The detection of homogeneous EBV molecules indicates that the tumour cells are a clonal expansion of a single cell that was infected with EBV.

In addition, in some tumour samples, particularly from endemic areas, faint ladder arrays of fragments representing linear genomes are also detected. This suggests that in occasional cells within the tumour the virus may be reactivated from latent infection. It is likely that this sporadic replication is the source of the antigenic stimulus that provokes the elevated antibody response to viral replicative antigens.

As episomal EBV DNA is present in multiple copies in nasopharyngeal carcinoma, it is difficult to determine whether EBV is also integrated; however, evidence of EBV integration was detected by pulse field analysis in four of 17 samples of nasopharyngeal carcinoma (Kripalani-Joshi & Law, 1994). Although integration may promote changes in cellular or viral expression that could contribute to tumour development, it is probably not a necessary event for development of this tumour.

4.5.1.2 *EBV expression*

EBV expression in nasopharyngeal carcinoma is different in several striking respects from that detected in transformed lymphocytes. The first studies of viral expression in samples of nasopharyngeal carcinomas revealed that sequences from the *Eco*RI-*Dhet* and the *Bam*HI K fragments were consistently transcribed (Raab-Traub *et al.*, 1983). These sequences were subsequently shown to encode the LMPs and EBNA-1, respectively, Surprisingly, the sequences that encode EBNA-2, a viral protein essential for transformation of lymphocytes, are not transcribed in nasopharyngeal carcinoma.

The pattern of gene expression suggested by transcriptional studies has for the most part been confirmed by the identification of specific viral proteins with monoclonal antibodies. LMP-1 and EBNA-1 have been detected, while EBNA-2, -3A, -3B, -3C and -LP are usually not found (Fåhraeus *et al.*, 1988; Young *et al.*, 1988). LMP-1 is not always detected in all cells or in all tumours on immunoblots, and EBNA-2 has not been identified in tumour samples, possibly due to a lack of suitable reagents (Stewart & Arrand, 1993). The BZLF1 protein has been detected in a few cells in some nasopharyngeal carcinomas, and spliced *BZLF1* mRNA and other mRNAs encoding lytic cytoproteins can also be detected by RT-PCR (Cochet *et al.*, 1993; Martel-Renoir *et al.*, 1995).

As the mRNAs that encode EBNAs all initiate at the W or C promoter in lymphocytes, it was of interest to determine the structure of the *EBNA-1* mRNA in nasopharyngeal carcinomas. Intriguingly, it was shown to initiate from sequences within *Bam*HI Q at a previously unidentified promoter, Q (Smith *et al.*, 1993; Tsai *et al.*, 1995). The Q promoter is complex and is apparently both autoregulated and governed by interferon regulatory factors (Sample *et al.*, 1992; Sung *et al.*, 1994).

In nasopharyngeal carcinoma, two different mRNAs encode LMP-1: the well-characterized, 2.8-kb *LMP-1* mRNA and a second, 3.7-kb mRNA (Gilligan *et al.*, 1990a). The 3.7-kb mRNA initiates from a promoter within the terminal repeat unit, which is transactivated *in vitro* by the SP1 transcription factor (Sadler & Raab-Traub, 1995b). Consistent transcription of *LMP-2* has been detected in nasopharyngeal carcinomas by northern blot analysis and RT-PCR (Busson *et al.*, 1992a). One study suggested that the *LMP-2B* or *TP2* transcript is preferentially expressed in nasopharyngeal carcinoma in comparison with matched B-cell lines (Smith & Griffin, 1991). Antibodies to LMP-2 were found highly specifically in patients with nasopharyngeal carcinoma (Frech *et al.*, 1993; Lennette *et al.*, 1995), suggesting that this protein is expressed in the tumour *in vivo*. In nasopharyngeal carcinoma, *EBNA-2*, *-3A*, *-3B*, *-3C* and *-LP* are not expressed, yet the promoters for *LMP-1* and *LMP-2* are apparently active, as the genes are transcribed. It is presently unclear how expression of these genes is regulated in the absence of the EBNA-2 and EBNA-3 proteins. Several studies have shown that methylation of the viral genome is an important regulatory element. The viral genome in nasopharyngeal carcinoma cells is extensively methylated, with the exception of *oriP* and regulatory elements for the *LMP-1* and *LMP-2* promoter (Ernberg *et al.*, 1989; Minarovits, 1991, 1994b; Hu *et al.*, 1991b). It is particularly striking that although the coding sequences for *LMP-1* are methylated, the promoter region is methylated only in those tumours in which the LMP-1 protein is not detected.

In addition to these RNAs, which are known to encode protein, a family of intricately spliced mRNAs was originally identified in samples of nasopharyngeal carcinomas (Hitt *et al.*, 1989; Gilligan *et al.*, 1990b, 1991). Sequence analysis of cDNA revealed that the RNAs are 3' co-terminal but differentially spliced (Smith *et al.*, 1993; Sadler & Raab-Traub, 1995a). The various RNAs contain different exons, forming novel ORFs at least one of which has been shown to encode a protein (Gilligan *et al.*, 1991; Fries *et al.*, 1997) that has been detected in most nasopharyngeal carcinoma samples. Although these RNAs are most abundant in nasopharyngeal carcinoma, they have also been identified in transformed lymphocytes and in Burkitt's lymphoma (Brooks *et al.*, 1993a).

The most abundant RNAs in EBV-infected cells are small nuclear RNAs transcribed by RNA polymerase III (Lerner *et al.*, 1981; Arrand & Rymo, 1982). These *EBERs* are present at approximately 10^6 copies per cell but are not necessary for lymphocyte transformation (Swaminathan *et al.*, 1991). They are, however, expressed in all of the malignancies associated with EBV and presumably contribute in some way to the maintenance of latency *in vivo* (Wu *et al.*, 1991). Interestingly, expression of the *EBERs* seems to be down-regulated during differentiation. Thus, samples of nasopharyngeal carcinoma that show differing degrees of differentiation lack EBER expression in differentiated areas (Pathmanathan *et al.*, 1995a). Extensive screening showed the presence of EBERs in 4 of 5 (80%) subtype 1, 71 of 73 (97%) subtype 2 and 71 of 73 (97%) subtype 3 tumours. In the subtype 1, EBER expression was largely confined to the basal area and was not detected in regions of differentiation (Tsai *et al.*, 1996b). The EBERs are also not detected in the permissive EBV infection, hairy leukoplakia (Gilligan *et al.*, 1990b).

These findings indicate that nasopharyngeal carcinoma represents a latent infection with EBV involving consistent expression of specific viral genes in a pattern distinct from that found in transformed B lymphocytes. The properties of the viral proteins expressed in nasopharyngeal carcinoma are reviewed in sections 1 and 4.1 This unique state of latency is also found in several other malignancies linked to EBV, including Hodgkin's disease and T-cell lymphoma, and has been called type II latency (Rickinson & Kieff, 1996; see Section 1).

4.5.1.3 *Phenotype and cellular gene expression*

The presence of an intense lymphoid stroma is a characteristic feature of undifferentiated carcinomas of the nasopharyngeal type; the functional role of this stroma has been a matter of debate. Recent studies have suggested that in undifferentiated carcinomas the stroma is required to support tumour cell growth (Busson *et al.*, 1987; Agathanggelou *et al.*, 1995). The difficulty in establishing nasopharyngeal carcinoma xenografts in nude mice may reflect loss of the infiltrating stroma (Busson *et al.*, 1987).

Expression of many markers, including potential factor receptors, usually expressed in B cells has been described in primary nasopharyngeal carcinoma samples. These include CD23, CD24, Ia antigen and Cdw70 (Ebbers *et al.*, 1985; Billaud *et al.*, 1989; Rousselet & Tursz, 1992; Niedobitek *et al.*, 1992c; Karran *et al.*, 1995). The expression of adhesion molecules is also altered, with elevated expression of ICAM-1 (CD54) and reduced expression of LFA3 (CD58) in comparison with EBV-immortalized B cells

(Busson *et al.*, 1992b). These effects are probably due to expression of *LMP-1* in nasopharyngeal carcinoma cells.

Interestingly, clinical analysis of nasopharyngeal carcinomas with and without LMP-1 expression suggested that the LMP-positive tumours grew more rapidly but that the LMP-1-negative tumours were more likely to recur and had an increased tendency to metastasize (Hu *et al.*, 1995). This difference may be due to the fact that B7 (CD80/CD87) is expressed in LMP-1-positive but not in LMP-1-negative tumours (Agathanggelou *et al.*, 1995).

4.5.1.4 *EBV infection and transformation of epithelial cells* in vitro

Although EBV readily infects and transforms B lymphocytes, human epithelial cells cannot easily be infected or transformed; however, monkey epithelial cells were transformed by transfer of specific EBV DNA fragments cloned into cosmid vectors (Griffin & Karran, 1984). The fragments that had transforming ability included the *Bam*HI A region of the genome which has since been shown to be expressed in nasopharyngeal carcinomas (Hitt *et al.*, 1989; Gilligan *et al.*, 1990b). Some attempts to infect human epithelial cells directly have resulted in morphological transformation but not permanent transformation or EBNA expression (Desgranges & de Thé, 1977; Huang *et al.*, 1978a). In another study of epithelial cells infected *in vitro*, evidence of EBV replication was detected only in wild-type strains obtained directly from throat washings, suggesting distinct biological properties of some strains (Sixbey *et al.*, 1983).

The difficulty of infecting epithelial cells may be due to differences in expression of the EBV receptor, CD21 or CR2. Evidence for expression of the receptor is conflicting, depending on the monoclonal antibody used and the source of epithelium (Young *et al.*, 1989c; Sixbey *et al.*, 1987). Transfer of CD21 into an epithelial cell line facilitated infection, and a high level of infected cells was detected early after infection with the Akata strain of EBV (Li *et al.*, 1992). The genome was rapidly lost from these cells, and some cells spontaneously entered the replicative cycle; however, cell clones could be established that retained the EBV genome in episomal form and expressed *EBNA-1* and *LMP-1* (Knox *et al.*, 1996). The stably infected clones seemed to be impaired in their ability to differentiate or to allow viral replication. Clones that lacked EBV were also impaired in differentiation, suggesting that impairment in differentiation influences the ability of EBV to establish a latent, transforming infection.

The mode of viral entry may also influence the outcome of infection. It has been shown that secretory IgA facilitates the entry of EBV into epithelial cells (Sixbey & Yao, 1992). The cellular organization also modulates EBV infection, such that EBV is transcytosed by EBV-specific IgA directly through the epithelium in polarized cultures, but EBV replicative gene products are detected in unpolarized cultures (Gan *et al.*, 1997).

In some epithelial cell lines, LMP-1 has been reported to inhibit differentiation and cause morphological transformation, with decreased cytokeratin expression (Dawson *et al.*, 1990; Fåhraeus *et al.*, 1990a; Niedobitek *et al.*, 1992d). Decreased expression of E-cadherin and increased invasive ability has also been described in epithelial lines expressing LMP-1 (Fåhraeus *et al.*, 1992). In transgenic mice in which LMP-1 was

expressed in skin, epithelial hyperplasia and altered expression of keratin 6 were observed (Wilson et al., 1990).

LMP-1 expression induces expression of the epidermal growth factor receptor, specifically in epithelial cells, and elevated levels of epidermal growth factor receptor are also detected in nasopharyngeal carcinoma (Zheng et al., 1994c; Miller et al., 1995b). As discussed in section 4.1, two domains have been identified in the carboxy terminus of LMP-1 that can activate NFκB (Huen et al., 1995). A recent study indicated that the TRAF interacting domain is responsible for induction of expression of the epidermal growth factor receptor (Miller et al., 1997). This suggests that LMP-1 activates two distinct signalling pathways: one that activates NFκB and a second pathway mediated by TRAF activation that induces expression of genes like EGFR and also activates NFκB.

Several EBV replicative proteins have been shown to have different effects in epithelial cells and lymphoid cells or to have transforming properties in rodent cell lines. Although only BZLF1 can disrupt latency in lymphoid cell lines, in infected epithelial cell lines such as NPC-KT and D98/HR1, the immediate early gene BRFL1 can also disrupt latency (Zalani et al., 1996). In addition, BZLF1 has been shown to interact with p53 (Zhang et al., 1994b), the p65 component of NFκB (Gutsch et al., 1994), and induce expression of TGFβ. The EBV early gene, BHRF1, is homologous to the bcl2 gene and can block apoptosis induced by various agents (Henderson et al., 1993). BHRF1 can also complement transformation by the adenovirus E1A gene (Theodorakis et al., 1996). Another early gene encoded by the BARF1 ORF can transform rodent fibroblasts and induce tumorigenicity in the EBV-negative Louckes Burkitt's lymphoma cell line (Wei et al., 1994). As expression of EBV replicative gene products occurs in only a few percent of nasopharyngeal carcinoma cells in vivo, it is unlikely, however, that they contribute to malignant growth.

4.5.1.5   Detection of EBV infection in normal, premalignant and malignant naso-pharyngeal tissues

The site and state of infection in normal nasopharynx and in populations at high risk for developing nasopharyngeal carcinoma have been investigated in several studies. In patients with infectious mononucleosis, EBV DNA and mRNA encoding EA were detected in sloughed epithelial cells (Lemon et al., 1978; Sixbey et al., 1984). This suggested that the nasopharyngeal epithelial cells were the source of the infectious virus that is detected in saliva during infectious mononucleosis. Evidence of EBV replication has also been detected in epithelial cells in parotid tissue, where high copy numbers of EBV genomes were detected by in-situ hybridization (Wolf et al., 1984). A recent study also showed evidence of EBV replication in epithelial cells adjacent to an EBV-positive T-cell lymphoma (Wen et al., 1997). The epithelial cells had high copy numbers of EBV DNA and were BZLF1-positive but EBER-negative, while the malignant lymphocytes were positive for EBER and LMP-1 expression. In contrast, studies of normal naso-pharyngeal mucosa have shown evidence of EBV only in lymphocytes in which EBER-positive and BZLF1-positive cells were occasionally detected (Tao et al., 1995). EBV-infected lymphocytes were detected in nasal polyps, and some were positive for BZLF1

expression (Tao *et al.*, 1996). Similarly, Karajannis *et al.* (1997) detected isolated EBV-positive B cells but no EBV-positive epithelial cells in throat washings from patients with infectious mononucleosis, suggesting that lymphocytes are the source of virus secreted into the oropharynx in reactivated infection.

Comprehensive screening of a large Chinese population resulted in the identification of 1267 individuals with IgA to VCA. Of the 203 from whom a biopsy sample was taken, 46 had early nasopharyngeal carcinoma and an additional 12 had detectable nasopharyngeal carcinoma within 12 months. EBV was detected by Southern blot hybridization in 14 samples from individuals without detectable nasopharyngeal carcinoma (Desgranges *et al.*, 1982), but it was not determined whether the tissues were normal or whether the patients had occult nasopharyngeal carcinoma.

Attempts to culture transformed or EBV-infected cells taken at biopsy resulted in the establishment of EBV-positive lymphoid lines from nasopharyngeal tissues from patients with and without nasopharyngeal carcinoma and from adenoidal tissue from other patients; however, epithelial cell lines were not established (de Thé *et al.*, 1970; Takimoto *et al.*, 1989). Further, attempts led to the development of cell lines in which EBV genomes were initially detected but were lost over time (Gu *et al.*, 1983; Lin *et al.*, 1993b). It is unclear whether these cell lines represent the tumour cells.

EBV markers for EBNA or latent or replicative genes were not detected in samples of normal tissue from patients without histological evidence of nasopharyngeal carcinoma, and were found only in samples with nests of tumour cells (Huang *et al.*, 1978b; Sam *et al.*, 1993). Subsequent screening showed EBV in all biopsy samples of nasopharyngeal carcinoma, but only the genome was detected in a subset of cells from carcinoma *in situ* (Yeung *et al.*, 1993). Samples from carcinomas *in situ* with microinvasion had a higher proportion of EBV-positive cells. An extensive screening of 5326 biopsy samples from the ear-nose-and-throat clinic at the University of Malaya resulted in the detection of nasopharyngeal carcinoma in 1744 samples; 56 samples had carcinoma *in situ* and invasive carcinoma, and only 11 samples showed dysplasia or carcinoma *in situ* without adjacent invasive carcinoma. In these samples, EBER and LMP-1 were detected in all cells, and analysis of the EBV termini revealed clonal EBV (Pathmanathan *et al.*, 1995a,b).

Some studies have suggested that EBV is detected only in discrete areas within a nasopharyngeal carcinoma, while in other studies homogeneous infection is detected throughout the samples (Wu *et al.*, 1991; Lin *et al.*, 1994; Pathmanathan *et al.*, 1995a,b). This variation may reflect differences in the method of detection or the state of infection. EBV DNA or EBER expression has been looked for as an indicator of the presence of EBV in many studies, but EBV DNA may not be detected in cells with a very low copy number and EBER expression may vary, as EBERs are not expressed in the permissive infection, hairy leukoplakia, or within areas of differentiation in tumours of mixed histological type (Gilligan *et al.*, 1990a; Pathmanathan *et al.*, 1995a,b). [The Working Group noted that the role of EBV in primary infection of epithelial cells is controversial.]

Taken together, these studies indicate that EBV infection of epithelial cells is rare. It may occur during primary infection but, in normal asymptomatic infection, EBV is

harboured in lymphoid cells. The rare event that leads to the development of nasopharyngeal carcinoma may enable establishment of a latent EBV infection in epithelium. The changes that predispose the cells to this event may have already induced changes in growth properties, which were originally suggested by Lenoir and de Thé (1978). The impaired differentiation observed by Knox et al. (1996) may have a correlate in vivo in the dysplasia described by Yeung et al. (1993). Furthermore, the study of Pathmanathan et al. (1995b) suggests that the combination of EBV oncogene expression and pre-existing cellular changes results in the rapid proliferation of an infected epithelial cell, which rapidly develops into malignant, invasive carcinoma (Raab-Traub, 1992a,b).

4.5.1.6 Strain variation

Many of the cancers associated with EBV, and particularly nasopharyngeal carcinoma, occur in distinct populations with endemic patterns of incidence. As a possible contributing factor is the prevalence of specific variants of EBV in distinct geographical locations with unique biological or pathological properties, strain variation has been studied continuously (Bornkamm et al., 1980; Raab-Traub et al., 1980). Most biological studies of EBV have involved virus obtained from Burkitt's lymphomas (HR1, W91, Akata, AG876) or from a patient with infectious mononucleosis (B95-8); one viral strain was obtained from a nasopharyngeal carcinoma biopsy sample that was co-cultured with lymphocytes (Crawford et al., 1979). Viral replication could be induced in some of the lymphoid cell lines, and these studies revealed that virus from nasopharyngeal carcinomas is both replication and transformation competent. This isolate, the MABA strain, was further compared by restriction enzyme analysis and shown to be largely identical to other EBV strains from cases of infectious mononucleosis and Burkitt's lymphoma (Bornkamm et al., 1980; Polack et al., 1984b). EBV DNA in nasopharyngeal carcinoma biopsy samples was also shown to be similar to that in laboratory strains (Raab-Traub et al., 1987). A second nasopharyngeal carcinoma strain was obtained by direct fusion of nasopharyngeal carcinoma tumour cells with a human adenoidal epithelial cell line (Takimoto et al., 1985). This cell line, NPC-KT, produces virus that can transform lymphocytes and can spread during cell-to-cell fusion (Sato et al., 1989; Yoshizaki et al., 1994).

Specific genetic variations in the viral genome have been looked for by restriction enzyme polymorphism, and specific strains were found to be prevalent in different populations. Lung et al. (1990) and Lung and Chang (1992) first described a common Chinese variant marked by loss of the BamHI site between the W1' and I1' fragments (designated C variant), frequently with an additional BamHI site in the F fragment (f variant). This Cf variant was detected in 86% of nasopharyngeal carcinoma biopsy samples from southern China, while healthy subjects usually had the C variation without the f polymorphism. The f variant was more frequently detected in the nasopharyngeal carcinoma biopsy samples than in peripheral blood lymphocytes or throat washings (Lung et al., 1992). The C variation was also prevalent in Japanese populations; however, this variant lacked the f polymorphism (Tamura et al., 1993). A polymorphism at an XhoI site was also detected in most Chinese samples (Hu et al., 1991a; Miller et al., 1994b).

Abdel-Hamid *et al.* (1992) performed similar analyses on epithelial and lymphoid malignancies from endemic and non-endemic regions, including Chinese, Mediterranean and American populations, determining the restriction enzyme polymorphisms and EBV type. The prevalent strain in Southeast Asia was a variant of EBV-1, which had the *Bam*HI and *Xho*I polymorphisms described above. In nasopharyngeal carcinomas from areas of middle or low incidence, a distinctive EBV-1 subtype, originally identified in the nasopharyngeal carcinoma-derived MABA strain, was found in approximately 50% of the tumours, and an additional 25% had the Chinese polymorphisms. In the Alaskan Inuit population, a new variant of EBV-2, with the polymorphisms from the right end of the genome that distinguish the Chinese EBV-1 strain, was identified in nasopharyngeal carcinomas and carcinomas of the parotid gland. In contrast, the standard EBV-1 genome, lacking the above-mentioned polymorphisms, was detected in lymphomas from central Africa (Burkitt's lymphoma), Egypt and the United States.

The presence of specific restriction enzyme polymorphisms suggested that EBV strains might have consistent sequence variation. As EBV *LMP-1* is essential for transformation and is considered to be the EBV oncogene, many studies have focused on strain variation in the *LMP-1* gene. The sequence of a Chinese isolate, Cao, revealed several amino-acid changes and deletion of 10 amino acids within LMP-1 (Hu *et al.*, 1991a). The sequence of 50 Taiwanese isolates revealed that a particular strain was prevalent in Taiwan and had greater transforming ability in BALB/c 3T3 cells than LMP-1 from B95-8 (Chen *et al.*, 1992a). A similar comparison of the transforming ability of B95-8 and Cao in the RHEK epithelial cell line showed that both increased proliferation, with higher colony-forming efficiency and decreased differentiation, marked by decreased involucrin expression. The B95-8 and Cao transformants differed morphologically, and only the Cao transformants induced tumours in nude mice (Hu *et al.*, 1993; Zheng *et al.*, 1994c).

Sample *et al.* (1994) found that the *LMP-1* sequence in the prototype EBV-1 and EBV-2 strains were nearly identical. A comprehensive analysis of *LMP-1* sequence variation in nasopharyngeal carcinoma isolates from several geographical regions revealed that consistent base-pair changes, including the 10-amino acid deletion, marked a Chinese EBV-1 strain. In contrast, the Alaskan strain had many of the same base-pair changes but was undeleted and represented EBV-2. This study also showed that the number of small-repeat elements within *LMP-1* can vary, such that viral replication could produce progeny with differing numbers of the small *LMP-1* repeat (Miller *et al.*, 1994a). In a comparison of the EBV strain in nasopharyngeal carcinoma, T-cell lymphoma, normal nasopharynx and throat washings in Taiwan, the Chinese prototype strain was detected in 48/48 nasopharyngeal carcinoma samples, 23/25 samples of normal nasopharynx and 37/44 throat washings, indicating the widespread prevalence of this strain in Taiwan (Chang *et al.*, 1995).

Many studies have focused on the small deletion in *LMP-1*. The effect of the deletion on BALB/c 3T3 cell transformation was analysed by producing chimaeric proteins (Li *et al.*, 1996). It was found that the enhanced transforming ability of the Chinese strain in BALB/c 3T3 cells was transferred with the carboxy terminus that included the 10-amino acid deletion. Deletion of the 10 amino acids in B95-8 LMP-1 resulted in the ability to

induce transformation in BALB/c 3T3 cells and tumorigenicity, while insertion of the 10 amino acids into the Chinese strain eliminated transformation and tumorigenicity. As B95-8 *LMP-1* itself transforms in rodent fibroblasts, the significance of these differences in the transforming ability of *LMP-1* in cell lines remains unclear.

The presence or absence of the deleted form of *LMP-1* and multiple sequence changes in various disease states have also been evaluated (Knecht *et al.*, 1996). Some studies suggest that strains with deleted *LMP-1* are found in more aggressive tumours, and others indicate that these changes reflect the prevalence of a given strain within a population (Knecht *et al.*, 1993; Cheung *et al.*, 1996: Khanim *et al.*, 1996). In epithelial tumours, specific amino-acid changes have been detected in EBNA-1 which are suggested to influence its biological properties (Snudden *et al.*, 1995).

Trivedi *et al.* (1994) studied the effect of *LMP-1* on the immunogenicity of mouse mammary carcinoma cells. Immunization with B95-8 *LMP-1*-transfected tumour cells protected against challenge with B95-8 LMP-1-positive tumour cells, whereas immunization with Cao LMP-1 did not. This difference could be due to an inability of this particular mouse strain to present the MHC class-I or class-II epitopes of the Cao strain. It is possible that similar differences in immune response occur in distinct human populations.

These studies suggest that different EBV strains predominate in different geographical areas and that some variants may be detected preferentially in nasopharyngeal carcinoma. The prevalence of these distinct strains in epithelial malignancies could reflect an epithelial cell tropism in which particular variants of EBV preferentially establish latent infection in epithelial cells, or it may reflect differences in immunogenicity. It is also possible that strains that encode *LMP-1* with increased transforming properties have increased pathogenicity *in vivo*.

4.5.2   Contribution of environmental and genetic factors

4.5.2.1   Dietary cofactors

(a)   Experiments in rodents

Groups of 10 male and 10 female Wistar WA rats were fed salted fish for six months and observed for one to two years. Four female rats developed carcinomas in the nasal or paranasal regions, while none was observed in the six control rats (Huang *et al.*, 1978c).

Salted fish purchased weekly in streetside markets in Hong Kong was fed to 111 male and 110 female Wistar-Kyoto rats aged 21 days, immediately after weaning in order to recreate the human experience. The animals were randomized by sex into one of three experimental groups: groups of 37 males and 37 females were fed a powdered diet consisting of one part steamed salted fish and three or five parts certified rat chow for the first 18 months; 37 males and 36 females were given rat chow only throughout the experiment. After 18 months, all rats were given rat chow pellets for the remainder of their lifespan. Four malignant nasal cavity tumours developed among the treated rats: the first three tumours (one undifferentiated carcinoma, one moderately differentiated squamous-cell carcinoma and one spindle-cell carcinoma) all occurred in the group at the

high dose; the fourth tumour was a spindle-cell tumour (not otherwise specified) in a rat at the low dose. No tumours of the upper respiratory tract were observed among control rats, consistent with the rarity of reports of such spontaneous tumours in rats. The rate of tumour occurrence among treated rats was statistically different from the base rate of zero (p = 0.02). The levels of salted fish fed to the rats were close to the range of human consumption, i.e. the amounts fed to Cantonese babies during weaning, as determined in a survey of mothers in Guangzhou, China (Yu et al., 1989b).

In a two-year experiment, 16 pregnant Sprague-Dawley rats were randomized into four groups. Four rats were fed a diet consisting of 10% by weight steamed salted fish (purchased in Guangzhou, China) throughout pregnancy and lactation, and the 41 off-spring continued to be fed the diet after weaning. Four pregnant rats were fed standard rat chow during pregnancy and lactation, but their 41 offspring were fed the 10% fish diet after weaning. Rats in the third group received a diet consisting of 5% salted fish by weight, as for the first group. Rats in the last group served as controls, both the mothers and the 40 offspring being fed a standard rat chow diet throughout the experiment. Seven nasal tumours were observed among the rats fed salted fish, with four in group 1 (one squamous-cell carcinoma, one poorly differentiated carcinoma, one fibrosarcoma and one adenocarcinoma), two in group 2 (one squamous-cell carcinoma and one rhabdo-myosarcoma) and one soft-tissue sarcoma in group 3; no respiratory-tract tumours were seen in the controls. The difference in the occurrence of nasal tumours in the four experimental groups was statistically significant (p for linear trend = 0.04) (Zheng et al., 1994d).

(b) High-risk populations

Low levels (subparts per million) of several volatile nitrosamines, including N-nitro-sodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-propylamine, N-nitrosodi-n-butylamine and N-nitrosomorpholine, have been detected in samples of Chinese salted fish (Huang et al., 1981; Tannenbaum et al., 1985). Most of these volatile nitrosamines can induce nasal tumours in animals (Haas et al., 1973; Pour et al., 1973; Althoff et al., 1974; Lijinsky & Taylor, 1978). Bacterial mutagens were detected in Chinese salted fish that had been exposed to a nitrosating agent under simulated gastric conditions (Tannenbaum et al., 1985; Weng et al., 1992). Directly acting genotoxic substances have also been found in extracts of this food (Poirier et al., 1989). In addition, samples of Chinese salted fish were found to contain substance(s) capable of activating EBV in latently infected Raji cells (Shao et al., 1988; Poirier et al., 1989).

Low levels (parts per billion) of several volatile nitrosamines have been detected in samples of Chinese mustard green, chung choi and fermented soya-bean paste and in Tunisian quaddid and touklia (Poirier et al., 1987). N-Nitrosodimethylamine was detected in quaddid, touklia, salted mustard greens and chung choi; N-nitrosopiperidine in touklia and salted mustard greens; and N-nitrosopyrrolidine in quaddid, touklia, salted mustard greens, chung choi and fermented soya-bean paste. Directly acting genotoxic substances have been found in samples of Chinese salted shrimp and fermented soya-bean paste and in Tunisian quaddid, touklia and harissa (Poirier et al., 1989). In addition, samples of harissa and quaddid were shown to contain substances capable of activating

EBV in latently infected Raji cells (Shao *et al.*, 1988; Poirier *et al.*, 1989). These substances have been characterized as including macromolecular lignin which can activate the *ZEBRA* EBV latent gene (Bouvier *et al.*, 1995).

Zheng *et al.* (1993) compared the levels of urinary nitrate and four nitrosamino acids in 77 healthy subjects in two villages in southern China in which there is a 10-fold range in the incidence of nasopharyngeal carcinoma. The five urinary metabolites were measured in 12-h samples of urine collected in the absence of treatment, after ingestion of L-proline in the urine of subjects from the high-risk village or after ingestion of L-proline in combination with vitamin C. The total urinary nitrosamino acids in the urine of subjects from the high-risk village was significantly increased above the baseline level after proline treatment, while no difference from background levels was noted after treatment with both proline and vitamin C. No such variation in nitrosamino acid levels was found in subjects in the low-risk village. The mean urinary nitrate levels were significantly higher among subjects in the high-risk than the low-risk village in both treated and untreated urine samples. These results demonstrate a higher potential for endogenous nitrosation in subjects living in areas of high risk for nasopharyngeal carcinoma and suggest the presence of nitrosation inhibitors in the diet of southern Chinese living in areas at relatively low risk for nasopharyngeal carcinoma. These results also support the hypothesis that dietary precursors of nitroso compounds are involved in the pathogenesis of nasopharyngeal carcinoma.

4.5.2.2 Genetic factors

The role of HLA-related genes in nasopharyngeal carcinoma is discussed in section 2.4.3. Several HLA types have been associated with development of the disease, and familial aggregation has been linked to the HLA region (Chan *et al.*, 1983b; Lu *et al.*, 1990). A recent study revealed that the CTL presentation of LMP-2 was restricted to HLA A2.1 (Murray *et al.*, 1992b), and an inverse association between risk for nasopharyngeal carcinoma and the HLA A2 type has been described (Burt *et al.*, 1994). It has also been suggested that patients with nasopharyngeal carcinoma have impaired T-cell immunity to EBV in general (Tamura *et al.*, 1992); however, if impaired T-cell immunity and HLA-restricted presentation contribute to the development of nasopharyngeal carcinoma, an elevated incidence of this tumour would be expected in patients with HIV, which has not been observed to date (Melbye *et al.*, 1996).

High-risk families in which multiple cases of nasopharyngeal carcinoma have occurred have been described in both high- and low-risk populations (for details, see section 2.4.3.3). Familial clustering of nasopharyngeal carcinoma is likely to be the result of genetic constitution and environmental exposures.

Genetic changes characteristic of other malignancies, such as *c-myc* rearrangement, *p53* mutation, *Rb* alterations or *ras* mutations, would not necessarily contribute to altered growth, and such mutations have not been detected in nasopharyngeal carcinomas in Chinese, American or Arab populations (Effert *et al.*, 1992; Spruck *et al.*, 1992; Sun *et al.*, 1993; Nasrin *et al.*, 1994; Sun *et al.*, 1995). Unmutated p53 protein is, however, detected at high levels in nasopharyngeal carcinomas and *p53* expression has been shown

to be induced by NFκB, a transcription factor that is activated by *LMP-1* (Niedobitek *et al.*, 1993b; Chen & Cooper, 1996). In studies to investigate whether an EBV protein interferes with some aspect of *p53* function and eliminates a selection for inactivating mutations, it was found that the EBV BZLF1 protein binds p53 and inhibits p53-dependent transcriptional *trans*-activation, however, *BZLF1* is expressed in rare cells in nasopharyngeal carcinomas and would be unlikely to influence the selection of inactivating mutations in *p53* in the majority of nasopharyngeal carcinoma cells (Zhang *et al.*, 1994b). In latent infections, EBV does not interfere with the ability of *p53* to arrest cells in G1 after DNA damage by inducing expression of the *p21* cyclin kinase inhibitor. Two studies have shown, however, that LMP-1 inhibits p53-mediated apoptosis induced by serum withdrawal (Okan *et al.*, 1995; Fries *et al.*, 1996). This property could be due to induction of *bcl2*, which has been described in lymphoid cell lines (Henderson *et al.*, 1991); but elevated *bcl2* expression is not linked to EBV infection in epithelial tumours (Lu *et al.*, 1993) and *bcl2* was not induced in epithelial cell lines expressing LMP-1 that were protected from apoptosis (Fries *et al.*, 1996). Specific protection from p53-mediated apoptosis was conferred by the A20 protein, which is induced by LMP-1 expression and protects against apoptosis induced by tumour necrosis factor or serum withdrawal. Protection against p53-mediated apoptosis is likely to be responsible for the lack of *p53* mutations in EBV-associated cancers.

Recent studies have identified areas of loss of heterozygosity on several chromosomes, including the regions 3p24 and 9p21 (Huang *et al.*, 1991, 1994). Deletions at 3p24 have also been detected in nasopharyngeal carcinomas in Indian populations (Kumari *et al.*, 1995). The *p16* gene at 9p21 is completely deleted in some samples of nasopharyngeal carcinoma (Lo *et al.*, 1995). Although *p16* is not deleted in the C15 tumour passaged in nude mice, it is not expressed (Sun *et al.*, 1995). The *p16* gene is a critical regulator of cell-cycle progression through G1, and mutations in the *Rb* gene, amplification of cyclins or inactivation of cyclin-dependent kinases can all inactivate this component of cellular control. Deletion of *p16* and repression of *p16* expression in nasopharyngeal carcinomas suggests that this pathway is affected in this tumour, as in many others. The identification of other specific genes in the regions that show chromosomal loss may help in finding the critical cellular genes that contribute to the development of nasopharyngeal carcinoma in high-risk populations or a gene that is affected by mutagenic environmental factors.

4.6 Other malignancies, including lymphoepithelial carcinomas

Latent EBV infection has been detected in gastric lymphoepithelial carcinoma, and EBV is also found in a subset of gastric adenocarcinomas in all geographic areas in which it has been studied (Shibata & Weiss, 1992; Rowlands *et al.*, 1993; Selves *et al.*, 1996a). Clonal EBV is detected in the tumours, while immunohistochemical studies suggest that EBNA-1 is expressed in the absence of detectable EBNA-2 or LMP-1 in both adenocarcinomas and lymphoepithelial carcinomas (Rowlands *et al.*, 1993; Fukayama *et al.*, 1994; Imai *et al.*, 1994a; Ott *et al.*, 1994; Murray *et al.*, 1996; Selves *et al.*, 1996b). This suggests a type-I EBV latency rather than the type-II latency found in

nasopharyngeal carcinomas. These results were confirmed recently at the transcriptional level by RT-PCR, which showed that EBNA-1 is expressed from the Q promoter, as in types-I and -II latency (Sugiura et al., 1996). Consistent with the results of previous immunohistochemical studies, EBNA-2 and LMP-1 mRNA were not detectable; however, transcripts derived from the BamHI A fragment of the viral genome were detected in all cases, and a proportion of cases had detectable levels of LMP-2A mRNA (Sugiura et al., 1996). In a preliminary immunohistochemical study with new LMP-2A-specific monoclonal antibodies, no staining was detected in EBV-positive gastric carcinomas (Niedobitek et al., 1997c). This suggests that carcinomas may represent a form of type-I latency, with possible additional expression of LMP-2A in a proportion of cases.

Expression of the BZLF1 protein and of BZLF1 transcripts has also been reported in a few scattered cells in a proportion of gastric carcinomas (Niedobitek et al., 1992b; Rowlands et al., 1993), while Sugiura et al. (1996) did not detect lytic cycle RNA transcripts by RT-PCR. This suggests that viral replicative genes are expressed in a very small fraction of tumour cells; however, it is presently unclear whether this leads to the production of infectious virions. In a single case, Niedobitek et al. (1992b) reported the absence of detectable gp350 expression in a lymphoepithelial carcinoma of the stomach.

Multiple other types of epithelial cancer have also been linked to EBV. Most are rare undifferentiated carcinomas with a prominent lymphoid stroma (lymphoepithelial carcinomas), reminiscent of nasopharyngeal carcinoma. EBV has been associated with lymphoepithelial carcinoma of the parotid gland (Saemundsen et al., 1982; Saw et al., 1986; Huang et al., 1988; Raab-Traub et al., 1991), which develops at relatively high incidence among Inuit populations and in Chinese (Saw et al., 1986; Huang et al., 1988). Other epithelial salivary-gland tumours have been shown to be EBV-negative (Wen et al., 1997). In a Japanese study, EBV was detected in lymphoepithelial carcinoma samples but not in benign lymphoepithelial lesions (Nagao et al., 1996), suggesting that EBV infection is the pivotal step to malignancy. Like nasopharyngeal carcinoma, undifferentiated parotid tumours contain clonal EBV and express EBERs, LMP-1 and BamHI A transcripts (Raab-Traub et al., 1991).

Other rare cancers are linked to EBV infection. These include a subset of undifferentiated sinonasal carcinomas in Italian and Chinese populations (Gallo et al., 1995; Leung et al., 1995b), lymphoepithelial cholangiocarcinoma (Hsu et al., 1996) and smooth-muscle tumours in HIV-infected children and transplant recipients (Morel et al., 1996). The smooth-muscle tumours express EBER-1 RNA and EBNA-2 but not LMP-1 (Lee et al., 1995a). In all samples, identification of the EBV termini showed that the infection was non-permissive and clonal with regard to EBV. Genes expressed in latency type II are found in most tumours, although the detection is variable and some tumours, such as gastric carcinoma, may express only EBNA-1. The detection of EBV in tumours of diverse cell types indicates that EBV may gain entry into different cell types under specific circumstances and can potently affect cell growth. The clonality of EBV suggests that establishment of a latent transforming infection is the rare event that triggers cancer development.

4.7 Immune responses and EBV-associated malignancies

A common denominator of virus-associated tumours is the persistence in the malignant cells of all or parts of the viral genetic material and the continued expression of viral proteins that are the potential targets of tumour-specific rejection. The various patterns of viral gene expression identified in EBV-associated malignancies (see Table 3) and the diverse cellular origin of the malignant cells themselves could provide different types of challenge to the host immune system. Thus, failures or specific changes in the host immune response are likely to play different roles in the pathogenesis of these tumours. Characteristic changes in the pattern of antibodies to the latent antigens and to antigens associated with the productive cycle occur in patients with EBV-associated tumours. These antibody patterns reflect the load of infectious virus and/or viral antigen-expressing cells, and their role in limiting the growth of virus-infected cells is uncertain. With the exception of immunosuppressed patients who develop EBV-associated lymphomas, little is known about the status of cell-mediated virus-specific immune responses in patients with EBV-carrying tumours.

4.7.1 B-Cell lymphoma and other tumours associated with severe immunosuppression

The nine viral antigens that are typically detected in lymphoblastoid cell lines are also expressed in B-cell lymphomas arising in immunosuppressed patients (see Table 3). The phenotypic similarity of these lymphomas to lymphoblastoid cell lines suggests that the lymphomas represent virus-derived lesions growing out opportunistically in the absence of immune control. Indeed, regression has been reported after cessation of immunosuppressive therapy in renal transplant recipients (Starzl *et al.*, 1984), and passive transfer of blood lymphocytes or activated EBV-specific CTL cultures *in vitro* has been successfully used in the treatment of lymphomas arising in bone-marrow recipients (see section 1.5.3; Papadopoulos *et al.*, 1994; Rooney *et al.*, 1995). Immunosuppression could also play a role in the pathogenesis of EBV-positive leiomyosarcomas, since these tumours appear to express at least one highly immunogenic viral antigen (see Table 3).

4.7.2 Burkitt's lymphoma

Although transient impairment of EBV-specific responses has been demonstrated during acute malaria (Whittle *et al.*, 1984), normal levels of EBV-specific CTL precursors were demonstrated in a single study of the inhibition of autologous B-cell transformation in Burkitt's lymphoma patients (Rooney *et al.*, 1997). Even so, disturbance of the antiviral response is likely to play an important role in the pathogenesis of endemic Burkitt's lymphoma. EBV infection early in life and recurrent episodes of severe malaria were shown to increase the risk for the lymphoma in African children (reviewed by Magrath, 1990). Malarial infection is associated with chronic B-cell stimulation and follicular hyperplasia, which is likely to favour the establishment of a large pool of latently infected B lymphocytes from which cytogenetically altered cells may arise during the rapid proliferative phase of follicular reactions. A similar mechanism could also explain the high incidence of Burkitt's lymphoma in HIV-positive patients, in whom chronic stimulation of the B-cell compartment is a common feature in the pre-AIDS

stage. EBV-positive Burkitt's lymphoma lines that have maintained *in vitro* the pheno-typic characteristics of the tumour *in vivo* are insensitive to lysis by EBV-specific MHC class I-restricted CTLs (Rooney *et al.*, 1995). This observation is consistent with the finding that the viral antigens that are recognized by these effectors are down-regulated and only EBNA-1 is expressed in the tumour. EBNA-1 may be protected from MHC class I-restricted rejection responses by an inhibitory effect of the glycine–alanine repeat on antigen processing (Levitskaya *et al.*, 1995). MHC class II-restricted EBNA-1-specific CTLs have been demonstrated *in vitro* (Khanna *et al.*, 1996), but the capacity of these cells to recognize the tumour is not firmly established. In addition to down-regu-lation of viral antigens, Burkitt's lymphoma cells consistently show low expression of adhesion molecules and MHC class I-restricted antigens and a selective loss of certain class-I alleles, with HLA A11 as the most consistent example (Masucci *et al.*, 1987; Andersson *et al.*, 1991). These features are characteristic of both EBV-positive and EBV-negative tumours and may therefore represent a phenotypic feature of the Burkitt's lym-phoma precursor (Torsteinsdottir *et al.*, 1989). At least some may be specifically induced by the constitutive activation and overexpression of *c-myc* that characterizes this tumour (Polack *et al.*, 1996). More recently, down-regulation of the transporters associated with antigen presentation (Khanna *et al.*, 1995; Rowe *et al.*, 1995) and defects of antigen pro-cessing (Frisan *et al.*, 1996) were reported to be consistently associated with the Burkitt's lymphoma-cell phenotype.

4.7.3   Hodgkin's disease

The role of specific immunosuppression in the pathogenesis of Hodgkin's disease has been discussed extensively(Slivnick *et al.*, 1990). A decrease in EBV-specific responses, as assessed by the regression assay, was suggested in earlier studies; however, recent reports clearly demonstrate the presence of EBV-specific CTL precursors in the blood of Hodgkin's disease patients, independently of the EBV status of the tumour (Dolcetti *et al.*, 1995; Frisan *et al.*, 1995). A comparative study of EBV-specific responses in patients with EBV-positive and EBV-negative Hodgkin's disease failed to demonstrate virus-specific cytotoxicity within the tumour-infiltrating lymphocytes of six EBV-posi-tive cases, while EBV-specific effectors were readily detected (Frisan *et al.*, 1995). The detection of CTLs in the blood of patients with EBV-positive Hodgkin's disease suggests that virus-specific reactivity is selectively suppressed in the tumour. The finding of multiple sequence variations in the *LMP-1* gene in EBV isolates from cases of Hodgkin's disease and nasopharyngeal carcinoma supports the possibility that the tumour cells present altered peptides that act as antagonists of EBV-specific responses. Alternatively, production of IL-10 in the LMP-1-positive tumours (Herbst *et al.*, 1996b) may mediate local inhibition of CTL responses.

4.7.4   Nasopharyngeal carcinoma

Nasopharyngeal carcinoma arises in apparently immunocompetent individuals, but the frequency is not increased in patients with recognized forms of immunosuppression. Lymphocytes from nasopharyngeal carcinoma patients maintain the capacity to inhibit

the proliferation of autologous virus-infected cells in regression assays, although a significant impairment in virus-specific T-cell immunity is shown in newly diagnosed patients when compared with either long-term survivors or controls (Moss et al., 1983b). It is therefore quite surprising that the continuous expression of at least two highly immunogenic viral antigens, LMP-1 and LMP-2, does not lead to rejection. A characteristic feature of tumours expressing LMP-1 is the presence of abundant lymphoid infiltrate, which may be indicative of an on-going reaction to viral and/or tumour antigens. Indeed, LMP-1 was shown to increase the stimulatory capacity of EBV-negative Burkitt's lymphoma lines in allogeneic mixed-lymphocyte cultures (Cuomo et al., 1990), to up-regulate the expression of adhesion molecules (Wang et al., 1990) and to enhance the presentation of endogenous and exogenous antigens (de Campos-Lima et al., 1993b; Rowe et al., 1995). It is not known whether LMP-1 has similar effects on the immunogenic phenotype of epithelial cells, and the sensitivity of these cells to EBV-specific CTLs has not been investigated. Several surface molecules expressed in undifferentiated nasopharyngeal carcinoma cells, such as CD70, CD80 and CD86, may be involved in the induction of T-cell activation, and T cells with an activated phenotype have been detected in the tumour; however, their role in the pathogenesis of the disease remains unclear (Agathenanggelou et al., 1995). A study of the expression of the T-cell receptor repertoire of tumour-infiltrating lymphocytes from nasopharyngeal carcinomas showed a significantly lower representation of certain V families (Vα10, Vα11, Vα13, Vα14, Vβ14 and Vβ20) than in control biopsies. Some of these differences were also observed in peripheral blood (Chen et al., 1995b). Of particular interest is the observation that the frequencies of Vα17 ($p = 0.01$) and Vα18 were significantly lower ($p = 0.04$) in HLA B46-positive nasopharyngeal carcinoma patients than in B46-positive controls. It is presently unknown whether specific EBV peptides are present through HLA B46. Taken together, these results suggest that the expression of certain HLA alleles (see section 1.1.5) and the absence of certain T-cell receptor Vα/β families is important in the pathogenesis of nasopharyngeal carcinoma.

An additional clue is provided by comparisons of the immunogenicity of a nasopharyngeal carcinoma-derived LMP-1 with LMP-1 from the standard EBV laboratory strain B95.8 in a transfectant mammary carcinoma model in mice. While transfectants expressing the B95.8-derived LMP-1 became immunogenic and were rejected by syngeneic animals, the nasopharyngeal carcinoma-derived polypeptide failed to induce rejection (Trivedi et al., 1994). Conceivably, the tumour-derived protein lacks antigenic epitopes that are relevant for rejection or fails to induce a phenotypic shift that would increase immunogenicity.

5. Summary of Data Reported and Evaluation

5.1 Virus–host interactions

Epstein-Barr virus (EBV) is a gamma-1 herpesvirus found throughout all human populations, with a prevalence of over 90% in adults. Primary infection usually occurs in early childhood and is asymptomatic, whereas delayed primary infection may cause a

self-limiting lymphoproliferative disease, infectious mononucleosis. Infection results in the establishment of a life-long carrier state characterized by the persistence of antibodies to several viral gene products and the secretion of infectious virus in saliva. Saliva is the usual vehicle of transmission. EBV can induce growth transformation of human and primate B-lymphocytes *in vitro*, and it causes malignant lymphomas in certain New World primates. B-cell growth transformation is initiated by binding of the virus to the complement receptor CD21 and is associated with the expression of a restricted set of viral genes which encode two non-translated small RNAs (EBER-1 and -2), six nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, -3B, -3C and EBNA-LP) and three latent membrane proteins (LMP-1, -2A and -2B). Three forms of latent infection, referred to as latency types I–III, have been demonstrated in EBV-carrying B-cell lines and EBV-carrying tumour biopsy samples. In type-I latency, only EBNA-1 is expressed, while in type-II latency EBNA-1 is expressed together with LMP-1, -2A and -2B; in type III latency, the latent membrane proteins are expressed with all six EBNAs. Only a small fraction of latently infected B lymphocytes spontaneously enters the productive cycle, which is characterized by the co-ordinate expression of immediate early genes that encode viral transactivators, early genes that encode enzymes involved in viral replication and late genes that encode structural proteins.

Cellular and perhaps humoral immune responses contribute to the control of primary EBV infection and mediate the transition to asymptomatic persistence of the virus in healthy carriers. The humoral responses include the production of neutralizing antibodies directed to the CD21-binding protein gp350/320 and antibodies directed against lytic and latent gene products. Cells expressing type-III latency, which are detected during primary infection, are promptly eliminated by CD8$^+$ major histocompatibility class (MHC) I-restricted cytotoxic T lymphocytes which recognize epitopes from all the latent viral proteins except EBNA-1. Accordingly, only EBNA-1 appears to be expressed in the latently infected B cells that persist in healthy virus carriers. The failure of this protein to elicit cytotoxic T-lymphocyte responses could be instrumental in allowing persistence of virus-infected cells in immunocompetent hosts. CD4$^+$ MHC class II-restricted effectors could also play a role by producing growth inhibitory cytokines.

Few drugs are available that prevent viral replication without significant toxicity. Acyclovir and a number of related compounds have been used successfully to reduce viral replication but with no significant effect on persistent infection. Prophylactic, post-infection and therapeutic EBV vaccination strategies are currently being developed with recombinant subunit viral proteins and live recombinant virus vectors. The success of this endeavour will depend on a better understanding of the EBV life cycle and the immune responses generated by natural infection in humans.

5.2   Human carcinogenicity

Since the large majority of the world's population is latently infected with EBV, the mere presence of the virus in tumour tissue is insufficient evidence for its etiological role. In particular, since B lymphocytes are a normal reservoir for latent infection, the presence of EBV-infected lymphocytes in tumour tissue may be either incidental or an

effect of the tumour rather than its cause. Therefore in addition to the usual criteria (stated in the Preamble) by which epidemiologists judge the causality of association, for EBV, other factors should be considered: (1) the proportion of EBV-positive cases in a given tumour entity, (2) the proportion of tumour cells that carry the virus in any given case, (3) the monoclonality of EBV in the tumour (suggesting the presence of latent infection prior to expansion of the malignant clone); and (4) the expression of EBV proteins.

5.2.1   Burkitt's lymphoma

Early case–control studies indicated that African patients with Burkitt's lymphoma had much higher titres of antibodies to EB viral capsid antigen and early antigen than normal subjects. Moreover, a large cohort study in the West Nile district of Uganda showed that patients who developed Burkitt's lymphoma had significantly higher titres of antibodies to viral capsid antigen than control children between seven months and six years before diagnosis.

The viral DNA is present in Burkitt's lymphoma cells in monoclonal form. The expression of viral proteins is almost entirely restricted to EBNA-1. The frequency of this association between EBV and Burkitt's lymphoma varies geographically. c-myc immunoglobulin gene translocations are invariably seen in Burkitt's lymphoma.

The importance of malaria as a cofactor in the development of Burkitt's lymphoma in Africa was demonstrated by the coincidence of the distribution of hyperendemic and holoendemic malaria and Burkitt's lymphoma in different geographic areas, in different population subgroups and over time and by the reduction in the frequency of both diseases after chloroquine prophylaxis.

The importance of EBV in the causation of Burkitt's lymphoma varies in different regions and population groups, but appears to be greatest when infection occurs in the early years of life. The evidence suggests that EBV is an important pathogenic factor for the development of Burkitt's lymphoma.

5.2.2   Non-Hodgkin's lymphomas

EBV has particular importance in non-Hodgkin's lymphomas occurring in immuno-suppressed individuals, who are at increased risk for these malignancies. Non-Hodgkin's lymphomas in transplant recipients and in patients with congenital immunodeficiency are nearly always EBV-positive. In HIV-positive subjects, EBV is uniformly associated with primary central nervous system lymphoma and is frequently associated with systemic lymphoma (although cases of all histological subtypes can be EBV-positive or EBV-negative).

EBV is strongly associated with some uncommon types of non-Hodgkin's lymphoma, on the basis of frequent EBV positivity, a high prevalence of EBV in tumour cells, EBV monoclonality in tumours and expression of EBV proteins. Sinonasal angiocentric T-cell lymphoma appears to be a malignant proliferation of EBV-infected T cells. A fraction of other peripheral T-cell lymphomas may be EBV-related, although EBV-positive and

EBV-negative cases are not distinguishable on the basis of histology or site of involvement.

The associations with lymphomas occurring in immunosuppressed individuals and sinonasal angiocentric T-cell lymphoma indicate a causal role of EBV in these forms of non-Hodgkin's lymphoma. The evidence for other types of non-Hodgkin's lymphoma is as yet inadequate.

5.2.3   Hodgkin's disease

In multiple specimens of Hodgkin's disease from case series, molecular evidence of clonal EBV genome with specifically restricted expression of latent viral proteins in the Reed-Sternberg cells is found in 30–50% of cases. EBV genome status appears to be uniform in involved nodes within patients and over time in those patients studied longitudinally.

The consistency of the finding of clonal EBV and the expression of LMP-1 in about half of Hodgkin's disease cases in many patient populations throughout the world argues strongly against a passenger role for the virus in these cases. Seroepidemiological findings in multiple case–control studies and two cohort studies show that patients with Hodgkin's disease can be distinguished by an altered antibody profile to EBV. The evidence indicates that EBV is a causal factor in the etiology of Hodgkin's disease.

5.2.4   Nasopharyngeal carcinoma

Nasopharyngeal carcinoma is a rare malignancy in most populations, although very high rates are seen in populations in southern China and more moderate rates in Inuit populations, in other parts of Southeast Asia and in North Africa. The incidence of nasopharyngeal carcinoma bears no relationship to age at infection (on the basis of the prevalence of antibodies to EBV in children and adolescents) either between or within countries.

One cause of nasopharyngeal carcinoma in high-risk populations identified previously is Chinese-style salted fish, a carcinogen in Group 1 (IARC, 1993). Other preserved foods and cigarette smoking have also been implicated.

Elevated immunoglobulin A antibodies to Epstein-Barr viral capsid and early antigens are a well-established feature of undifferentiated nasopharyngeal carcinoma. EBV DNA and viral products are regularly detected in malignant cells but not in normal nasopharyngeal epithelium.

The consistency of the molecular evidence strongly implicates EBV as a causative factor in the etiology of nasopharyngeal carcinoma: all undifferentiated nasopharyngeal carcinomas are EBV-positive, they are monoclonal with regard to EBV, and virtually all cells in each tumour also contain EBV DNA and/or EBV proteins.

5.2.5   Other tumours

EBV has been detected in the vast majority of gastric lymphoepithelial carcinomas and in a high proportion of lymphoepithelial carcinomas of the lung and salivary gland.

A smaller proportion of gastric adenocarcinomas is also EBV-associated. EBV DNA has been detected occasionally in epithelial tumours at a wide variety of other anatomical sites. An etiological role for EBV in lymphoepithelial and adenocarcinomas has not been conclusively established.

Smooth-muscle tumours in immunosuppressed individuals uniformly contain EBV, indicating a possible causal role for the virus in this setting.

5.3 Studies of cancer in animals

The available studies in non-human primates and rodents on the pathogenesis of EBV showed that New World primates (cotton-topped tamarins, owl monkeys and squirrel monkeys), which are naturally free of EBV-like viruses, can be infected by EBV. The pathogenesis of transforming EBV in such non-human primates varied from latent infection, to benign lymphoproliferation, to malignancy in lymphoid tissues; non-transforming EBV failed to induce tumours. Old World primates, which carry their own EBV-like viruses, did not develop clinical manifestations or tumours when inoculated with EBV. SCID mice engrafted with EBV-positive B cells developed tumours that expressed EBV proteins. The experimentally induced tumours were either oligoclonal or monoclonal.

Non-human primate lymphocryptoviruses from a variety of Old World primates have close homology to EBV. Each species of Old World primate may carry its own EBV-like herpesvirus. These herpesviruses are B-lymphotropic and transform human and monkey B cells. *Herpesvirus papio* is the only one that has been widely studied. It is commonly distributed in species of baboons and is horizontally transmitted; animals become infected early in life, and the virus becomes latent. *Herpesvirus papio* does not apparently induce tumours in the natural host or in experimentally infected non-human primates. *Herpesvirus papio* and EBV-like herpesviruses from apes (gorillas, chimpanzees and orangutans) and monkeys (rhesus, African green and cynomolgus) share antigenic cross-reactivity among themselves and with EBV. Experimental infection of such animals with EBV is therefore difficult.

A lymphoblastoid cell line derived from a lymph node of a cynomolgus monkey contained an EBV-like transforming herpesvirus. In another study, a herpesvirus (HVMF-1) isolated from cynomolgus monkeys was found to share antigenic and molecular properties with EBV. HVMF-1 remained latent in cynomolgus monkeys after infection. When such latently infected monkeys were inoculated with simian immunodeficiency virus 1, some animals developed malignant B-cell lymphomas that contained EBV-like virus. This experimental lymphoma may offer a good model for the study of EBV tumorigenesis in patients with AIDS.

5.4 Other relevant data

EBV infection of primary B lymphocytes *in vitro* efficiently induces cell immortalization into permanent cell lines. This is the only system available for the identification, characterization and functional analysis of virally encoded proteins associated with

immortalization. Altogether, 13 viral genes have been found to be expressed in the immortalized lymphocytes. The phenotype of the EBV-transformed B lymphocyte suggests that the effect of viral protein expression mimics that of antigen-driven lymphocyte activation. The immortalization-associated viral proteins regulate maintenance of episomal viral DNA and viral gene expression, drive cellular proliferation and block apoptosis.

5.4.1 Burkitt's lymphoma

The experimental evidence strongly suggests that malaria and early EBV infection are both important factors in increasing the risk for Burkitt's lymphoma in young African children.

Cell proliferation in Burkitt's lymphoma is driven by constitutive c-myc activation due to translocations involving the proto-oncogene and one of the immunoglobulin loci. The translocation is the consequence of an aberrant recombination process, which can occur in conjunction with VDJ rearrangement or immunoglobulin switching. EBV-driven cell proliferation, immune dysregulation caused by malaria, including chronic T-helper-2 stimulation of B cells, and chronic antigen exposure all contribute to the B-cell hyperplasia seen in people at risk for endemic Burkitt's lymphoma. Chronic B-lymphocyte hyperplasia increases the target cell population for aberrant recombination. The results of studies in experimental animals support a role for malaria as a cofactor in tumorigenesis.

Similar considerations apply to the pathogenesis in patients with AIDS, although the cofactor malaria is replaced by HIV infection leading to immune dysregulation. The resulting B-cell hyperplasia increases the risk for translocations at the premalignant stage. As in other Burkitt's lymphomas, c-myc–immunoglobulin translocations are invariably present in AIDS-associated Burkitt's lymphoma, whereas the prevalence of EBV infection varies. The EBV-positive cases express a type I latency programme.

The Burkitt's lymphoma cell has the phenotype of a germinal-centre cell. The constitutive c-myc expression prevents its potential entry into a resting state. The EBV type I latency programme and down-regulation of MHC class I ensure immune evasion.

5.4.2 Non-Hodgkin's lymphomas and lymphoproliferation

The risk of acquiring EBV-positive B-cell lymphomas increases dramatically in patients who are immunosuppressed due to primary genetic disorders, immunosuppressive therapy in transplant recipients and patients with AIDS. These lymphomas include non-Hodgkin's lymphomas of the diffuse, large B-cell type and, less frequently, of the Burkitt's lymphoma type. In these disorders, a range of conditions has been described, from polyclonal lymphoproliferation to oligoclonal and monoclonal malignancies. This may reflect a progressive situation, with an initial EBV-driven proliferation leading eventually to the outgrowth of fully malignant lymphoma. In the predominant large B-cell lymphoma types, types-II and -III latency are the most commonly detected patterns of EBV gene expression. It is therefore conceivable that the viral transforming genes drive cell proliferation and contribute to subversion of apoptosis in proli-

feration or malignancies. In the development of monoclonal conditions, additional cellular genetic changes have been observed at variable frequencies, which contribute to tumour progression. These include activation of autocrine and paracrine loops with the cytokines interleukin-6 and interleukin-10. The complex immunosuppression seen in these conditions is instrumental in pathogenesis and progression.

The T-cell lymphomas present a heterogeneous situation. EBV is detected at high frequency in many types of T-cell lymphomas. Monoclonality has been demonstrated in particular in sinonasal angiocentric T-cell lymphoma, providing one piece of evidence for a role of EBV in their pathogenesis. In other types, EBV gene expression is seen in only a minority of the cells in the tumour. There are few experimental data on the role of EBV in T-cell malignancies, including how and when EBV enters such cells and the effects of EBV transformation-associated proteins on T-cell proliferation and survival.

5.4.3 Hodgkin's disease

In-situ hybridization has disclosed the presence of the virus in virtually all tumour cells in EBV-positive cases, consistent with the detection of monoclonal EBV genomes in DNA extracted from most Hodgkin's disease tissues. These findings indicate that EBV infection of Hodgkin-Reed-Sternberg cells takes place before clonal expansion. The presence of EBV and the expression of type II-latency genes in these cells has been correlated with increased expression of lymphocyte activation antigens and with decreased expression of CD20 B-cell antigen. Of particular relevance is the association of EBV infection with the expression of cytokines such as interleukins 6 and 10 and in Hodgkin-Reed-Sternberg cells. Although Hodgkin's disease patients with EBV-positive tumours show a good, EBV-specific cytotoxic T-lymphocyte response in blood lymphocytes, the tumour-infiltrating lymphocytes lack EBV-specific activity and may thus be suppressed by the tumour cells. The available evidence strongly implicates the virus as a factor in the pathogenesis of EBV-positive Hodgkin's disease.

5.4.4 Nasopharyngeal carcinoma

EBV is detected in all undifferentiated nasopharyngeal carcinomas, and viral DNA is present in a monoclonal form in every malignant cell. The tumour cells show a type-II pattern of latency of gene expression; however, LMP-1 is not detected in all tumours. Some evidence has been provided for infection of nasopharyngeal carcinoma in situ. The viral genes expressed affect cell growth and differentiation, but tumour progression must involve one or several additional changes in cellular genes. The establishment of predominantly non-permissive infection in epithelium could be an important event that leads to the development of nasopharyngeal carcinoma.

Studies in experimental animals strongly support the epidemiological finding that a diet containing salted fish is an important cofactor, but the mechanism is unknown. Several additional somatic genetic alterations have been described in nasopharyngeal carcinoma, which may contribute to the predominance of a specific clone or to tumour progression. Cases of familial aggregation have been well documented, but susceptibility genes for this cancer have not been investigated.

5.4.5   *Other malignancies, including lymphoepithelial carcinomas*

EBV is strongly associated with some other tumours. These include lymphoepithelial carcinomas of the stomach, the salivary glands and the lungs. EBV has also been identified in a small but consistent proportion of gastric adenocarcinomas. In all cases analysed, monoclonal viral genomes have been found, indicating the presence of EBV before the onset of proliferation of the malignant cell clone. In contrast, the patterns of EBV latent gene expression are variable, type-I latency being prevalent in the gastric carcinomas and type-II latency in lymphoepithelial carcinomas at other sites. This implies different contributions of EBV to these neoplastic processes.

5.5   Evaluation

There is *sufficient evidence* for the carcinogenicity of EBV in the causation of Burkitt's lymphoma, sinonasal angiocentric T-cell lymphoma, immunosuppression-related lymphoma, Hodgkin's disease and nasopharyngeal carcinoma.

EBV is *carcinogenic to humans (Group 1)*.

6.   References

Abbot, S.D., Rowe, M., Cadwallader, K., Ricksten, A., Gordon, J., Wang, F., Rymo, L. & Rickinson, A.B. (1990) Epstein-Barr virus nuclear antigen 2 induces expression of the virus-encoded latent membrane protein. *J. Virol.*, **64**, 2126–2134

Abdel-Hamid, M., Chen, J.-J., Constantine, N., Massoud, M. & Raab-Traub, N. (1992) EBV strain variation: Geographical distribution and relation to disease state. *Virology*, **190**, 168–175

Ablashi, D.V., de Thé, G., Easton, J.M., Liabeuf, A., Levine, P.H. & Armstrong, G.R. (1974) Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. *Biomedicine*, **20**, 288–293

Ablashi, D.V., Easton, J.M. & Pearson, G.R. (1977) Transformation of squirrel monkey lymphocytes by Epstein-Barr virus: Effect of donor age. *Cancer Lett.*, **3**, 319–324

Ablashi, D.V., Glaser, R., Easton, J.M., Nonoyama, M. & Armstrong, G.R. (1978) Athymic nude mice: Induction of tumors containing Epstein-Barr virus using Burkitt's-related cell lines. *Exp. Hematol.*, **6**, 365–374

Abo, W., Takada, K., Kamada, M., Imamura, M., Motoya, T., Iwanga, M., Aya, T., Yano, S., Nakao, T. & Osato, T. (1982) Evolution of infectious mononucleosis into Epstein-Barr virus carrying monoclonal malignant lymphoma. *Lancet*, **i**, 1272–1276

Abramson, J.H., Pridan, H., Sacks, M.I., Avitzour, M. & Peritz, E. (1978) A case–control study of Hodgkin's disease in Israel. *J. natl Cancer Inst.*, **61**, 307–314

Adams, A. & Lindahl, T. (1975) Epstein-Barr virus genomes with properties of circular DNA molecules in carrier cells. *Proc. natl Acad. Sci. USA*, **72**, 1477–1481

Adams, J.M., Gerondakis, S., Webb, E., Corcoran, L.M. & Cory, S. (1983) Cellular *myc* onco-gene is altered by chromosome translocation to an immunoglobulin locus in murine plasma-cytomas and is rearranged similarly in human Burkitt lymphomas. *Proc. natl Acad. Sci. USA*, **80**, 1982–1986

Aderele, W.I., Seriki, O. & Osunkoya, B.O. (1975) Pleural effusion in Burkitt's lymphoma. *Br. J. Cancer*, **32**, 745–746

Adldinger, H.K., Delius, H., Freese, U.K., Clarke, J. & Bornkamm, G.W. (1985) A putative transforming gene of Jijoye virus differs from that of Epstein-Barr virus prototypes. *Virology*, **141**, 221–234

Afonso, L.C.C., Scharton, T.M., Vieira, L.Q., Wysocka, M., Trinchieri, G. & Scott, P. (1994) The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. *Science*, **263**, 235–237

Agathanggelou, A., Niedobitek, G., Chen, R., Nicholls, J., Yin, W. & Young, L.S. (1995) Expression of immune regulatory molecules in Epstein-Barr virus-associated nasopharyngeal carcinomas with prominent lymphoid stroma. Evidence for a functional interaction between epithelial tumor cells and infiltrating lymphoid cells. *Am. J. Pathol.*, **147**, 1152–1160

Agrba, V.Z., Yakovleva, L.A., Lapin, B.A., Sangulija, I.A., Timanovskaya, V.V., Markaryan, D.S., Chuvirov, G.N. & Salmanova, E.A. (1975) The establishment of continuous lympho-blastoid suspension cell culture from hematopoietic organs of baboon (*P. hamadryas*) with malignant lymphoma. *Exp. Pathol.*, **10**, 318–332

Ahearn, J.M., Hayward, S.D., Hickey, J.C. & Fearon, D.T. (1988) Epstein-Barr virus (EBV) infection of murine L cells expressing recombinant human EBV/C3d receptor. *Proc. natl Acad. Sci. USA*, **85**, 9307–9311

Aitken, C., Sengupta, S.K., Aedes, C., Moss, D.J. & Sculley, T.B. (1994) Heterogeneity within the Epstein-Barr virus nuclear antigen 2 gene in different strains of Epstein-Barr virus. *J. gen. Virol.*, **75**, 95–100

Albeck, H., Nielsen, N.H., Hansen, H.E., Bentzen, J., Ockelmann, H.H., Bretlau, P. & Hansen, H.S. (1992) Epidemiology of nasopharyngeal and salivary gland carcinoma in Greenland. *Arct. med. Res.*, **51**, 189–195

Albeck, H., Bentzen, J., Ockelmann, H.H., Nielsen, N.H., Bretlau, P. & Hansen, H.S. (1993) Familial clusters of nasopharyngeal carcinoma and salivary gland carcinomas in Greenland natives. *Cancer*, **72**, 196–200

Albrecht, J.-C., Nicholas, J., Biller, D., Cameron, K.R., Biesinger, B., Newman, C., Wittmann, M.A., Craxton, M.A., Coleman, B., Fleckenstein, B. & Honess, R.W. (1992) Primary structure of the herpesvirus saimiri genome. *J. Virol.*, **66**, 5047–5058

Alexander, F.E., McKinney, P.A., Williams, J., Ricketts, T.J. & Cartwright, R.A. (1991a) Epide-miological evidence for the 'two-disease hypothesis' in Hodgkin's disease. *Int. J. Epide-miol.*, **20**, 354–361

Alexander, F.E., Ricketts, T.J., McKinney, P.A. & Cartwright, R.A. (1991b) Community life-style characteristics and incidence of Hodgkin's disease in young people. *Int. J. Cancer*, **48**, 10–14

Alfieri, C., Birkenbach, M. & Kieff, E. (1991) Early events in Epstein-Barr virus infection of human B lymphocytes. *Virology*, **181**, 595–608

Al-Idrissi, H.Y. (1990) Head and neck cancer in Saudi Arabia: Retrospective analysis of 65 patients. *J. int. med. Res.*, **18**, 515–519

Alkan, S., Ross, C.W., Hanson, C.A. & Schnitzer, B. (1995) Epstein-Barr virus and bcl-2 protein overexpression are not detected in the neoplastic cells of nodular lymphocyte predominance Hodgkin's disease. *Mod. Pathol.*, **8**, 544–547

Allan, G.J., Inman, G.J., Parker, B.D., Rowe, D.T. & Farrell, P.J. (1992) Cell growth effects of Epstein-Barr virus leader protein. *J. gen. Virol.*, **73**, 1547–1551

Allday, M.J., Crawford, D.H. & Griffin, B.E. (1989) Epstein-Barr virus latent gene expression during the initiation of B cell immortalization. *J. gen. Virol.*, **70**, 1755–1764

Allday, M.J. & Farrell, P.J. (1994) Epstein-Barr virus nuclear antigen EBNA3C/6 expression maintains the level of latent membrane protein 1 in G_1-arrested cells. *J. Virol.*, **68**, 3491–3498

Allday, M.J., Crawford, D.H. & Thomas, J.A. (1993) Epstein-Barr virus (EBV) nuclear antigen 6 induces expression of the EBV latent membrane protein and an activated phenotype in Raji cells. *J. gen. Virol.*, **74**, 361–369

Allday, M.J., Sinclair, A., Parker, G., Crawford, D.H. & Farrell, P.J. (1995) Epstein-Barr virus efficiently immortalizes human B cells without neutralizing the function of p53. *EMBO J.*, **14**, 1382–1391

Allison, A.C. (1963) Inherited factors in blood conferring resistance to protozoa. In: Garnham, P.C.C., Pierce, A.E. & Roitt, I., eds, *Immunity to Protozoa*, Oxford, Blackwell Scientific Publications, p. 109

Allison, A.C. & Byars, N.E. (1986) An adjuvant formulation that selectively elicits the formation of antibodies of protective isotype and of cell-mediated immunity *J. immunol. Meth.*, **95**, 157–168

Allison, A.C. & Byars, N.E. (1987) Vaccine technology: Adjuvants for increased efficiency. *Biotechnology*, **5**, 1041–1045

Althoff, J., Mohr, U., Page, N. & Reznik, G. (1974) Carcinogenic effect of dibutylnitrosamine in European hamsters (*Cricetus cricetus*). *J. natl Cancer Inst.*, **53**, 795–800

Altiok, E., Klein, G., Zech, L., Uno, M., Henriksson, B.E., Battat, S., Ono, Y. & Ernberg, I. (1989) Epstein-Barr virus-transformed pro-B cells are prone to illegitimate recombination between the switch region of the mu chain gene and other chromosomes. *Proc. natl Acad. Sci. USA*, **86**, 6333–6337

Åman, P., Lewin, N., Nordström, M. & Klein, G. (1986) EBV-activation of human B-lymphocytes. *Curr. Top. Microbiol. Immunol.*, **132**, 266–271

Ambinder, R.F., Shah, W.A., Rawlins, D.R., Hayward, G.S. & Hayward, S.D. (1990) Definition of the sequence requirements for binding of the EBNA-1 protein to its palindromic target sites in Epstein-Barr virus DNA. *J. Virol.*, **64**, 2369–2379

Ambinder, R.F., Mullen, M.A., Chang, Y.-N., Hayward, G.S. & Hayward, S.D. (1991) Functional domains of Epstein-Barr virus nuclear antigen EBNA-1. *J. Virol.*, **65**, 1466–1478

Ambinder, R.F., Browning, P.J., Lorenzana, I., Leventhal, B.G., Cosenza, H., Mann, R.B., MacMahon, E.M.E., Medina, R., Cardona, V., Grufferman, S., Olshan, A., Levin, A., Petersen, E.A., Blattner, W. & Levine, P.H. (1993) Epstein-Barr virus and childhood Hodgkin's disease in Honduras and the United States. *Blood*, **81**, 462–467

Ambinder, R.F., Robertson, K.D., Moore, S.M. & Yang, J. (1996) Epstein-Barr virus as a therapeutic target in Hodgkin's disease and nasopharyngeal carcinoma. *Sem. Cancer Biol.*, **7**, 217–226

d'Amore, F., Johansen, P., Houmand, A., Weisenburger, D.D. & Mortensen, L.S. (1996) Epstein-Barr virus genome in non-Hodgkin's lymphomas occurring in immunocompetent patients: Highest prevalence in nonlymphoblastic T-cell lymphoma and correlation with a poor prognosis. *Blood*, **87**, 1045-1055

Anagnostopoulos, I., Herbst, H., Niedobitek, G. & Stein, H. (1989) Demonstration of mono-clonal EBV genomes in Hodgkin's disease and Ki-1-positive anaplastic large cell lymphoma by combined Southern blot and in situ hybridization. *Blood*, **74**, 810–816

Anagnostopoulos, I., Hummel, M., Kreschel, C. & Stein, H. (1995) Morphology, immuno-phenotype, and distribution of latently and/or productively Epstein-Barr virus-infected cells in acute infectious mononucleosis: Implications for the interindividual infection route of Epstein-Barr virus. *Blood*, **85**, 744–750

Anagnostopoulos, I., Hummel, M., Kaudewitz, P., Korbjuhn, P., Leoncini, L. & Stein, H. (1996) Low incidence of Epstein-Barr virus presence in primary cutaneous T-cell lymphoproli-ferations. *Br. J. Dermatol.*, **134**, 276-281

Anderson, E.N., Jr, Anderson, M.L. & Ho, H.C. (1978) Environmental backgrounds of young Chinese nasopharyngeal carcinoma patients. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 231–239

Andersson, J. & Ernberg, I. (1988) Management of Epstein-Barr virus infections. *Am. J. Med.*, **85** (Suppl. 2A), 107–115

Andersson, M.L., Klein, G., Ziegler, J.L. & Henle, W. (1976) Association of Epstein–Barr viral genomes with American Burkitt lymphoma. *Nature*, **260**, 357–359

Andersson, M.L., Stam, N.J., Klein, G., Ploegh, H.L. & Masucci, M.G. (1991) Aberrant expression of HLA class-I antigens in Burkitt lymphoma cells. *Int. J. Cancer*, **47**, 544–550

Angel, C.A., Slater, D.N., Royds, J.A., Nelson, S.N.P. & Bleehen, S.S. (1996) Absence of Epstein-Barr viral encoded RNA (EBER) in primary cutaneous T-cell lymphoma. *J. Pathol.*, **178**, 173-175

Anon. (1982) The non-Hodgkin's lymphoma pathologic classification project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage. *Cancer,* **49**, 2112–2135

Anwar, N., Kingma, D.W., Bloch, A.R., Mourad, M., Raffeld, M., Franklin, J., Magrath, I., El Bolkainy, N. & Jaffe, E.S. (1995) The investigation of Epstein-Barr viral sequences in 41 cases of Burkitt's lymphoma from Egypt. Epidemiologic correlations. *Cancer*, **76**, 1245–1252

Araujo, I., Foss, H.-D., Bittencourt, A., Hummel, M., Demel, G., Mendonca, N., Herbst, H. & Stein, H. (1996) Expression of Epstein-Barr virus gene products in Burkitt's lymphoma in Northeast Brazil, *Blood*, **87**, 5279–5286

Arber, D.A., Weiss, L.M., Albújar, P.F., Chen, Y.-Y. & Jaffe, E.S. (1993) Nasal lymphomas in Peru. High incidence of T-cell immunophenotype and Epstein-Barr virus infection. *Am. J. surg. Pathol.*, **17**, 392-399

Arber, D.A., Kamel, O.W., van de Rijn, M., Davis, R.E., Medeiros, L.J., Jaffe, E.S. & Weiss, L.M. (1995) Frequent presence of the Epstein-Barr virus in inflammatory pseudotumor. *Hum. Pathol.*, **26**, 1093–1098

Armitage, J.M., Kormos, R.L., Stuart, R.S., Fricker, F.J., Griffith, B.P., Nalesnik, M., Hardesty, R.L. & Dummer, J.S. (1991) Posttransplant lymphoproliferative disease in thoracic organ transplant patients: Ten years of cyclosporine-based immunosuppression. *J. Heart Lung Transplant.*, **10**, 877–886

Armstrong, R.W. & Armstrong, M.J. (1983) Environmental risk factors and nasopharyngeal carcinoma in Selangor, Malaysia: A cross-ethnic perspective. *Ecol. Dis.*, **2**, 185–198

Armstrong, D., Henle, G. & Henle, W. (1966) Complement fixation tests with cell lines derived from Burkitt's lymphoma and acute leukemias. *J. Bacteriol.*, **19**, 1257–1262

Armstrong, R.W., Kannan Kutty, M. & Armstrong, M.J. (1978) Self-specific environments associated with nasopharyngeal carcinoma in Selangor, Malaysia. *Soc. Sci. Med.*, **12D**, 149–156

Armstrong, R.W., Kannan Kutty, M., Dharmalingam, S.K. & Ponnudurai, J.R. (1979) Incidence of nasopharyngeal carcinoma in Malaysia, 1968–1977. *Br. J. Cancer*, **40**, 557–567

Armstrong, R.W., Armstrong, M.J., Yu, M.C. & Henderson, B.E. (1983) Salted fish and inhalants as risk factors for nasopharyngeal carcinoma in Malaysian Chinese. *Cancer Res.*, **43**, 2967–2970

Armstrong, A.A., Weiss, L.M., Gallagher, A., Jones, D.B., Krajewski, A.S., Angus, B., Brown, G., Jack, A.S., Wilkins, B.S., Onions, D.E. & Jarrett, R.F. (1992) Critera for the definition of EBV association in Hodgkin's disease. *Leukemia*, **6**, 869–874

Arrand, J.R. & Rymo, L. (1982) Characterization of the major Epstein-Barr virus-specific RNA in Burkitt lymphoma-derived cells. *J. Virol.*, **41**, 376–389

Arribas, J.R., Clifford, D.B., Fichtenbaum, C.J., Roberts, R.L., Powderly, W.G. & Storch, G.A. (1995) Detection of Epstein-Barr virus DNA in cerebrospinal fluid for diagnosis of AIDS-related central nervous system lymphoma. *J. clin. Microbiol.*, **33**, 1580-1583

Arvanitakis, L., Yaseen, N. & Sharma, S. (1995) Latent membrane protein-1 induces cyclin D2 expression, pRb hyperphosphorylation, and loss of TGF-beta 1-mediated growth inhibition in EBV-positive B cells. *J. Immunol.*, **155**, 1047-1056

Asada, H., Okada, N., Hashimoto, K., Yamanishi, K., Sairenji, T., Hirota, S., Nomura, S., Kitamura, Y. & Yoshikawa, K. (1994) Establishment and characterization of the T-cell line, EBT-8 latently infected with Epstein-Barr virus from large granular lymphocyte leukemia. *Leukemia*, **8**, 1415-1423

Askew, D.S., Ashmun, R.A., Simmons, B.C. & Cleveland, J.L. (1991) Constitutive c-*myc* expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene*, **6**, 1915–1922

Austin, P.J., Flemington, E., Yandava, C.N., Strominger, J.L. & Speck, S.H. (1988) Complex transcription of the Epstein-Barr virus *Bam*HI fragment H rightward open reading frame 1 (BHRF1) in latently and lytically infected B lymphocytes. *Proc. natl Acad. Sci. USA*, **85**, 3678–3682

Axelsen, S.M. & Stamp, I.M. (1995) Lymphoepithelioma-like carcinoma of the vulvar region. *Histopathology*, **27**, 281–283

Bacchi, M.M., Bacchi, C.E., Alvarenga, M., Miranda, R, Chen, Y.-Y. & Weiss, L.M. (1996a) Burkitt's lymphoma in Brazil: Strong association with Epstein-Barr virus. *Mod. Pathol.*, **9**, 63–67

Bacchi, C.E., Bacchi, M.M., Rabenhorst, S.H., Soares, F.A., Fonseca, L.E., Jr, Barbosa, H.S., Weiss, L.M. & Gown, A.M. (1996b) AIDS-related lymphoma in Brazil. Histopathology, immunophenotype, and association with Epstein-Barr virus. *Am. J. clin. Pathol.*, **105**, 230-237

Baer, R., Bankier, A.T., Biggin, M.D., Deininger, P.L., Farrell, P.J., Gibson, T.J., Hatfull, G., Hudson, G.S., Satchwell, S.C., Séguin, C., Tuffnell, P.S. & Barrell, B.G. (1984) DNA sequence and expression of the B95-8 Epstein-Barr virus genome. *Nature*, **310**, 207-211

Bai, M.C., Jiwa, N.M., Horstman, A., Vos, W., Kluin, P.H., Van Der Valk, P., Mullink, H., Walboomers, J.M.M. & Meijer, C.J.L.M. (1994) Decreased expression of cellular markers in Epstein-Barr virus-positive Hodgkin's disease. *J. Pathol.*, **174**, 49-55

Baichwal, V.R. & Sugden, B. (1988) Transformation of Balb 3T3 cells by the BNLF-1 gene of Epstein-Barr virus. *Oncogene*, **2**, 461-467

Baichwal, V.R. & Sugden, B. (1989) The multiple membrane-spanning segments of the BNLF-1 oncogene from Epstein-Barr virus are required for transformation. *Oncogene*, **4**, 67-74

Baikie, A.G., Kinlen, L.J. & Pike, M.C. (1972) Detection and assessment of case clustering in Burkitt's lymphoma and Hodgkin's disease. *Recent Results Cancer Res.*, **39**, 201-209

Balakrishnan, V. (1975) An additional younger-age peak for cancer of the nasopharynx. *Int. J. Cancer*, **15**, 651-657

Ballerini, P., Gaidano, G., Gong, J.Z., Tassi, V., Saglio, G., Knowles, D.M. & Dalla-Favera, R. (1993) Multiple genetic lesions in acquired immunodeficiency syndrome-related non-Hodgkin's lymphoma. *Blood*, **81**, 166-176

Banchereau, J. & Rousset, F. (1991) Growing human B lymphocytes in the CD40 system, *Nature*, **353**, 678-679

Banchereau, J., de Paoli, P., Vallé, A., Garcia, E. & Rousset, F. (1991) Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science*, **251**, 70-72

Bashir, R.M., Hochberg, F.H., Harris, N.L. & Purtilo, D. (1990) Variable expression of Epstein-Barr virus genome as demonstrated by in situ hybridization in central nervous system lymphomas in immunocompromised patients. *Mod. Pathol.*, **3**, 429-434

Bashir, R., McManus, B., Cunningham, C., Weisenburger, D. & Hochberg, F.H. (1994) Detection of EBER-1 RNA in primary brain lymphomas in immunocompetent and immuno-compromised patients. *J. Neuro-Oncol.*, **20**, 47-53

Baskies, A.M., Chretien, P.B., Yang, C.-S., Wolf, G.T., Makuch, R.W., Tu, S.-M., Hsu, M.-M., Lynn, T.-C., Yang, H.-M., Weiss, J.F. & Spiegel, H.E. (1979) Serum glycoproteins and immunoglobulins in nasopharyngeal carcinoma: Correlations with Epstein-Barr virus associated antibodies and clinical tumor stage. *Am. J. Surg.*, **138**, 478-488

Bayliss, G.J. & Wolf, H. (1980) Epstein-Barr virus-induced cell fusion. *Nature*, **287**, 164-165

Bayliss, G.J. & Wolf, H. (1981) An Epstein-Barr virus early protein induces cell fusion. *Proc. natl Acad. Sci. USA*, **78**, 7162-7165

Beaufils, P., Choquet, D., Mamoun, R.Z. & Malissen, B. (1993) The $(YXXL/I)_2$ signalling motif found in the cytoplasmic segments of the bovine leukaemia virus envelope protein and Epstein-Barr virus latent membrane protein 2A can elicit early and late lymphocyte activation events. *EMBO J.*, **12**, 5105-5112

Beigel, A., Peulen, J.F. & Westphal, E. (1983) The spectrum of histocompatibility antigens (HLA) in tumours of the nasopharynx. *Arch. Otorhinolaryngol.*, **237**, 285-288 (in German)

Beisel, C., Tanner, J., Matsuo, T., Thorley-Lawson, D., Kezdy, F. & Kieff, E. (1985) Two major outer envelope glycoproteins of Epstein-Barr virus are encoded by the same gene. *J. Virol.*, **54**, 665–674

Bejarano, M.T., Masucci, M.G., Morgan, A., Morein, B., Klein, G. & Klein, E. (1990) Epstein-Barr virus (EBV) antigens processed and presented by B cells, B blasts and macrophages trigger T-cell-mediated inhibition of EBV-induced B-cell transformation. *J. Virol.*, **64**, 1398–1401

Bell, R.B. & Maguda, T.A. (1970) Nasopharyngeal carcinoma in Caucasian siblings: Report of two cases. *J. Tennessee med. Assoc.*, **63**, 753-754

Benjamin, D., Magrath, I.T., Maguire, R., Janus, C,. Todd, H.D. & Parsons, R.G. (1982) Immunoglobulin secretion by cell lines derived from African and American undifferentiated lymphomas of Burkitt's and non-Burkitt's type. *J. Immunol.*, **129**, 1336–1342

Benjamin, D., Magrath, I.T., Triche, T.J., Schroff, R.W., Jensen, J.P. & Korsmeyer, S.J. (1984) Induction of plasmacytoid differentiation by phorbol ester in B-cell lymphoma cell lines bearing 8;14 translocations. *Proc. natl Acad Sci USA*, **81**, 3547–3551

Ben-Sasson, S.A. & Klein, G. (1981) Activation of the Epstein-Barr virus genome by 5-azacytidine in latently infected human lymphoid lines. *Int. J. Cancer*, **28**, 131–135

Beral, V., Peterman, T., Berkelman, R. & Jaffe, H. (1991) AIDS-associated non-Hodgkin lymphoma. *Lancet*, **337**, 805–809

Berard, C., O'Connor, G.T., Thomas, L.B. & Torloni, H. (1969) Histopathological definition of Burkitt's tumour. *Bull. World Health Organ.*, **40**, 601–607

Berger, F. & Delecluse, H.J. (1993) Lymphomas in immunocompromised hosts. *Rev. Prat.*, **43**, 1661–1664 (in French)

Bergmann, M., Blasius, S., Bankfalvi, A. & Mellin, W. (1995/96) Primary non-Hodgkin lymphomas of the CNS: Proliferation, oncoproteins and Epstein-Barr virus. *Gen. diagn. Pathol.*, **141**, 235-242

Bernard, S.M., Cartwright, R.A., Bird, C.C., Richards, I.D.G., Lauder, I. & Roberts, B.E. (1984) Aetiologic factors in lymphoid malignancies: A case–control epidemiological study. *Leukemia Res.*, **8**, 681–689

Bernard, S.M., Cartwright, R.A., Darwin, C.M., Richards, I.D.G., Roberts, B.E., O'Brien, C.O. & Bird, C.C. (1987) Hodgkin's disease: Case control epidemiological study in Yorkshire. *Br. J. Cancer*, **55**, 85–90

Bernheim, A. & Berger, R. (1988) Cytogenetic studies of Burkitt lymphoma–leukemia in patients with acquired immunodeficiency syndrome. *Cancer Genet. Cytogenet.*, **32**, 67–74

Bernheim, A., Berger, R. & Lenoir, G.M. (1981) Cytogenetic studies on African Burkitt's lymphoma cell lines: t(8;14), t(2;8) and t(8;22) translocations. *Cancer Genet. Cytogenet.*, **3**, 307–315

Betuel, H., Camoun, M., Colombani, J., Day, N.E., Ellouz, R. & de Thé, G. (1975) The relationship between nasopharyngeal carcinoma and the HL-A system among Tunisians. *Int. J. Cancer*, **16**, 249–254

Bhagat, S.K.M., Medeiros, L.J., Weiss, L.M., Wang, J., Raffeld, M. & Stetler-Stevenson, M. (1993) *Bcl-2* expression in Hodgkin's disease: Correlation with the t(14;18) translocation and Epstein-Barr virus. *Am. J. clin. Pathol.*, **99**, 604–608

Bhatia, K., Gutiérrez, M.I., Huppi, K., Siwarski, D. & Magrath, I.T. (1992) The pattern of *p53* mutations in Burkitt's lymphoma differs from that of solid tumors. *Cancer Res.*, **52**, 4273–4276

Bhatia, K., Spangler, G., Gaidano, G., Hamdy, N., Dalla-Favera, R. & Magrath, I. (1994) Mutations in the coding region of c-myc occur frequently in acquired immunodeficiency syndrome-associated lymphomas. *Blood*, **84**, 883–888

Bhatia, K., Spangler, G., Hamdy, N., Neri, A., Brubaker, G., Levin, A. & Magrath, I.T. (1995) Mutations in the coding region of c-myc occur independently of mutations in the regulatory regions and are predominantly associated with myc/Ig translocations. *Curr. Top. Microbiol. Immunol.*, **194**, 389–398

Bhatia, K., Raj, A., Guitiérrez, M.I., Judde, J.G., Spangler, G., Venkatesh, H. & Magrath, I.T. (1996) Variation in the sequence of Epstein Barr virus nuclear antigen 1 in normal peripheral blood lymphocytes and in Burkitt's lymphomas. *Oncogene*, **13**, 177–181

Biberfeld, P., Christensson, B., Andersson-Anvret, M., Ernberg, I., Lewensohn, R., Ekman, M., Johansson, B. & Tribukait, B. (1981) An EBV-associated, Swedish case of Burkitt-type malignant lymphoma. *Acta pathol. microbiol. scand. Sect. A*, **89**, 417–424

Bienzle, U., Guggenmoos-Holzmann, I. & Luzzatto, L. (1981) Plasmodium falciparum malaria and human red cells. I. A genetic and clinical study in children. *Int. J. Epidemiol.*, **10**, 9–15

Biggar, R.J. & Nkrumah, F.K. (1979) Burkitt's lymphoma in Ghana: Urban–rural distribution, time–space clustering and seasonality. *Int. J. Cancer*, **23**, 330–336

Biggar, R.J., Henle, W., Fleisher, G., Böcker, J., Lennette, E.T. & Henle, G. (1978) Primary Epstein-Barr virus infection in African infants. I. Decline of maternal antibodies and time of infection. *Int. J. Cancer*, **22**, 239–243

Biggar, R.J., Gardiner, C., Lennette, E.T., Collins, W.E., Nkrumah, F.K. & Henle, W. (1981) Malaria, sex and place of residence as factors in antibody response to Epstein-Barr virus in Ghana, West Africa. *Lancet*, **ii**, 115–118

Bignon, Y.-J., Bernard, D., Curé, H., Fonck, Y., Pauchard, J., Travade, P., Legros, M., Dastugue, B. & Plagne, R. (1990) Detection of Epstein-Barr viral genomes in lymph-nodes of Hodgkin's disease patients. *Mol. Carcinog.*, **3**, 9–11

Bignon, Y.-J., Clavelou, P., Ramos, F., Jouvet, A., Tommasi, M., Tournilhac, M., Dastugue, B. & Plagne, R. (1991) Detection of Epstein-Barr virus sequences in primary brain lymphoma without immunodeficiency. *Neurology*, **41**, 1152-1153

Billaud, M., Busson, P., Huang, D., Mueller-Lantzch, N., Rousselet, G., Pavlish, O., Wakasugi, H., Seigneurin, J.M., Tursz, T. & Lenoir, G.M. (1989) Epstein-Barr virus (EBV)-containing nasopharyngeal carcinoma cells express the B-cell activation antigen Blast 2/CD23 and low levels of the EBV receptor CR2. *J. Virol.*, **63**, 4121–4128

Billaud, M., Rousset, F., Calender, A., Cordier, M., Aubry, J.P., Laisse, V. & Lenoir, G.M. (1990) Low expression of lymphocyte function-associated antigen (LFA)-1 and LFA-3 adhesion molecules is a common trait in Burkitt's lymphoma associated with and not associated with Epstein-Barr virus. *Blood*, **75**, 1827–1833

Birkenbach, M., Liebowitz, D., Wang, F., Sample, J. & Kieff, E. (1989) Epstein-Barr virus latent infection membrane protein increases vimentin expression in human B-cell lines. *J. Virol.*, **63**, 4079–4084

Birkenbach, M., Tong, X., Bradbury, L.E., Tedder, T.F. & Kieff, E. (1992) Characterization of an Epstein-Barr virus receptor on human epithelial cells. *J. exp. Med.*, **176**, 1405–1414

Birkenbach, M., Josefsen, K., Yalamanchili, R., Lenoir, G.M. & Kieff, E. (1993) Epstein-Barr virus-induced genes: First lymphocyte-specific G protein-coupled peptide receptors. *J. Virol.*, **67**, 2209–2220

Birx, D.L., Redfield, R.R. & Tosato, G. (1986) Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. *New Engl. J. Med.*, **314**, 874–879

Black, F.L., Woodall, J.P., Evans, A.S., Liebhaber, H. & Henle, G. (1970) Prevalence of antibody against viruses in the Tiriyo, an isolated Amazon tribe. *Am. J. Epidemiol.*, **91**, 430–438

Blair, A., Stewart, P.A., O'Berg, M., Gaffey, W., Walrath, J., Ward, J., Bales, R., Kaplan, S. & Cubit, D. (1986) Mortality among industrial workers exposed to formaldehyde. *J. natl Cancer Inst.*, **76**, 1071–1084

Blair, A., Stewart, P.A., Hoover, R.N., Fraumeni, J.F., Jr, Walrath, J., O'Berg, M. & Gaffey, W. (1987) Cancers of the nasopharynx and oropharynx and formaldehyde exposure (Letter to the Editor). *J. natl Cancer Inst.*, **78**, 191–192

Blasco, J., Guido, M., Carestia, M., Power, C., Drut, M. & Drut, R. (1996) Gastric undifferentiated lymphoepithelial carcinoma with Epstein-Barr virus. *Medicina*, **56**, 287–290 (in Spanish)

Blazar, B., Patarroyo, M., Klein, E. & Klein, G. (1980) Increased sensitivity of human lymphoid lines to natural killer cells after induction of the Epstein-Barr viral cycle by superinfection or sodium butyrate. *J. exp. Med.*, **151**, 614–627

Bochkarev, A., Barwell, J.A., Pfuetzner, R.A., Furey, W., Jr, Edwards, A.M. & Frappier, L. (1995) Crystal structure of the DNA-binding domain of the Epstein-Barr virus origin-binding protein EBNA1. *Cell*, **83**, 39–46

Böcker, J.F., Tiedemann, K.H., Bornkamm, G.W. & zur Hausen, H. (1980) Characterization of an EBV-like virus from African green monkey lymphoblasts. *Virology*, **101**, 291–295

Bodescot, M. & Perricaudet, M. (1986) Epstein-Barr virus mRNAs produced by alternative splicing. *Nucleic Acids Res.*, **14**, 7103–7114

Bodescot, M., Brison, O. & Perricaudet, M. (1986) An Epstein-Barr virus transcription unit is at least 84 kilobases long. *Nucleic Acids Res.*, **14**, 2611–2620

Bogedain, C., Wolf, H., Modrow, S., Stuber, G. & Jilg, W. (1995) Specific cytotoxic T lymphocytes recognize the immediate-early transactivator Zta of Epstein-Barr virus. *J. Virol.*, **69**, 4872–4879

Bogger-Goren, S., Zaizov, R., Vogel, R., Leventon-Kriss, S., Sayar, Y. & Gotlieb-Stematsky, T. (1983) Clinical and virogical observations in childhood Hodgkin's disease in Israel. *Isr. J. med. Sci.*, **19**, 989–991

Boiocchi, M., Carbone, A., De Re, V. & Dolcetti, R. (1989) Is the Epstein-Barr virus involved in Hodgkin's disease? *Tumori*, **75**, 345–350

Boiocchi, M., De Re, V., Dolcetti, R., Volpe, R., Gloghini, A., Tavian, M., Viel, A., Maestro, R. & Carbone, A. (1990) Pathogenesis of malignant lymphomas in intravenous drug-abuser, HIV-infected patients. *Cancer Detect. Prev.*, **14**, 661-668

Boiocchi, M., Dolcetti, R., De Re, V., Gloghini, A. & Carbone, A. (1993a) Demonstration of a unique Epstein-Barr virus-positive cellular clone in metachronous multiple localizations of Hodgkin's disease. *Am. J. Pathol.*, **142**, 33–38

Boiocchi, M., De Re, V., Gloghini, A., Vaccher, E., Dolcetti, R., Marzotto, A., Bertola, G. & Carbone, A. (1993b) High incidence of monoclonal EBV episomes in Hodgkin's disease and anaplastic large-cell KI-1-positive lymphomas in HIV-1-positive patients. *Int. J. Cancer*, **54**, 53–59

Bonelli, L., Vitale, V., Bistolfi, F., Landucci, M. & Bruzzi, P. (1990) Hodgkin's disease in adults: Association with social factors and age at tonsillectomy. A case–control study. *Int. J. Cancer*, **45**, 423–427

Booth, K., Burkitt, D.P., Bassett, D.J., Cooke, R.A. & Biddulph, J. (1967) Burkitt lymphoma in Papua, New Guinea. *Br. J. Cancer*, **21**, 657–664

Borisch, B., Kirchner, T., Marx, A. & Müller-Hermelink, H.K. (1990) Absence of the Epstein-Barr virus genome in the normal thymus, thymic epithelial tumors, thymic lymphoid hyperplasia in a European population. *Virchows Arch. B. Cell Pathol.*, **59**, 359–365

Borisch, B., Finke, J., Hennig, I., Delacrétaz, F., Schneider, J., Heitz, P.U. & Laissue, J.A. (1992a) Distribution and localization of Epstein-Barr virus subtypes A and B in AIDS-related lymphomas and lymphatic tissue of HIV-positive patients. *J. Pathol.*, **168**, 229-236

Borisch, B., Hennig, I., Horber, F., Bürki, K. & Laissue, J. (1992b) Enteropathy-associated T-cell lymphoma in a renal transplant patient with evidence of Epstein-Barr virus involvement. *Virchows Arch. A Pathol.*, **421**, 443–447

Borisch-Chappuis, B., Nezelof, C., Müller, H. & Müller-Hermelink, H.K. (1990a) Different Epstein-Barr virus expression in lymphomas from immunocompromised and immunocompetent patients. *Am. J. Pathol.*, **136**, 751–758

Borisch-Chappuis, B., Müller, H., Stutte, J., Hey, M.M., Hübner, K. & Müller-Hermelink, H.K. (1990b) Identification of EBV-DNA in lymph nodes from patients with lymphadenopathy and lymphomas associated with AIDS. *Virchows Arch. B Cell Pathol.*, **58**, 199–205

Bornkamm, G.W., Stein, H., Lennert, K., Rüggeberg, F., Bartels, F. & zur Hausen, H. (1976) Attempts to demonstrate virus-specific sequences in human tumors. IV. EB viral DNA in European Burkitt lymphoma and immunoblastic lymphadenopathy with excessive plasma-cytosis. *Int. J. Cancer*, **17**, 177–181

Bornkamm, G.W., Delius, H., Zimber, U., Hudewentz, J. & Epstein, M.A. (1980) Comparison of Epstein-Barr virus strains of different origin by analysis of the viral DNAs. *J. Virol.*, **35**, 603–618

Bornkamm, G.W., Hudewentz, J., Freese, U.K. & Zimber, U. (1982) Deletion of the non-transforming Epstein-Barr virus strain P3HR-1 causes fusion of the large internal repeat to the DS_L region. *J. Virol.*, **43**, 952–968

Bornkamm, G.W., Polack, A. & Eick, D. (1988) c-myc deregulation by chromosomal translocation in Burkitt's lymphoma. In: Klein, G., ed., *Cellular Oncogene Activation*, New York, M. Dekker, pp. 223–273

Borras, A.M., Strominger, J.L. & Speck, S.H. (1996) Characterization of the ZI domains in the Epstein-Barr virus BZLF1 gene promoter: Role in phorbol ester induction. *J. Virol.*, **70**, 3894–3901

van den Bosch, C., Griffin, B.E., Kazembe, P., Dziweni, C. & Kadzamira, L. (1993) Are plant factors a missing link in the evolution of endemic Burkitt's lymphoma? *Br. J. Cancer*, **68**, 1232–1235

Bouvier, G., Hergenhahn, M., Polack, A., Bornkamm, G.W., de Thé, G. & Bartsch, H. (1995) Characterization of macromolecular lignins as Epstein-Barrr virus inducer in foodstuff associated with nasopharyngeal carcinoma risk. *Carcinogenesis*, **16**, 1879–1885

Boyle, M.J., Sewell, W.A., Sculley, T.B., Apolloni, A., Turner, J.J., Swanson, C.E., Penny, R. & Cooper, D.A. (1991) Subtypes of Epstein-Barr virus in human immunodeficiency virus-associated non-Hodgkin's lymphoma. *Blood*, **78**, 3004-3011

Boyle, M.J., Vasak, E., Tschuchnigg, M., Turner, J.J., Sculley, T.B., Penny, R., Cooper, D.A., Tindall, B. & Sewell, W.A. (1993) Subtypes of Epstein-Barr virus (EBV) in Hodgkin's disease: Association between B-type EBV and immunocompromise. *Blood*, **81**, 468–474

Brichácek, B., Hirsch, I., Šíbl, O., Vilikusová, E. & Vonka, V. (1983) Association of some supraglottic laryngeal carcinomas with EB virus. *Int. J. Cancer.*, **32**, 193–197

Brichácek, B., Hirsch, I., Šíbl, O., Vilikusová, E. & Vonka, V. (1984) Presence of Epstein-Barr virus DNA in carcinomas of the palatine tonsil. *J. natl Cancer Inst.*, **72**, 809–815

Brielmeier, M., Mautner, J., Laux, G. & Hammerschmidt, W. (1996) The latent membrane protein 2 gene of Epstein-Barr virus is important for efficient B cell immortalization. *J gen. Virol.*, **77**, 2807–2818

Brocksmith, D., Angel, C.A., Pringle, J.H. & Lauder, I. (1991) Epstein-Barr viral DNA in Hodgkin's disease: Amplification and detection using the polymerase chain reaction. *J. Pathol.*, **165**, 11–15

Brooks, L.A., Yao, Q.Y., Rickinson, A.B. & Young, L.S. (1992) Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: Co-expression of EBNA1, LMP1 and LMP2 transcripts. *J. Virol.*, **66**, 2689–2697

Brooks, L.A., Lear, A.L., Young, L.S. & Rickinson, A.B. (1993a) Transcripts from the Epstein-Barr virus *Bam*HI A fragment are detectable in all three forms of virus latency. *J. Virol.*, **67**, 3182–3190

Brooks, J.M., Murray, R.J., Thomas, W.A., Kurilla, M.G. & Rickinson, A.B. (1993b) Different HLA-B27 subtypes present the same immunodominant Epstein-Barr virus peptide. *J. exp. Med.*, **178**, 879–887

Brousset, P., Chittal, S., Schlaifer, D., Icart, J., Payen, C., Rigal-Huguet, F., Voigt, J.-J. & Delsol, G. (1991) Detection of Epstein-Barr virus messenger RNA in Reed-Sternberg cells of Hodgkin's disease by in situ hybridization with biotinylated probes on specially processed modified acetone methyl benzoate xylene (ModAMeX) sections. *Blood*, **77**, 1781–1786

Brousset, P., Butet, V., Chittal, S., Selves, J. & Delsol, G. (1992) Comparison of in situ hybridization using different nonisotopic probes for detection of Epstein-Barr virus in nasopharyngeal carcinoma and immunohistochemical correlation with anti-latent membrane protein antibody. *Lab. Invest.*, **67**, 457–464

Brousset, P., Knecht, H., Rubin, B., Drouet, E., Chittal, S., Meggetto, F., Al Saati, T., Bachmann, E., Denoyel, G., Sergeant, A. & Delsol, G. (1993) Demonstration of Epstein-Barr virus replication in Reed-Sternberg cells of Hodgkin's disease. *Blood*, **82**, 872–876

Brousset, P., Schlaifer, D., Meggetto, F., Bachmann, E., Rothenberger, S., Pris, J., Delsol, G. & Knecht, H. (1994) Persistence of the same viral strain in early and late relapses of Epstein-Barr virus-associated Hodgkin's disease. *Blood*, **84**, 2447–2451

Brown, T.M., Jr, Heath, C.W., Jr, Lang, R.M., Lee, S.K. & Whalley, B.W. (1976) Nasopharyngeal cancer in Bermuda. *Cancer*, **37**, 1464–1468

Brown, N.A., Liu, C., Garcia, C.R., Wang, Y.-F., Griffith, A., Sparkes, R.S. & Calame, K.L. (1986) Clonal origins of lymphoproliferative disease induced by Epstein-Barr virus. *J. Virol.*, **58**, 975–978

Brubaker, G., Geser, A. & Pike, M.C. (1973) Burkitt's lymphoma in the North Mara district of Tanzania 1964–70: Failure to find evidence of time–space clustering in a high risk isolated rural area. *Br. J. Cancer*, **28**, 469–472

Brubaker, G., Levin, A.G., Steel, C.M., Creasey, G., Cameron, H.M., Linsell, C.A. & Smith, P.G. (1980) Multiple cases of Burkitt's lymphoma and other neoplasms in families in the North Mara district of Tanzania. *Int. J. Cancer*, **26**, 165–170

Buckmaster, A.E., Scott, S.D., Sanderson, M.J., Boursnell, M.E.G., Ross, N.L.J. & Binns, M.M. (1988) Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: Implication for herpesvirus classification. *J. gen. Virol.*, **69**, 2033–2042

Buell, P. (1973) Race and place in the etiology of nasopharyngeal cancer: A study based on California death certificates. *Int. J. Cancer*, **11**, 268–272

Buell, P. (1974) The effect of migration on the risk of nasopharyngeal cancer among Chinese. *Cancer Res.*, **34**, 1189–1191

Burdin, N., Péronne, C., Banchereau, J. & Rousset, F. (1993) Epstein-Barr virus transformation induces B lymphocytes to produce human interleukin 10. *J. exp. Med.*, **177**, 295–304

Burke, A.P., Yen, T.S.B., Shekitka, K.M. & Sobin, L.H. (1990) Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. *Mod. Pathol.*, **3**, 377–380

Burkhardt, A.L., Bolen, J.B., Kieff, E. & Longnecker, R. (1992) An Epstein-Barr virus transformation-associated membrane protein interacts with *src* family tyrosine kinases. *J. Virol.*, **66**, 5161–5167

Burkitt, D.P. (1958) A sarcoma involving the jaws in African children. *Br. J. Surg.*, **46**, 218–223

Burkitt, D.P. (1962a) Determining the climatic limitations of a children's cancer common in Africa. *Br. med. J.*, **ii**, 1019–1023

Burkitt, D. (1962b) A children's cancer dependent on climatic factors. *Nature*, **194**, 232–234

Burkitt, D. (1962c) A 'tumour safari' in East and Central Africa. *Br. J. Cancer*, **16**, 379–386

Burkitt, D.P. (1963) A lymphoma syndrome dependent on environment. 1. Clinical aspects. In: *Symposium on Lymph Tumours in Africa*, Paris, Karger, pp. 80–93

Burkitt, D.P. (1967) Burkitt's lymphoma outside the known endemic areas of Africa and New Guinea. *Int. J. Cancer*, **2**, 562–565

Burkitt, D.P. (1969) Etiology of Burkitt's lymphoma — An alternative hypothesis to a vectored virus. *J. natl Cancer Inst.*, **42**, 19–28

Burkitt, D.P. (1970a) General features and facial tumours. In: Burkitt, D.P. & Wright, D.H., eds, *Burkitt's Lymphoma*, Edinburgh, E. & S. Livingstone, pp. 6–15

Burkitt, D.P. (1970b) Geographical distribution. In: Burkitt, D.P. & Wright, D.H., eds, *Burkitt's Lymphoma*, Edinburgh, E. & S. Livingstone, pp. 186–197

Burkitt, D.P. (1985) The beginnings of the Burkitt's lymphoma story. In: Lenoir, G.M., O'Connor, G. & Olweny, C.L.M., eds, *Burkitt's Lymphoma: A Human Cancer Model* (IARC Scientific Publications No. 60), Lyon, IARC, pp. 11–15

Burkitt, D.P. & Davies, J.N.P. (1961) Lymphoma syndrome in Uganda and tropical Africa. *Med. Press*, **245**, 367–369

Burkitt, D.P. & Wright, D.H. (1963) A lymphoma syndrome in tropical Africa. *Int. Rev. exp. Pathol.*, 2, 67–138

Burkitt, D.P. & Wright, D.H. (1966) Geographical and tribal distribution of the African lymphoma in Uganda. *Br. med. J.*, i, 569–573

Burrows, S.R., Misko, I.S., Sculley, T.B., Schmidt, C. & Moss, D.J. (1990a) An Epstein-Barr virus-specific cytotoxic T-cell epitope present on A- and B-type transformants. *J. Virol.*, 64, 3974–3976

Burrows, S.R., Sculley, T.B., Misko, I.S., Schmidt, C. & Moss, D.J. (1990b) An Epstein-Barr virus-specific cytotoxic T cell epitope in EBV nuclear antigen 3 (EBNA 3). *J. exp. Med.*, 171, 345–349

Burrows, S.R., Khanna, R., Burrows, J.M. & Moss, D.J. (1994) An alloresponse in humans is dominated by cytotoxic T lymphocytes (CTL) cross-reactive with a single Epstein-Barr virus CTL epitope: Implications for graft-versus-host disease. *J. exp. Med.*, 179, 1155–1161

Burrows, J.M., Khanna, R., Sculley, T.B., Alpers, M.P., Moss, D.J. & Burrows, S.R. (1996a) Identification of a naturally occurring recombinant Epstein-Barr virus isolate from New Guinea that encodes both type 1 and type 2 nuclear antigen sequences. *J. Virol.*, 70, 4829–4833

Burrows, J.M., Burrows, S.R., Poulsen, L.M., Sculley, T.B., Moss, D.J. & Khanna, R. (1996b) Unusually high frequency of Epstein-Barr virus genetic variants in Papua New Guinea that can escape cytotoxic T-cell recognition: Implications for virus evolution. *J. Virol.*, 70, 2490–2496

Burt, R.D., Vaughan, T.L. & McKnight, B. (1992) Descriptive epidemiology and survival analysis of nasopharyngeal carcinoma in the United States. *Int. J. Cancer*, 52, 549–556

Burt, R.D., Vaughan, T.L., Nisperos, B., Swanson, M. & Berwick, M. (1994) A protective association between the HLA-A2 antigen and nasopharyngeal carcinoma in US Caucasians. *Int. J. Cancer*, 56, 465–467

Busson, P., Braham, K., Ganem, G., Thomas, F., Grausz, D., Lipinski, M., Wakasugi, H. & Tursz, T. (1987) Epstein-Barr virus-containing epithelial cells from nasopharyngeal carcinoma produce interleukin 1 alpha. *Proc. natl Acad. Sci. USA*, 84, 6262–6266

Busson, P., Ganem, G., Flores, P., Mugneret, F., Clausse, B., Caillou, B., Braham, K., Wakasugi, H., Lipinski, M. & Tursz, T. (1988) Establishment and characterization of three transplantable EBV-containing nasopharyngneal carcinomas. *Int. J. Cancer*, 42, 599–606

Busson, P., McCoy, R., Sadler, R., Gilligan, K., Tursz, T. & Raab-Traub, N. (1992a) Consistent transcription of the Epstein-Barr virus LMP2 gene in nasopharyngeal carcinoma. *J. Virol.*, 66, 3257–3262

Busson, P., Zhang, Q., Guillon, J.-M., Gregory, C.D., Young, L.S., Clausse, B., Lipinski, M., Rickinson, A.B. & Tursz, T. (1992b) Elevated expression of ICAM1 (CD54) and minimal expression of LFA3 (CD58) in Epstein-Barr-virus-positive nasopharyngeal carcinoma cells. *Int. J. Cancer*, 50, 863–867

Butler, A.E., Colby, T.V., Weiss, L. & Lombard, C. (1989) Lymphoepithelioma-like carcinoma of the lung. *Am. J. surg. Pathol.*, 13, 632–639

Byars, N.E., Nakano, G., Welch, M., Lehman, D. & Allison, A.C. (1991) Improvement of hepatitis B vaccine by the use of a new adjuvant. *Vaccine*, 9, 309–318

Callan, M.F.C., Steven, N., Krausa, P., Wilson, J.D.K., Moss, P.A.H., Gillespie, G.M., Bell, J.I., Rickinson, A.B. & McMichael, A.J. (1996) Large clonal expansions of CD8⁺ T cells in acute infectious mononucleosis. *Nature Med.*, 2, 906–911

Calnek, B.W. (1986) Marek's disease — A model for herpesvirus oncology. *CRC crit. Rev. Microbiol.*, 12, 293–320

Calnek, B.W. & Witter, R.L. (1991) Marek's disease. In: Calnek, B.W., Barnes, H.J., Beard, C.W., Reid, W.M. & Yoder, H.W., Jr, eds, *Diseases of Poultry*, Ames, Iowa, Iowa State University Press, pp. 342–385

Calnek, B.W., Adldinger, H.K. & Kahn, D.E. (1970) Feather follicle epithelium: A source of enveloped and infectious cell-free herpesvirus from Marek's disease. *Avian Dis*, 14, 219–233

Cammoun, M., Vogt Hoerner, G. & Mourali, N. (1974)Tumors of the nasopharynx in Tunisia. An anatomic and clinical study based on 143 cases. *Cancer*, 33, 184–192

de Campos-Lima, P.-O., Gavioli, R., Zhang, Q.-J., Wallace, L.E., Dolcetti, R., Rowe, M., Rickinson, A.B. & Masucci, M.G. (1993a) HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11⁺ population. *Science*, 260, 98–100

de Campos-Lima, P.-O., Torsteinsdóttir, S., Cuomo, L., Klein, G., Sulitzeanu, D. & Masucci, M.G. (1993b) Antigen processing and presentation by EBV-carrying cell lines: Cell-phenotype dependence and influence of the EBV-encoded LMP1. *Int. J. Cancer*, 53, 856–862

de Campos-Lima, P.-O., Levitsky, V., Brooks, J., Lee, S.P., Hu, L.F., Rickinson, A.B. & Masucci, M.G. (1994) T Cell responses and virus evolution: Loss of HLA A11-restricted CTL epitopes in Epstein-Barr virus isolates from highly A11-positive populations by selective mutation of anchor residues. *J. exp. Med.*, 179, 1297–1305

de Campos-Lima, P.-O., Levitskaya, J., Frisan, T. & Masucci, M.G. (1996) Strategies of immunoescape in Epstein-Barr virus persistence and pathogenesis. *Semin. Virol.*, 7, 75–82

Carbone, A., Gloghini, A., Zanette, I., Canal, B. & Volpe, R. (1993a) Demonstration of Epstein-Barr viral genomes by in situ hybridization in acquired immune deficiency syndrome-related high grade and anaplastic large cell CD30⁺ lymphomas. *Am. J. clin. Pathol.*, 99, 289-297

Carbone, A., Tirelli, U., Gloghini, A, Volpe, R. & Boiocchi, M. (1993b) Human immunodeficiency virus-associated systemic lymphomas may be subdivided into two main groups according to Epstein-Barr viral latent gene expression. *J. clin. Oncol.*, 11, 1674-1681

Carbone, A., Gloghini, A., Zanette, I., Canal, B., Rizzo, A. & Volpe, R. (1993c) Co-expression of Epstein-Barr virus latent membrane protein and vimentin in 'aggressive' histological subtypes of Hodgkin's disease. *Virchows Arch. A Pathol. Anat.*, 422, 39–45

Carbone, A., Dolcetti, R., Gloghini, A., Maestro, R., Vaccher, E., Di Luca, D., Tirelli, U. & Boiocchi, M. (1996) Immunophenotypic and molecular analyses of acquired immune deficiency syndrome-related and Epstein-Barr virus-associated lymphomas: A comparative study. *Hum. Pathol.*, 27, 133-146

Carr, K.A., Bulengo, S., Weiss, L.M. & Nickoloff, B.J. (1992) Lymphoepitheliomalike carcinoma of the skin. A case report with immunophenotypic analysis and in situ hybridization for Epstein-Barr viral genome. *Am. J. surg. Pathol.*, 16, 909–913

Carter, C.D., Brown, T.M., Jr, Herbert, J.T. & Heath, C.W., Jr (1977) Cancer incidence following infectious mononucleosis. *Am. J. Epidemiol.*, 105, 30–36

Çavdar, A.O., Yavuz, G., Babacan, E., Gözdasoglu, S., Ünal, E., Ertem, U., Pamir, A., Yücesan, S., Gökcora, H., Uluoglu, Ö. & Ikinciogullari, A. (1994) Burkitt's lymphoma in Turkish children: Clinical, viral [EBV] and molecular studies. *Leukemia Lymphoma*, 14, 323–330

Cesarman, E., Moore, P.S., Rao, P.H., Inghirami, H., Knowles, D.M. & Chang, Y. (1995) In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. *Blood*, **86**, 2708–2714

Chadburn, A., Suciu-Foca, N., Cesarman, E., Reed, E., Michler, R.E. & Knowles, D.M. (1995a) Post-transplantation lymphoproliferative disorders arising in solid organ transplant recipients are usually of recipient origin. *Am. J. Pathol.*, **147**, 1862–1870

Chadburn, A., Cesarman, E., Liu, Y.F., Addonizio, L., Hsu, D., Michler, R.E. & Knowles, D.M. (1995b) Molecular genetic analysis demonstrates that multiple posttransplantation lympho-proliferative disorders occurring in one anatomic site in a single patient represent distinct primary lymphoid neoplasms. *Cancer*, **75**, 2747–2756

Chan, S.H., Levine, P.H., de Thé, G., Mulroney, S.E., Lavoué, M.F., Glen, S.P.P., Goh, E.H., Khor, T.H. & Connelly, R.R. (1979a) A comparison of the prognostic value of antibody-dependent lymphocyte toxicity and other EBV antibody assays in Chinese patients with nasopharyngeal carcinoma. *Int. J. Cancer*, **23**, 181–185

Chan, S.H., Wee, G.B., Srinivasan, N., Glen, S.P.P., Cheng, P., Vengadasalam, D., Alano, F.A. & Simons, M.J. (1979b) HLA antigens in three common populations in South East Asia — Chinese, Malay and Filipino. *Tissue Antigens*, **13**, 361–368

Chan, S.H., Day, N.E., Khor, T.H. & Kunaratnam, N. & Chia, K.B. (1981) HLA markers in the development and prognosis of NPC in Chinese. In: Grundmann, E., Krueger, G.R.F. & Ablashi, D.V., eds, *Cancer Campaign*, Vol. 5, *Nasopharyngeal Carcinoma*, Stuttgart, Gustav Fischer Verlag, pp. 205–211

Chan, S.H., Day, N.E., Kunaratnam, N., Chia, K.B. & Simons, M.J. (1983a) HLA and naso-pharyngeal carcinoma in Chinese — A further study. *Int. J. Cancer*, **32**, 171–176

Chan, S.H., Wee, G.B., Kunaratnam, N., Chia, K.B. & Day, N.E. (1983b) HLA locus B and DR antigen associations in Chinese NPC patients and controls. In: Prasad, U., Ablashi, D.V., Levine, P.H. & Pearson, G.R., eds, *Nasopharyngeal Carcinoma: Current Concepts*, Kuala Lumpur, University of Malaya Press, pp. 307–312

Chan, S.H., Chew, C.T., Prasad, U., Wee, G.B., Srinivasan, N. & Kunaratnam, N. (1985) HLA and nasopharyngeal carcinoma in Malays. *Br. J. Cancer*, **51**, 389–392

Chan, S.H., Chew, C.T., Chandanayingyong, D., Vootiprux, V., Sangruchi, S., Zhu, X.N. & Chen, R. (1986) Nasopharyngeal carcinoma: Joint report. In: Aizawa, M., ed., *HLA in Asia-Oceania, 1986, Proceedings of the 3rd Asia–Oceania Histocompatibility Workshop Conference*, Sapporo, Hokkaido University Press, pp. 396–400

Chan, C.K., Mueller, N., Evans, A., Harris, N.L., Comstock, G.W., Jellum, E., Magnus, K., Orentreich, N., Polk, F. & Vogelman, J. (1991) Epstein-Barr virus antibody patterns prece-ding the diagnosis of nasopharyngeal carcinoma. *Cancer Causes Control*, **2**, 125–131

Chan, J.K.C., Yip, T.T.C., Tsang, W.Y.W., Ng, C.S., Lau, W.-H., Poon, Y.F., Wong, C. S.C. & Ma, V.W.S. (1994a) Detection of Epstein-Barr viral RNA in malignant lymphomas of the upper aerodigestive tract. *Am. J. surg. Pathol.*, **18**, 938-946

Chan, J.K.C., Yip, T.T.C., Tsang, W.Y.W., Poon, Y.F., Wong, C.S.C. & Ma, V.W.S. (1994b) Specific association of Epstein-Barr virus with lymphoepithelial carcinoma among tumors and tumorlike lesions of the salivary gland. *Arch. Pathol. Lab. Med.*, **118**, 994–997

Chan, J.K.C., Tsang, W.Y.M., Ng, C.S., Wong, C.S.C. & Lo, E.S.F. (1995a) A study of the association of Epstein-Barr virus with Burkitt's lymphoma occurring in a Chinese population. *Histopathology*, **26**, 239–245

Chan, J.K.C., Yip, T.T.C., Tsang, W.Y.M., Lau, W.-H., Wong, C.S.C. & Ma, V.W.S. (1995b) Detection of Epstein-Barr virus in Hodgkin's disease occurring in an oriental population. *Hum. Pathol.*, **26**, 314–318

Chang, Y.-N., Dong, D.L.-Y., Hayward, G.S. & Hayward, S.D. (1990) The Epstein-Barr virus Zta transactivator: A member of the bZIP family with unique DNA-binding specificity and a dimerization domain that lacks the characteristic heptad leucine zipper motif. *J. Virol.*, **64**, 3358–3369

Chang, K.L., Flaris, N., Hickey, W.F., Johnson, R.M., Meyer, J.S. & Weiss, L.M. (1993a) Brain lymphomas of immunocompetent and immunocompromised patients: Study of the association with Epstein-Barr virus. *Mod. Pathol.*, **6**, 427–432

Chang, K.L., Albújar. P.F., Chen.Y.-Y., Johnson, R.M. & Weiss, L.M. (1993b) High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of Hodgkin's disease occurring in Peru. *Blood*, **81**, 496–501

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M. & Moore, P.S. (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*, **266**, 1865–1869

Chang, Y.-S., Su, I.-J., Chung, P.J,. Shu, C.-H., Ng, C.-K., Wu, S.-J. & Liu, S.-T. (1995) Detection of an Epstein-Barr-virus variant in T-cell-lymphoma tissues identical to the distinct strain observed in nasopharyngeal carcinoma in the Taiwanese population. *Int. J. Cancer*, **62**, 673–677

Chao, T.-Y., Chow, K.-C., Chang, J.-Y., Wang, C.-C., Tsao, T.-Y., Harn, H.-J. & Chi, K.-H. (1996) Expression of Epstein-Barr virus-encoded RNAs as a marker for metastatic undifferentiated nasopharyngeal carcinoma. *Cancer*, **78**, 24–29

Chapman, C.J., Mockridge, C.I., Rowe, M., Rickinson, A.B. & Stevenson, F.K. (1995) Analysis of V_H genes used by neoplastic B cells in endemic Burkitt's lymphoma shows somatic hypermutation and intraclonal heterogeneity. *Blood*, **85**, 2176–2181

Chen, W. & Cooper, N.R. (1996) Epstein-Barr virus nuclear antigen 2 and latent membrane protein independently transactivate p53 through induction of NFκB activity. *J. Virol.*, **70**, 4849–4853

Chen, J.-Y., Chen, C.-J., Liu, M.-Y., Cho, S.-M., Hsu, M.-M., Lynn, T.-C., Shieh, T., Tu, S.-M., Lee, H.H. & Kuo, S.L. (1987) Antibodies to Epstein-Barr virus-specific DNase in patients with nasopharyngeal carcinoma and control groups. *J. med. Virol.*, **23**, 11–21

Chen, C.-J., Chen, J.-Y. & Hsu, M.-M. (1988a) Epidemiological characteristics and early detection of nasopharyngeal carcinoma in Taiwan. In: Wolf, G.T. & Carey, T.E., eds, *Head and Neck Oncology Research*, Amsterdam, Kugler Publications, pp. 505–513

Chen, C.-J., Wang, Y.-F., Shieh, T., Chen J.-Y. & Liu, M.-Y. (1988b) Multifactorial etiology of nasopharyngeal carcinoma. Epstein-Barr virus, familial tendency and environmental cofactors. In: Wolf, G.T. & Carey, T.E., eds, *Head and Neck Oncology Research*, Amsterdam, Kugler Publications, pp. 469–476

Chen, J.-Y., Chen, C.-J., Liu, M.-Y., Cho, S.-M., Hsu, M.-M., Lynn, T.-C., Shieh, T., Tu, S.-M., Beasley, R.P., Hwang, L.-Y., Lee, H.-H., Kuo, S.-L. & Yang, C.-S. (1989) Antibody to Epstein-Barr virus-specific DNase as a marker for field survey of patients with naso-pharyngeal carcinoma in Taiwan. *J. med. Virol.*, **27**, 269–273

Chen, M.-L., Tsai, C.-N., Liang, C.-L., Shu, C.-H., Huang, C.-R., Sulitzeanu, D., Liu, S.-T. & Chang, Y.-S. (1992a) Cloning and characterization of the latent membrane protein (LMP) of a specific Epstein-Barr virus variant derived from the nasopharyngeal carcinoma in the Taiwanese population. *Oncogene*, **7**, 2131–2140

Chen, H.-F., Kevan-Jah, S., Suentzenich, K.-O., Grässer, F.A. & Mueller-Lantzsch, N. (1992b) Expression of the Epstein-Barr virus latent membrane protein (LMP) in insect cells and detection of antibodies in human sera against this protein. *Virology*, **190**, 106–115

Chen, H.-L., Lung, M.M.L., Sham, J.S.T., Choy, D.T.K., Griffin, B.E. & Ng, M.H. (1992c) Transcription of *Bam*HI-A region of the EBV genome in NPC tissues and B cells. *Virology*, **191**, 193–201

Chen, F., Zou, J.-Z., di Renzo, L., Winberg, G., Hu, L.-F., Klein, E., Klein, G. & Ernberg, I. (1995a) A subpopulation of normal B cells latently infected with Epstein-Barr virus re-sembles Burkitt lymphoma cells in expressing EBNA-1 but not EBNA-2 or LMP1. *J. Virol.*, **69**, 3752–3758

Chen, Y., Chew, C.T. & Chan, S.H. (1995b) T-Cell receptor gene expression in tumour-infil-trating lymphocytes and peripheral blood lymphocytes of patients with nasopharyngeal carci-noma. *Br. J. Cancer*, **72**, 117–122

Cheng, Y.-C., Chen, J.-Y., Glaser, R. & Henle, W. (1980) Frequency and levels of antibodies to Epstein-Barr virus-specific DNase are elevated in patients with nasopharyngeal carcinoma. *Proc. natl Acad. Sci. USA*, **77**, 6162–6165

Cheng, Y.-C., Huang, E.-S., Lin, J.-C., Mar, E.-C., Pagano, J.S., Dutschman, G.E. & Grill, S.P. (1983) Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine against herpesviruses *in vitro* and its mode of action against herpes simplex virus type 1. *Proc. natl Acad. Sci. USA*, **80**, 2767–2770

Cheng, H.-M., Foong, Y.-T., Mathew, A., Sam, C.-K., Dillner, J. & Prasad, U. (1993) Screening for nasopharyngeal carcinoma with an ELISA using the Epstein-Barr virus nuclear antigen, EBNA 1: A complementary test to the IgA/VCA immunofluorescence assay. *J. virol. Meth.*, **42**, 45–52

Cheung, S.-T., Lo, K.-W., Leung, S.F., Chan, W.-Y., Choi, P.H.K., Johnson, P.J., Lee, J.C.K. & Huang, D.P. (1996) Prevalence of LMP1 deletion variant of Epstein-Barr virus in naso-pharyngeal carcinoma and gastric tumors in Hong Kong (Letter to the Editor). *Int. J. Cancer*, **66**, 711–712

Chi, T. & Carey, M. (1993) The ZEBRA activation domain: Modular organization and mecha-nism of action. *Mol. cell. Biol.*, **13**, 7045–7055

Chinn, T.W,. Lai, H.M. & Choy, P.P., eds (1969) *A History of the Chinese in California. A Syllabus*, San Francisco, Chinese Historical Society of America

Cho, M.-S., Bornkamm, G.W. & zur Hausen, H. (1984) Structure of defective DNA molecules in Epstein-Barr virus preparations from P3HR-1 cells. *J. Virol.*, **51**, 199–207

Chow, W.-H., McLaughlin, J.K., Hrubec, Z., Nam, J.-M. & Blot, W.J. (1993) Tobacco use and nasopharyngeal carcinoma in a cohort of US veterans. *Int. J. Cancer*, **55**, 538–540

Christensen, S., Kodoyianni, V., Bosenberg, M., Friedman, L. & Kimble, J. (1996) *Lag-1*, a gene required for *lin-12* and *glp*-1 signalling in *Caenorhabditis elegans*, is homologous to human CBF1 and *Drosophila* Su(H). *Development*, 122, 1373–1383

Churchill, A.E. & Biggs, P.M. (1967) Agent of Marek's disease in tissue culture. *Nature*, 215, 528–530

Churchill, A.E. & Biggs, P.M. (1968) Herpes-type virus isolated in cell culture from tumors of chickens with Marek's disease. II. Studies *in vivo*. *J. natl Cancer Inst.*, 41, 951–956

Churchill, A.E., Payne, L.N. & Chubb, R.C. (1969) Immunization against Marek's disease using a live attenuated virus. *Nature*, 221, 744–747

Cinque, P., Brytting, M., Vago, L., Castagna, A., Parravicini, C., Zanchetta, N., Monforte, A.D'A., Wahren, B., Lazzarin, A. & Linde, A. (1993) Epstein-Barr virus DNA in cerebro-spinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. *Lancet*, 342, 398-401

Cleary, M.L., Epstein, M.A., Finerty, S., Dorfman, R.F., Bornkamm, G.W., Kirkwood, J.K., Morgan, A.J. & Sklar, J. (1985) Individual tumors of multifocal EB virus-induced malignant lymphomas in tamarins arise from different B-cell clones. *Science*, 228, 722–724

Cleary, M.L., Smith, S.D. & Sklar, J. (1986) Cloning and structural analysis of cDNAs for *bcl*-2 and a hybrid *bcl*-2/immunoglobulin transcript resulting from the t(14;18) translocation. *Cell*, 47, 19–28

Clifford, P. (1965) Carcinoma of the nasopharynx in Kenya. *E. Afr. med. J.*, 42, 373 –396

Clifford, P. (1972) Carcinogens in the nose and throat: Nasopharyngeal carcinoma in Kenya. *Proc. R. Soc. Med.*, 65, 682–686

Coates, P.J., Slavin, G. & d'Ardenne, A.J. (1991a) Persistence of Epstein-Barr virus in Reed-Sternberg cells throughout the course of Hodgkin's disease. *J. Pathol.*, 164, 291–297

Coates, P.J., Mak, W.P., Slavin, G. & d'Ardenne, A.J. (1991b) Detection of single copies of Epstein-Barr virus in paraffin wax sections by non-radioactive in situ hybridisation. *J. clin. Pathol.*, 44, 487–491

Cochet, C., Martel-Renoir, D., Grunewald, V., Bosq, J., Cochet, G., Schwaab, G., Bernaudin, J.-F. & Joab, I. (1993) Expression of Epstein-Barr virus immediate early gene, BZLF1, in nasopharyngeal carcinoma tumor cells. *Virology*, 197, 358–365

Cohen, J.I. (1992) A region of herpes simplex virus VP16 can substitute for a transforming domain of Epstein-Barr virus nuclear protein 2. *Proc. natl Acad. Sci. USA*, 89, 8030–8034

Cohen, J.I. & Kieff, E. (1991) An Epstein-Barr virus nuclear protein 2 domain essential for transformation is a direct transcriptional activator. *J. Virol.*, 65, 5880–5885

Cohen, S. & McGregor, I.A. (1963) Gamma-globulin and acquired immunity to malaria. In: Garnam, P.C.C., Pierce, A.E. & Roitt, I., eds, *Immunity to Protozoa*, Oxford, Blackwell, pp. 123–159

Cohen, S., McGregor, I.A. & Carrington, S. (1961) Gamma-globulin and acquired immunity to human malaria. *Nature*, 192, 733–737

Cohen, B.M., Smetana, H.F. & Miller, R.W. (1964) Hodgkin's disease: Long survival in a study of 388 World War II army cases. *Cancer*, 17, 856–866

Cohen, J.I., Wang, F., Mannick, J. & Kieff, E. (1989) Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. *Proc. natl Acad. Sci. USA*, 86, 9558–9562

Cohen, J.I., Wang, F. & Kieff, E. (1991) Epstein-Barr virus nuclear protein 2 mutations define essential domains for transformation and transactivation. *J. Virol.*, **65**, 2545–2554

Cohen, J.I., Picchio, G.R. & Mosier, D.E. (1992) Epstein-Barr virus nuclear protein 2 is a critical determinant for tumor growth in SCID mice and for transformation *in vitro*. *J. Virol.*, **66**, 7555–7559

Colby, B.M., Shaw, J.E., Elion, G.B. & Pagano, J.S. (1980) Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. *J. Virol.*, **34**, 560–568

Collins, J.J., Caporossi, J.C. & Utidjian, H.M.D. (1988) Formaldehyde exposure and nasopharyngeal cancer: Re-examination of the National Cancer Institute study and an update of one plant (Letter to the Editor). *J. natl Cancer Inst.*, **80**, 376–377

Connelly, R.R. & Christine, B.W. (1974) A cohort study of cancer following infectious mononucleosis. *Cancer Res.*, **34**, 1172–1178

Contreras-Brodin, B., Anvret, M., Imreh, S., Altiok, E., Klein, G. & Masucci, M.G. (1991) B Cell phenotype-dependent expression of the Epstein-Barr virus nuclear antigens EBNA-2 to EBNA-6: Studies with somatic cell hybrids. *J. gen. Virol.*, **72**, 3025–3033

Contreras-Salazar, B., Klein, G. & Masucci, M.G. (1989) Host cell-dependent regulation of growth transformation-associated Epstein-Barr virus antigens in somatic cell hybrids. *J. Virol.*, **63**, 2768–2772

Conway, M., Morgan, A.J. & Mackett, M. (1989) Expression of Epstein-Barr virus membrane antigen gp340/220 in mouse fibroblasts using a bovine papillomavirus vector. *J. gen. Virol.*, **70**, 729–734

Conway, E.J., Hudnall, S.D., Lazarides, A., Bahler, A., Fraire, A.E. & Cagle, P.T. (1996) Absence of evidence for an etiologic role for Epstein-Barr virus in neoplasms of the lung and pleura. *Mod. Pathol.*, **9**, 491–495

Correa, P. & O'Conor, G.T. (1971) Epidemiologic patterns of Hodgkin's disease. *Int. J. Cancer*, **8**, 192–201

Cox, M.A., Leahy, J. & Hardwick, J.M. (1990) An enhancer within the divergent promoter of Epstein-Barr virus responds synergistically to the R and Z transactivators. *J. Virol.*, **64**, 313–321

Cox, C., Chang, S., Karran, L., Griffin, B. & Wedderburn, N. (1996) Persistent Epstein-Barr virus infection in the common marmoset (*Callithrix jacchus*). *J. gen. Virol.*, **77**, 1173–1180

Cozen, W., Katz, J. & Mack, T.M. (1992) Risk patterns of Hodgkin's disease in Los Angeles vary by cell type. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 261–268

Craig, F.E., Gulley, M.L. & Banks, P.M. (1993) Posttransplantation lymphoproliferative disorders. *Am. J. clin. Pathol.*, **99**, 265–276

Crawford, D.H. & Ando, I. (1986) EB virus induction is associated with B-cell maturation. *Immunology*, **59**, 405–409

Crawford, D.H., Epstein, M.A., Bornkamm, G.W., Achong, B.G., Finerty, S. & Thompson, J.L. (1979) Biological and biochemical observations on isolates of EB virus from the malignant epithelial cells of two nasopharyngeal carcinomas. *Int. J. Cancer*, **24**, 294–302

Croce, C.M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G.G., Dolby, T.W. & Koprowski, H. (1979) Chromosomal location of the genes for human immunoglobulin heavy chains. *Proc. natl Acad. Sci. USA*, **76**, 3416–3419

Crompton, C.H., Cheung, R.K., Donjon, C., Miyazaki, I., Feinmesser, R., Hébert, D. & Dosch, H.-M. (1994) Epstein-Barr virus surveillance after renal transplantation. *Transplantation*, **57**, 1182–1189

Cuomo, L., Trivedi, P., Wang, F., Winberg, G., Klein, G. & Masucci, M.G. (1990) Expression of the Epstein-Barr virus (EBV)-encoded membrane antigen (LMP) increases the stimulatory capacity of EBV-negative B lymphoma lines in allogeneic mixed lymphocyte cultures. *Eur. J. Immunol.*, **20**, 2293–2299

Cuomo, L., Ramquist, T., Trivedi, P., Wang, F., Klein, G. & Masucci, M.G. (1992) Expression of the Epstein-Barr virus (EBV)-encoded membrane protein LMP-1 impairs the in vitro growth, clonability and tumorigenicity of an EBV-negative Burkitt lymphoma line. *Int. J. Cancer*, **51**, 949–955

Cwirla, S.E., Peters, E.A., Barrett, R.W. & Dower, W.J. (1990) Peptides on phage: A vast library of peptides for identifying ligands. *Proc. natl Acad. Sci. USA*, **87**, 6378–6382

Daibata, M., Speck, S.H., Mulder, C. & Sairenji, T. (1994) Regulation of the BZLF1 promoter of Epstein-Barr virus by second messengers in anti-immunoglobulin-treated B cells. *Virology*, **198**, 446–454

Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R.C. & Croce, C.M. (1982) Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. natl Acad. Sci. USA*, **79**, 7824–7827

Dalldorf, G. (1962) Lymphomas of African children with different forms or environmental influences. *J. Am. med. Assoc.*, **181**, 1026–1028

Dalldorf, G., Linsell, C.A., Barnhart, F.E. & Martyn, R. (1964) An epidemiological approach to the lymphomas of African children and Burkitt's sarcoma of the jaws. *Perspect. Biol. Med.*, **7**, 435–449

Dambaugh, T., Nkrumah, F.K., Biggar, R.J. & Kieff, E. (1979) Epstein-Barr virus RNA in Burkitt tumor tissue. *Cell*, **16**, 313–322

Dambaugh, T., Raab-Traub, N., Heller, M., Beisel, C., Hummel, M., Cheung, A., Fennewald, S., King, W. & Kieff, E. (1980) Variations among isolates of Epstein-Barr virus. *Ann. N.Y. Acad. Sci.*, **354**, 309–325

Dambaugh, T., Hennessy, K., Chamnankit, L. & Kieff, E. (1984) U2 region of Epstein-Barr virus DNA may encode Epstein-Barr nuclear antigen 2. *Microbiology*, **81**, 7632–7636

Daniel, L.W., Bauer, G. & zur Hausen, H. (1984) Effect of indomethacin on Epstein-Barr virus early antigen induction. *Cancer Res.*, **44**, 981–983

David, E.M. & Morgan, A.J. (1988) Efficient purification of Epstein-Barr virus membrane antigen gp340 by fast protein liquid chromatography. *J. immunol. Meth.*, **108**, 231–236

Davies, J.N.P. (1948) Reticuloendothelial tumours. *E. Afr. med. J.*, **25**, 117

Davies, J.N.P., Elmes, S., Hutt, M.S.R., Mtimavalye, L.A.R., Owor, R. & Shaper, L. (1964a) Cancer in an African community, 1897–1956. An analysis of the records of Mengo Hospital, Kampala, Uganda: Part 1. *Br. med. J.*, **i**, 259–264

Davies, J.N.P., Elmes, S., Hutt, M.S.R., Mtimavalye, L.A.R., Owor, R. & Shaper, L. (1964b) Cancer in an African community, 1897–1956. An analysis of the records of Mengo Hospital, Kampala, Uganda: Part 2. *Br. med. J.*, **i**, 336–341

Davis, C.L., Harrison, K.L., McVicar, J.P., Forg, P.J., Bronner, M.P. & Marsh, C.L. (1995) Antiviral prophylaxis and the Epstein Barr virus-related post-transplant lymphoproliferative disorder. *Clin. Transplant.*, **9**, 53–59

Davison, S.P., Habermann, T.M., Strickler, J.G., DeRemee, R.A., Earle, J.D. & McDonald, T.J. (1996) Nasal and nasopharyngeal angiocentric T-cell lymphomas. *Laryngoscope*, **106**, 139-143

Dawson, C.W., Rickinson, A.B. & Young, L.S. (1990) Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. *Nature*, **344**, 777-780

Deacon, E.M., Pallesen, G., Niedobitek, G., Crocker, J., Brooks, L., Rickinson, A.B. & Young, L.S. (1993) Epstein-Barr virus and Hodgkin's disease: Transcriptional analysis of virus latency in the malignant cells. *J. exp. Med.*, **177**, 339-349

Deamant, F.D., Albújar, P.F., Chen, Y.-Y. & Weiss, L.M. (1993) Epstein-Barr virus distribution in nonneoplastic lymph nodes. *Mod. Pathol.*, **6**, 729-732

DeAngelis, L.M., Wong, E., Rosenblum, M. & Furneaux, H. (1992) Epstein-Barr virus in acquired immunodeficiency syndrome (AIDS) and non-AIDS primary central nervous system lymphoma. *Cancer*, **70**, 1607-1611

Deinhardt, F. & Deinhardt, J. (1979) Oncogenic animal herpesviruses. In: Epstein, M.A. & Achong, B.G., eds, *Epstein-Barr Virus*, Berlin, Springer-Verlag, pp. 374-415

Deinhardt, F., Falk, L., Wolfe, L.G., Paciga, L. & Johnson, D.R. (1975) Response of marmosets to experimental infection with Epstein-Barr virus. In: de Thé, G., Epstein, M.A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II* (IARC Scientific Publications No. 11), Lyon, IARC, pp. 161-168

Deinhardt, F., Falk, L., Wolfe, L.G., Schudel, A., Nonoyama, M., Lai, P., Lapin, B. & Yakovleva, L. (1978) Susceptibility of marmosets to Epstein-Barr virus-like baboon herpesviruses. *Primate Med.*, **10**, 163-170

Delabie, J., Tierens, A., Gavriil, T., Wu, G., Weisenburger, D.D. & Chan, W.C. (1996) Phenotype, genotype and clonality of Reed-Sternberg cells in nodular sclerosis Hodgkin's disease: Results of a single-cell study. *Br. J. Haematol.*, **94**, 198-205

Delecluse, H.-J., Bartnizke, S., Hammerschmidt, W., Bullerdiek, J. & Bornkamm, G.W. (1993a) Episomal and integrated copies of Epstein-Barr virus coexist in Burkitt lymphoma cell lines. *J. Virol.*, **67**, 1292-1299

Delecluse, H.-J., Schüller, S. & Hammerschmidt, W. (1993b) Latent Marek's disease virus can be activated from its chromosomally integrated state in herpesvirus-transformed lymphoma cells. *EMBO J.*, **12**, 3277-3286

Delecluse, H.-J., Raphael, M., Magaud, J.P., Felman, P., Alsamad, I.A., Bornkamm, G.W. & Lenoir, G.M. (1993c) Variable morphology of human immunodeficiency virus-associated lymphomas with c-myc rearrangements. The French study group of pathology for human immunodeficiency virus-associated tumors. I. *Blood*, **82**, 552-563

Delecluse, H.-J., Kremmer, E., Rouault, J.-P., Cour, C., Bornkamm, G.W. & Berger, F. (1995a) The expression of Epstein-Barr virus latent proteins is related to the pathological features of post-transplant lymphoproliferative disorders. *Am. J. Pathol.*, **146**, 1113-1120

Delecluse, H.-J., Rouault, J.-P., Ffrench, M., Dureau, G., Magaud, J.P. & Berger, F. (1995b) Post-transplant lymphoproliferative disorders with genetic abnormalities commonly found in malignant tumours. *Br. J. Haematol.*, **89**, 90-97

Delsol, G., Brousset, P., Chittal, S. & Rigal-Huguet, F. (1992) Correlation of the expression of Epstein-Barr virus latent membrane protein and in situ hybridization with biotinylated *Bam*HI-W probes in Hodgkin's disease. *Am. J. Pathol.*, **140**, 247-253

Demers, P.A., Boffetta, P., Kogevinas, M., Blair, A., Miller, B.A., Robinson, C.F., Roscoe, R.J., Winter, P.D., Colin, D., Matos, E. & Vainio, H. (1995) Pooled reanalysis of cancer mortality among five cohorts of workers in wood-related industries. *Scand. J. Work Environ. Health*, **21**, 179–190

Desgranges, C. & de Thé, G. (1977) Epstein-Barr virus induces viral nuclear antigen in nasopharyngeal epithelial cells (Letter to the Editor). *Lancet*, **ii**, 1286–1287

Desgranges, C. & de Thé, G. (1978) Presence of Epstein-Barr virus specific IgA in saliva of nasopharyngeal carcinoma patients: Their activity, origin and possible clinical value. In: de Thé, G. & Ito, Y., *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 459–469

Desgranges, C., Wolf, H., de Thé, G., Shanmugaratnam, K., Cammoun, N., Ellouz, R., Klein, G., Lennert, K., Muñoz, N. & zur Hausen, H. (1975) Nasopharyngeal carcinoma. X. Presence of Epstein-Barr genomes in separated epithelial cells of tumours in patients from Singapore, Tunisia and Kenya. *Int. J. Cancer*, **16**, 7–15

Desgranges, C., Lenoir, G.M., de Thé, G., Seigneurin, J.-M., Hilgers, J. & Dubouch, P. (1976) In vitro transforming activity of EBV. 1. Establishment and properties of two EBV strain (M81 and M72) produced by immortalized Callithrix jacchus lymphocytes. *Biomedicine*, **25**, 349–352

Desgranges, C., de Thé, G., Ho, J.H.C. & Ellouz, R. (1977) Neutralizing EBV-specific IgA in throat washings of nasopharyngeal carcinoma (NPC) patients. *Int. J. Cancer*, **19**, 627–633

Desgranges, C., Bornkamm, G.W., Zeng, Y., Wang, P.C., Zhu, J.S., Shang, M. & de Thé, G. (1982) Detection of Epstein-Barr viral DNA internal repeats in the nasopharyngeal mucosa of Chinese with IgA/EBV-specific antibodies. *Int. J. Cancer*, **29**, 87–91

De Smet, M.P. (1956) Clinical observations of malignant tumours of reticuloendothelial and haemolymphopoietic tissues in the Congo. *Ann. Soc. belge Méd. trop.*, **36**, 53–70

Devergne, O., Hummel, M., Koeppen, H., Le Beau, M.M., Nathanson, E.C., Kieff, E. & Birkenbach, M. (1996) A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. *J. Virol.*, **70**, 1143–1153

DeVita, V.T., Hellman, S. & Rosenberg, S.A. (1993) *Cancer: Principles and Practice of Oncology*, 4th Ed., Philadelphia, J.B. Lippincott Co., pp. 1819–1852

Dictor, M., Cervin, A., Kalm, O. & Rambech, E. (1996) Sinonasal T-cell lymphoma in the differential diagnosis of lethal midline granuloma using in situ hybridization for Epstein-Barr virus RNA. *Mod. Pathol.*, **9**, 7–14

Diehl, V., Taylor, J.R., Parlin, J.A., Henle, G. & Henle, W. (1969) Infectious mononucleosis in East Africa. *E. Afr. med. J.*, **46**, 407–413

Dietl, J., Horny, H.-P. & Kaiserling, E. (1994) Lymphoepithelioma-like carcinoma of the vagina: A case report with special reference to the immunophenotype of the tumor cells and tumor-infiltrating lymphoreticular cells. *Int. J. gynecol. Pathol.*, **13**, 186–189

DiGiuseppe, J.A., Wu, T.-C. & Corio, R.L. (1994) Analysis of Epstein-Barr virus-encoded small RNA 1 expression in benign lymphoepithelial salivary gland lesions. *Mod. Pathol.*, **7**, 555–559

Dillner, J., Kallin, B., Alexander, H., Ernberg, I., Uno, M., Ono, Y., Klein, G. & Lerner, R.A. (1986) An Epstein-Barr virus (EBV)-determined nuclear antigen (EBNA5) partly encoded by the transformation-associated *Bam* WYH region of EBV DNA: Preferential expression in lymphoblastoid cell lines. *Proc. natl Acad. Sci. USA*, **83**, 6641–6645

Dillon, S.B., Demuth, S.G., Schneider, M.A., Weston, C.B., Jones, C.S., Young, J.F., Scott, M., Bhatnaghar, P.K., LoCastro, S. & Hanna, N. (1992) Induction of protective class I MHC-restricted CTL in mice by a recombinant influenza vaccine in aluminium hydroxide adjuvant. *Vaccine*, **10**, 309–318

Dimery, I.W., Lee, J.S., Blick, M., Pearson, G., Spitzer, G. & Hong, W.K. (1988) Association of the Epstein-Barr virus with lymphoepithelioma of the thymus. *Cancer*, **61**, 2475–2480

Diss, T.C., Wotherspoon, A.C., Speight, P., Pan, L. & Isaacson, P.G. (1995) B-Cell mono-clonality, Epstein-Barr virus, and t(14;18) in myoepithelial sialadenitis and low-grade B-cell MALT [mucosa-associated lymphoid tissue] lymphoma of the parotid gland. *Am. J. surg. Pathol.*, **19**, 531-536

Dix, S.P. & Wingard, J.R. (1996) Management of viral infections in bone marrow transplant recipients. *Clin. Immunother.*, **6**, 352–382

Dobner, T., Wolf, I., Emrich, T. & Lipp, M. (1992) Differentiation-specific expression of a novel G protein-coupled receptor from Burkitt's lymphoma. *Eur. J. Immunol.*, **22**, 2795–2799

Dobson, W.H. (1924) Cervical lymphosarcoma. *Chin. med. J.*, **38**, 786–787

Dolcetti, R., Frisan, T., Sjöberg, J., de Campos-Lima, P.O., Pisa, P., De Re, V., Gloghini, A., Rizzo, S., Masucci, M.G. & Boiocchi, M. (1995) Identification and characterization of an Epstein-Barr virus-specific T-cell response in the pathologic tissue of a patient with Hodgkin's disease. *Cancer Res.*, **55**, 3675–3681

Dolezal, M.V., Kamel, O.W., van de Rijn, M., Cleary, M.L., Sibley, R.K. & Warnke, R.A. (1995) Virus-associated hemophagocytic syndrome characterized by clonal Epstein-Barr virus genome. *Am. J. clin. Pathol.*, **103**, 189–194

Doll, R., Muir, C.S. & Waterhouse, J.A.H., eds (1970) *Cancer Incidence in Five Continents*, Vol. 2, Geneva, UICC

Dorfman, R.F. (1965) Childhood lymphosarcoma in St Louis, Missouri, clinically and histo-logically resembling Burkitt's tumor. *Cancer*, **18**, 418–430

Dotsika, E., Karagouni, E., Sundquist, B., Morein, B., Morgan, A. & Villacres-Eriksson, M. (1997) Influence of the Quillaja saponaria triterpenoid content on the immunomodulatory capacity of Epstein-Barr virus (EBV) iscoms. *Scand. J. Immunol.*, **45**, 261–268

Draper, C.C., Brubaker, G., Geser, A., Kilimali, V.A.E.B. & Wernsdorfer, W.H. (1985) Serial studies on the evolution of chloroquine resistance in an area of East Africa receiving inter-mittent malaria chemosuppression. *Bull. World Health Organ.*, **63**, 109–118

Drut, R.M., Day, S., Drut, R. & Meisner, L. (1994) Demonstration of Epstein-Barr viral DNA in paraffin-embedded tissues of Burkitt's lymphoma from Argentina using the polymerase chain reaction and in-situ hybridization. *Pediatr. Pathol.*, **14**, 101–109

DuBois, R.E., Seeley, J.K., Brus, I., Sakamoto, K., Ballow, M., Harada, S., Bechtold, T.A., Pearson, G. & Purtilo, D.T. (1984) Chronic mononucleosis syndrome. *South. med. J.*, **77**, 1376–1382

Durandy, A., Le Deist, F., Emile, J.-F., Debatin, K. & Fischer, A. (1997) Sensitivity of Epstein-Barr virus-induced B cell tumor to apoptosis mediated by anti-CD95/Apo-1/fas antibody. *Eur. J. Immunol.*, **27**, 538–543

Durodola, J.I. (1976) Burkitt's lymphoma presenting during lactation. *Int. J. Gynaecol. Obstet.*, **14**, 225–231

Dyck, J.A., Maul, G.G., Miller, W.H., Jr, Chen, J.D., Kakizuka, A. & Evans, R.M. (1994) A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor onco-protein. *Cell*, **76**, 333–343

Ebbers, J., Koldovsky, P. & Vosteen, K.-H. (1985) The expression of Ia-antigen on nasopharyngeal carcinomas xenografted into nude mice. *Arch. Otorhinolaryngol.*, **242**, 209–215

Edington, G.M. (1956) Malignant disease in the Gold Coast. *Br. J. Cancer*, **10**, 41–54

Edington, G.M. (1978) The pattern of cancer in the northern savannah of Nigeria with special reference to primary liver cell carcinoma and the Burkitt lymphoma. *Niger. med. J.*, **8**, 281–289

Edington, G.M. & Gilles, H.M.J. (1968) *Pathology in the Tropics*, London, Arnold

Edington, G.M. & MacLean, C.M.U. (1964) Incidence of the Burkitt tumour in Ibadan, western Nigeria. *Br. med. J.*, **i**, 264–266

Effert, P., McCoy, R., Abdel-Hamid, M., Flynn, K., Zhang, Q., Busson, P., Tursz, T., Liu, E. & Raab-Traub, N. (1992) Alterations of the p53 gene in nasopharyngeal carcinoma. *J. Virol.*, **66**, 3768–3775

Egeler, R.M., de Kraker, J., Slater, R. & Purtilo, D.T. (1992) Documentation of Burkitt lymphoma with t(8;14) (q24;q32) in X-linked lymphoproliferative disease. *Cancer*, **70**, 683–687

Emilie, D., Devergne, O., Raphael, M., Coumbaras, L.J. & Galanaud, P. (1992a) Production of interleukin-6 in high grade B lymphomas. *Curr. Top. Microbiol. Immunol.*, **182**, 349–355

Emilie, D., Touitou, R., Raphael, M., Peuchmaur, M., Devergne, O., Rea, D., Coumbraras, J., Crevon, M.-C., Edelman, L., Joab, I. & Galanaud, P. (1992b) In vivo production of interleukin-10 by malignant cells in AIDS lymphomas. *Eur. J. Immunol.*, **22**, 2937–2942

Emini, E.A., Luka, J., Armstrong, M.E., Banker, F.S., Provost, P.J. & Pearson, G.R. (1986) Establishment and characterization of a chronic infectious mononucleosis-like syndrome in common marmosets. *J. med. Virol.*, **18**, 369–379

Emini, E.A., Luka, J., Armstrong, M.E., Keller, P.M., Ellis, R.W. & Pearson, G.R. (1987) Identification of an Epstein-Barr virus glycoprotein which is antigenically homologous to the varicella-zoster virus glycoprotein II and the herpes simplex virus glycoprotein B. *Virology*, **157**, 552–555

Emini, E.A., Schleif, W.A., Armstrong, M.E., Silberklang, M., Schultz, L.D., Lehman, D., Maigetter, R.Z., Qualtière, L.F., Pearson, G.R. & Ellis, R.W. (1988) Antigenic analysis of the Epstein-Barr virus major membrane antigen (gp350/220) expressed in yeast and mammalian cells: Implications for the development of a subunit vaccine. *Virology*, **166**, 387–393

Emini, E.A., Schleif, W.A., Silberklang, M., Lehman, D. & Ellis, R.W. (1989) Vero cell-expressed Epstein-Barr virus (EBV) gp350/220 protects marmosets from EBV challenge. *J. med. Virol.*, **27**, 120–123

Epstein, M.A. (1985) Historical background; Burkitt's lymphoma and Epstein-Barr virus. In: Lenoir, G.M., O'Conor, G. & Olweny, C.L.M., eds, *Burkitt's Lymphoma: A Human Cancer Model* (IARC Scientific Publications No. 60), Lyon, IARC, pp. 17–27

Epstein, M.A. & Achong, B.G. (1968) Specific immunofluorescence test for the herpes-type EB virus of Burkitt lymphoblasts, authenticated by electron microscopy. *J. natl Cancer Inst.*, **40**, 593–607

Epstein, M.A., Achong, B.G. & Barr, Y.M. (1964) Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet*, **i**, 702–703

Epstein, M.A., Barr, Y.M. & Achong, B.G. (1965) The behaviour and morphology of a second tissue culture strain (EB2) of lymphoblasts from Burkitt's lymphoma. *Br. J. Cancer*, **19**, 108–114

Epstein, M.A., Hunt, R.D. & Rabin, H. (1973) Pilot experiments with EB virus in owl monkeys (*Aotus trivirgatus*). I. Reticuloproliferative disease in an inoculated animal. *Int. J. Cancer*, **12**, 309–318

Epstein, M.A., Morgan, A.J., Finerty, S., Randle, B.J. & Kirkwood, J.K. (1985) Protection of cottontop tamarins against Epstein-Barr virus-induced malignant lymphoma by a prototype subunit vaccine. *Nature*, **318**, 287–289

Ernberg, I. & Altiok, E. (1989) The role of Epstein-Barr virus in lymphomas of HIV-carriers. *Acta pathol. microbiol. immunol. scand.*, **8**, 58–61

Ernberg, I., Andersson-Anvret, M., Klein, G., Lundin, L. & Killanger, D. (1977) Relationship between amount of Epstein-Barr virus-determined nuclear antigen per cell and number of EBV-DNA copies per cell. *Nature*, **266**, 269–271

Ernberg, I., Falk, K. & Hansson, M. (1987) Progenitor and pre-B lymphocytes transformed by Epstein-Barr virus. *Int. J. Cancer*, **39**, 190–197

Ernberg, I., Falk, K., Minarovits, J., Busson, P., Tursz, T., Masucci, M.G. & Klein, G. (1989) The role of methylation in the phenotype-dependent modulation of Epstein-Barr nuclear antigen 2 and latent membrane protein genes in cells latently infected with Epstein-Barr virus. *J. gen. Virol.*, **70**, 2989–3002

Eshleman, J.L. (1966) A study of the relative incidence of malignant tumours seen at Shirati Hospital in Tanzania. *E. Afr. med. J.*, **43**, 274–283

Estrov, Z., Kurzrock, R., Pocsik, E., Pathak, S., Kantarjian, H.M., Zipf, T.F., Harris, D., Talpaz, M. & Aggarwal, B.B. (1993) Lymphotoxin is an autocrine growth factor for Epstein-Barr virus-infected B cell lines. *J. exp. Med.*, **177**, 763–774

Evan, G.I., Wyllie, A.H., Gilbert, C.S., Littlewood, T.D., Land, H., Brooks, M., Waters, C.M., Penn, L.Z. & Hancock, D.C. (1992) Induction of apoptosis in fibroblasts by c-myc protein. *Cell*, **69**, 119–128

Evans, A.S. (1972) Clinical syndromes associated with EB virus infection. *Adv. intern. Med.*, **18**, 77–93

Evans, A.S. (1993) Epstein-Barr vaccine: Use in infectious mononucleosis. In: Tursz, T., Pagano, J.S., Ablashi, D.V., de Thé, G., Lenoir, G. & Pearson, G.R., eds, *The Epstein-Barr Virus and Associated Diseases* (Colloque INSERM No. 225), Paris, INSERM/John Libbey Eurotext Ltd, pp. 593–598

Evans, A.S. & Gutensohn, N. (1984) A population-based case–control study of EBV and other viral antibodies among persons with Hodgkin's disease and their siblings. *Int. J. Cancer*, **34**, 149–157

Evans, A.S. & Kaslow, R.A. (1997) *Viral Infections in Humans*, 4th Ed., New York, Plenum (in press)

Evans, A.S. & Niederman, J.C. (1989) Epstein-Barr virus. In: Evans, A.S., ed., *Viral Infections of Humans*, 3rd Ed., New York, Plenum, pp. 265–292

Evans, A.S., Niederman, J.C. & McCollum, R.W. (1968) Seroepidemiological studies of infectious mononucleosis with EB virus. *New Engl. J. Med.*, **279**, 1121–1127

Evans, D.L., Barnett, J.W., Bowen, J.M. & Dmochowski, L. (1972) Antigenic relationship between the herpesviruses of infectious bovine rhinotracheitis, Marek's disease, and Burkitt's lymphoma. *J. Virol.*, 2, 277–287

Evans, A.S., Carvalho, R.P.S., Frost, P., Jamra, M. & Pozzi, D.H.B. (1978) Epstein-Barr virus infections in Brazil. II. Hodgkin's disease. *J. natl Cancer Inst.*, 61, 19–26

Evans, A.S., Kirchhoff, L.V., Pannuti, C.S., Carvalho, R.P.S. & McClelland, K.E. (1980) A case–control study of Hodgkin's disease in Brazil. II. Seroepidemiological studies in cases and family members. *Am. J. Epidemiol.*, 112, 609–618

Fåhraeus, R., Fu, H.L., Ernberg, I., Finke, J., Rowe, M., Klein, G., Falk, K., Nilsson, E., Yadav, M., Busson, P., Tursz, T. & Kallin, B. (1988) Expression of Epstein-Barr virus-encoded proteins in nasopharyngeal carcinoma. *Int. J. Cancer*, 42, 329–338

Fåhraeus, R., Rymo, L., Rhim, J.S. & Klein, G. (1990a) Morphological transformation of human keratinocytes expressing the LMP gene of Epstein-Barr virus. *Nature*, 345, 447–449

Fåhraeus, R., Jansson, A., Ricksten, A., Sjöblom, A. & Rymo, L. (1990b) Epstein-Barr virus-encoded nuclear antigen 2 activates the viral latent membrane protein promoter by modulating the activity of a negative regulatory element. *Proc. natl Acad. Sci. USA*, 87, 7390–7394

Fåhraeus, R., Chen, W., Trivedi, P., Klein, G. & Öbrink, B. (1992) Decreased expression of E-cadherin and increased invasive capacity in EBV-LMP-transfected human epithelial and murine adenocarcinoma cells. *Int. J. Cancer*, 52, 834–838

Fåhraeus, R., Jansson, A., Sjöblom, A., Nilsson, T., Klein, G. & Rymo, L. (1993) Cell phenotype-dependent control of Epstein-Barr virus latent membrane protein 1 gene regulatory sequences. *Virology*, 195, 71–80

Falk, L.A. (1979) A review of *Herpesvirus papio*, a B-lymphotropic virus of baboons related to EBV. *Comp. Immunol. Microbiol. infect. Dis.*, 2, 257–264

Falk, L.A., Wolfe, L.G., Sheramek, G. & Deinhardt, F. (1970) Herpesvirus saimiri neoplasia. *Proc. central soc. clin. Res.*, 43, 80–81

Falk, L., Deinhardt, F., Nonoyama, M., Wolfe, L.G., Bergholz, C., Lapin, B., Yakovleva, Agrba, V., Henle, G. & Henle, W. (1976) Properties of a baboon lymphotropic herpesvirus related to Epstein-Barr virus. *Int. J. Cancer*, 18, 798–807

Falk, L., Lindahl, T., Bjuvsell, G. & Klein, G. (1979) Herpesvirus papio: State and properties of intracellular viral DNA in baboon lymphoblastoid cell lines. *Int. J. Cancer*, 24, 75–79

Falk, K., Ernberg, I., Sakthivel, R., Davis, J., Christensson, B., Luka, J., Okano, M., Grierson, H.L., Klein, G. & Purtilo, D.T. (1990) Expression of Epstein-Barr virus-encoded proteins and B-cell markers in fatal infectious mononucleosis. *Int. J. Cancer*, 46, 976–984

Falk, M.H., Hültner, L., Milner, A., Gregory, C.D. & Bornkamm, G.W. (1993) Irradiated fibroblasts protect Burkitt lymphoma cells from apoptosis by a mechanism independent of *bcl-2*. *Int. J. Cancer*, 55, 485–491

Farrell, P.J., Allan, G.J., Shanahan, F., Vousden, K.H. & Crook, T. (1991) p53 is frequently mutated in Burkitt's lymphoma cell lines. *EMBO J.*, 10, 2879–2887

Farrell, P.J., Hollyoake, M., Niedobitek, G., Agathanggelou, A., Morgan, A. & Wedderburn, N. (1997) Direct demonstration of persistent Epstein-Barr virus gene expression in peripheral blood of infected common marmosets and analysis of virus-infected tissues *in vivo*. *J. gen. Virol.*, 78, 1417–1424

Feichtinger, H., Li, S.-L., Kaaya, E., Putkonen, P., Grünewald, K., Weyrer, K., Böttiger, D., Ernberg, I., Linde, A., Biberfeld, G. & Biberfeld, P. (1992) A monkey model for Epstein Barr virus-associated lymphomagenesis in human acquired immunodeficiency syndrome. *J. exp. Med.*, **176**, 281–286

Fellbaum, C., Hansmann, M.-L., Niedermeyer, H., Kraus, I., Alavaikko, M.J., Blanco, G., Aine, R., Busch, R., Pütz, B., Fischer, R. & Höfler, H. (1992) Influence of Epstein-Barr virus genomes on patient survival in Hodgkin's disease. *Am. J. clin. Pathol.*, **98**, 319–323

Fennewald, S., van Santen, V. & Kieff, E. (1984) Nucleotide sequence of an mRNA transcribed in latent growth-transforming virus infection indicates that it may encode a membrane protein. *J. Virol.*, **51**, 411–419

Ferrara, G. & Nappi, O. (1995) Lymphoepithelioma-like carcinoma of the lung. Two cases diagnosed in Caucasian patients. *Tumori*, **81**, 144–147

Filipovich, A.H., Mathur, A., Kamat, D. & Shapiro, R.S. (1992) Primary immunodeficiencies: Genetic risk factors for lymphoma. *Cancer Res.*, **52**, 5465s–5467s

Finerty, S., Scullion, F.T. & Morgan, A.J. (1988) Demonstration *in vitro* of cell-mediated immunity to Epstein-Barr virus in cotton-top tamarins. *Clin. exp. Immunol.*, **73**, 181–185

Finerty, S., Mackett, M., Arrand, J.R., Watkins, P.E., Tarlton, J. & Morgan, A.J. (1994) Immunisation of cotton-top tamarins and rabbits with a candidate vaccine against the Epstein-Barr virus based on the major viral envelope glycoprotein gp340 and alum. *Vaccine*, **12**, 1180–1184

Fingeroth, J.D., Weis, J.J., Tedder, T.F., Strominger, J.L., Biro, P.A. & Fearon, D.T. (1984) Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc. natl Acad. Sci. USA*, **81**, 4510–4514

Fingeroth, J.D., Clabby, M.L. & Strominger, J.D. (1988) Characterization of a T-lymphocyte Epstein-Barr virus/C3d receptor (CD21). *J. Virol.*, **62**, 1442–1447

Finke, J., Rowe, M., Kallin, B., Ernberg, I., Rosén, A., Dillner, J. & Klein, G. (1987) Monoclonal and polyclonal antibodies against Epstein-Barr virus nuclear antigen 5 (EBNA-5) detect multiple protein species in Burkitt's lymphoma and lymphoblastoid cell lines. *J. Virol.*, **61**, 3870–3878

Finlay, J., Luft, B., Yousem, S., Wood, G.S., Link, M., Arvin, A., Glader, B., Lennette, E., Shatsky, M., Olds, L., Borcherding, W., Hong, R. & Purtilo, D. (1986) Chronic infectious mononucleosis syndrome, pancytopenia, and polyclonal B-lymphoproliferation terminating in acute lymphoblastic leukemia. *Am. J. pediatr. Hematol. Oncol.*, **8**, 18–27

Fischer, A., Fischer, G.O. & Cooper, E. (1984) Familial nasopharyngeal carcinoma. *Pathology*, **16**, 23–24

Fleisher, G., Lennette, E.T., Henle, G. & Henle, W. (1979) Incidence of heterophil antibody responses in children with infectious mononucleosis. *J. Pediatr.*, **94**, 723–728

Floetmann, J.E., Ward, K., Rickinson, A.B. & Rowe, M. (1996) Cytostatic effect of Epstein-Barr virus latent membrane protein-1 analyzed using tetracycline-regulated expression in B cell lines. *Virology*, **223**, 29–40

Fortini, M.E. & Artavanis-Tsakonas, S. (1994) The suppressor of hairless protein participates in Notch receptor signaling. *Cell*, **79**, 273–282

Franken, M., Annis, B., Ali, A.N. & Wang, F. (1995) 5′ Coding and regulatory region sequence divergence with conserved function of the Epstein-Barr virus LMP2A homolog in Herpesvirus papio. *J. Virol.*, **69**, 8011–8019

Frappier, L. & O'Donnell, M. (1991) Overproduction, purification, and characterization of EBNA1, the origin binding protein of Epstein-Barr virus. *J. biol. Chem.*, **266**, 7819–7826

Frappier, L. & O'Donnell, M. (1992) EBNA1 distorts *oriP*, the Epstein-Barr virus latent replication origin. *J. Virol.*, **66**, 1786–1790

Fraumeni, J.F., Jr & Mason, T.J. (1974) Cancer mortality among Chinese Americans, 1950–69. *J. natl Cancer Inst.*, **52**, 659–665

Frech, B., Zimber-Strobl, U., Yip, T.T.C., Lau, W.H. & Mueller-Lantzsch, N. (1993) Characterization of the antibody response to the latent infection terminal proteins of Epstein-Barr virus in patients with nasopharyngeal carcinoma. *J. gen. Virol.*, **74**, 811–818

Freese, U.K., Laux, G., Hudewentz, J., Schwarz, E. & Bornkamm, G.W. (1983) Two distant clusters of partially homologous small repeats of Epstein-Barr virus are transcribed upon induction of an abortive or lytic cycle of the virus. *J. Virol.*, **48**, 731–743

Fresen, K.O., Cho, M.S., Gissman, L. & zur Hausen, H. (1980) NC37R1 EB virus: A possible recombinant between 'intracellular' NC37 viral DNA and superinfecting P3HR1 EBV. *Intervirology*, **12**, 303–310

Fries, K.L., Miller, W.E. & Raab-Traub, N. (1996) Epstein-Barr Virus latent membrane protein 1 blocks p53-mediated apoptosis through the induction of the A20 gene. *J. Virol.*, **70**, 8653–8659

Fries, K.L., Sculley, T.B., Webster-Cyriaque, J., Rajadurai, P., Sadler, R.H. & Raab-Traub, N. (1997) Identification of a novel protein encoded by the *Bam*HI A region of the Epstein-Barr virus. *J. Virol.*, **71**, 2765–2771

Frisan, T., Sjöberg, J., Dolcetti, R., Boiocchi, M., De Re, V., Carbone, A., Brautbar, C., Battat, S., Biberfeld, P., Eckman, M., Öst, Å., Christensson, B., Sundström, C., Björkholm, M., Pisa, P. & Masucci, M.G. (1995) Local suppression of Epstein-Barr virus (EBV)-specific cytotoxicity in biopsies of EBV-positive Hodgkin's disease. *Blood*, **86**, 1493–1501

Frisan, T., Zhang, Q.-J., Levitskaya, J., Coram, M., Kurilla, M.G. & Masucci, M.G. (1996) Defective presentation of MHC class I-restricted cytotoxic T-cell epitopes in Burkitt's lymphoma cells. *Int. J. Cancer*, **68**, 251–258

Fujimoto, K., Terato, K., Miyamoto, J., Ishiko, H,. Fujisaki, M., Cho, F. & Honjo, S. (1990) Establishment of a B-lymphoblastoid cell line infected with Epstein-Barr related virus from a cynomolgus monkey (Macaca fascicularis). *J. med. Primatol.*, **19**, 21–30

Fukayama, M., Hayashi, Y., Iwasaki, Y., Chong, J., Ooba, T., Takizawa, T., Koike, M., Mizutani, S., Miyaki, M. & Hirai, K. (1994) Epstein-Barr virus-associated gastric carcinoma and Epstein-Barr virus infection of the stomach. *Lab. Invest.*, **71**, 73–81

Funakoshi, S., Beckwith, M., Fanslow, W., Longo, D.L. & Murphy, W.J. (1995) Epstein-Barr virus-induced human B-cell lymphoma arising in HuPBL-SCID chimeric mice: Characterization and the role of CD40 stimulation in their treatment and prevention. *Pathobiology*, **63**, 133–142

Furukawa, M., Sakashita, H., Kato, C. & Umeda, R. (1990) Epstein-Barr virus infection of epithelial cells derived from primary cultures of adenoidal tissue. *Eur. Arch. Otorhinolaryngol.*, **247**, 109–113

Furukawa, T., Maruyama, S., Kawaichi, M. & Honjo, T. (1992) The Drosophila homolog of the immunoglobulin recombination signal-binding protein regulates peripheral nervous system development. *Cell*, **69**, 1191–1197

Gahn, T.A. & Sugden, B. (1995) An EBNA-1-dependent enhancer acts from a distance of 10 kilobase pairs to increase expression of the Epstein-Barr virus LMP gene. *J. Virol.*, **69**, 2633–2636

Gaidano, G. & Dalla-Favera, R. (1995) Molecular pathogenesis of AIDS-related lymphomas. *Adv. Cancer Res.*, **67**, 113–153

Gaidano, G., Ballerini, P., Gong, J.Z., Inghirami, G., Neri, A., Newcomb, E.W., Magrath, I.T., Knowles, D.M. & Dalla-Favera, R. (1991) p53 Mutations in human lymphoid malignancies: Association with Burkitt's lymphoma and chronic lymphocytic leukemia. *Proc. natl Acad. Sci. USA*, **88**, 5413–5417

Gaidano, G., Hauptschein, R.S., Parsa, N.Z., Offit, K., Rao, P.H., Lenoir, G., Knowles, D.M., Chaganti, R.S. & Dalla-Favera, R. (1992) Deletions involving two distinct regions of 6q in B-cell non-Hodgkin lymphoma. *Blood*, **80**, 1781–1787

Gaidano, G., Lo Coco, F., Ye, B.H., Shibata, D., Levine, A.M., Knowles, D.M. & Dalla-Favera, R. (1994) Rearrangements of the BCL-6 gene in acquired immunodeficiency syndrome-associated non-Hodgkin's lymphoma: Association with diffuse large-cell subtype. *Blood*, **84**, 397–402

Gajwani, B.W., Devereaux, J.M. & Beg, J.A. (1980) Familial clustering of nasopharyngeal carcinoma. *Cancer*, **46**, 2325–2327

Gal, A.A., Unger, E.R., Koss, M.N. & Yen, T.S. (1991) Detection of Epstein-Barr virus in lymphoepithelioma-like carcinoma of the lung. *Mod.-Pathol.*, **4**, 264–268

Gallagher, R.P. & Elwood, J.M. (1979) Cancer mortality among Chinese, Japanese, and Indians in British Columbia, 1964–73. *Natl Cancer Inst. Monogr.*, **53**, 89–94

Gallo, O., Di Lollo, S., Graziani, P., Gallina, E. & Baroni, G. (1995) Detection of Epstein-Barr virus genome in sinonasal undifferentiated carcinoma by use of in situ hybridization. *Otolaryngol. Head Neck Surg.*, **112**, 659–664

Gan, Y.-J., Chodosh, J., Morgan, A. & Sixbey, J.W. (1997) Epithelial cell polarization is a determinant in the infectious outcome of immunoglobulin A-mediated entry by Epstein-Barr virus. *J. Virol.*, **71**, 519–526

Garcia, C.R., Brown, N.A., Schreck, R., Stiehm, E.R. & Hudnall, S.D. (1987) B-cell lymphoma in severe combined immunodeficiency not associated with the Epstein-Barr virus. *Cancer*, **60**, 2941–2947

Gardner, M.J., Pannett, B., Winter, P.D. & Cruddas, A.M. (1993) A cohort study of workers exposed to formaldehyde in the British chemical industry: An update. *Br. J. ind. Med.*, **50**, 827–834

Garnier, J.-L., Cannon, M.J., Chargui, J., Touraine, J.-L. & Cooper, N.R. (1993) EBV human B-cell lymphomas in Scid mice: Description of the model and study of the role of anti-CD40 and anti-CD23 monoclonal antibodies in their development. *Transplant. Proc.*, **25**, 1387–1383

Garnier, J.-L., Lebranchu, Y., Dantal, J., Bedrossian, J., Cahen, R., Assouline, D., Jaccard, A., Fetissoff, F., Moreau, A., Martin, X., Delsol, G., Berger, F. & Touraine, J.-L.(1996) Hodgkin's disease after transplantation. *Transplantation*, **61**, 71–76

Gavioli, R., de Campos-Lima, P.O., Kurilla, M.G., Kieff, E., Klein, G. & Masucci, M.G. (1992) Recognition of the Epstein-Barr virus-encoded nuclear antigens EBNA-4 and EBNA-6 by HLA-A11-restricted cytotoxic T lymphocytes: Implications for down-regulation of HLA-A11 in Burkitt lymphoma. *Proc. natl Acad. Sci. USA*, **89**, 5862–5866

Gavioli, R., Kurilla, M.G., de Campos-Lima, P.O., Wallace, L.E., Dolcetti, R., Murray, R.J., Rickinson, A.B. & Masucci, M.G. (1993) Multiple HLA A11-restricted cytotoxic T-lymphocyte epitopes of different immunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. *J. Virol.*, **67**, 1572–1578

Geddes, J.F., Bhattacharjee, M.B., Savage, K. Scaravilli, F. & McLaughlin, J.E. (1992) Primary cerebral lymphoma: A study of 47 cases probed for Epstein-Barr virus genome. *J. clin. Pathol.*, **45**, 587-590

van Gelder, T., Jonkman, F.A.M., Niesters, H.G.M., Vuzevski, V.D., Spillenaar Bilgen, E.J. & Weimar, W. (1996) Absence of Epstein-Barr virus involvement in an adult heart transplant recipient with an epitheloid leiomyosarcoma (Letter to the Editor). *J. Heart Lung Transpl.*, **15**, 650–651

Gerber, P. & Birch, S. (1967) Complement-fixing antibodies in sera of human and non-human primates to viral antigens derived from Burkitt's lymphoma cells. *Proc. natl Acad. Sci. USA*, **58**, 478–484

Gerber, P., Nkrumah, F.K., Pritchett, R. & Kieff, E. (1976a) Comparative studies of Epstein-Barr virus strains from Ghana and the United States. *Int. J. Cancer*, **17**, 71–81

Gerber, P., Pritchett, R.F. & Kieff, E.D. (1976b) Antigens and DNA of chimpanzee agent related to Epstein-Barr virus. *J. Virol.*, **19**, 1090–1099

Gerber, P., Kalter, S.S., Schidlovsky, G., Peterson, W.D. & Daniel, M.D. (1977) Biologic and antigenic characteristics of Epstein-Barr virus related herpesviruses of chimpanzees and baboons. *Int. J. Cancer*, **20**, 448–459

Gergely, L., Klein, G. & Ernberg, I. (1971) Host cell macromolecular synthesis in cells containing EBV-induced early antigens, studied by combined immunofluorescence and radio-autography. *Virology*, **45**, 22–29

Gerhartz, H.H., Mittermüller, J., Raghavachar, A., Schmetzer, H., Clemm, C., Kolb, H.-J., Bartram, C.C. & Wolf, H. (1988) Epstein-Barr virus-positive recipient type B-cells survive in a 'complete chimera' after allogeneic bone-marrow transplantation. *Int. J. Cancer*, **42**, 672–676

Gerritsen, E.J.A., Stam, E.D., Hermans, J., van den Berg, H., Haraldsson, A., van Tol, M.J.D., van den Bergh, R.L., Waaijer, J.L.M., Kroes, A.C.M., Kluin, P.M. & Vossen, J.M. (1996) Risk factors for developing EBV-related B cell lymphoproliferative disorders (BLPD) after non-HLA-identical BMT in children. *Bone Marrow Transplant.*, **18**, 377–382

Geser, A., Charnay, N., Day, N.E., de Thé, G. & Ho, H.C. (1978) Environmental factors in the etiology of nasopharyngeal carcinoma: Report on a case–control study in Hong Kong. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 213–229

Geser, A., de Thé, G., Lenoir, G., Day, N.E. & Williams, E.H. (1982) Final case reporting from the Ugandan prospective study of the relationship between EBV and Burkitt's lymphoma. *Int. J. Cancer*, **29**, 397–400

Geser, A., Brubaker, G. & Draper, C.C. (1989) Effect of a malaria suppression program on the incidence of African Burkitt's lymphoma. *Am. J. Epidemiol.*, **129**, 740–752

Geysen, H.M., Rodda, S.J. & Mason, T.J. (1986) A priori delineation of a peptide which mimics a discontinuous antigenic determinant. *Mol. Immunol.*, **23**, 709–715

Ghosh, D. & Kieff, E. (1990) cis-Acting regulatory elements near the Epstein-Barr virus latent-infection membrane protein transcriptional start site. *J. Virol.*, **64**, 1855–1858

Gibbons, D.L., Rowe, M., Cope, A.P., Feldmann, M. & Brennan, F.M. (1994) Lymphotoxin acts as an autocrine growth factor for Epstein-Barr virus-transformed B cells and differentiated Burkitt lymphoma cell lines. *Eur. J. Immunol.*, **24**, 1879–1885

Gilligan, K., Rajadurai, P., Resnick, L. & Raab-Traub, N. (1990a) Epstein-Barr virus small nuclear RNAs are not expressed in permissively infected cells in AIDS-associated leuko-plakia. *Proc. natl Acad. Sci. USA*, **87**, 8790–8794

Gilligan, K., Sato, H., Rajadurai, P., Busson, P., Young, L., Rickinson, A., Tursz, T. & Raab-Traub, N. (1990b) Novel transcription from the Epstein-Barr virus terminal *EcoRI* fragment, DIJhet, in a nasopharyngeal carcinoma. *J. Virol.*, **64**, 4948–4956

Gilligan, K.J., Rajadurai, P., Lin, J.-C., Busson, P., Abdel-Hamid, M., Prasad, U., Tursz, T. & Raab-Traub, N. (1991) Expression of the Epstein-Barr virus *BamHI* A fragment in naso-pharyngeal carcinoma: Evidence for a viral protein expressed *in vivo*. *J. Virol.*, **65**, 6252–6259

Gilles, H.M.J. (1963) *Akeefe. An Environmental Study of a Nigerian Village Community*, Ibadan, Ibadan University Press

Glaser, S.L. (1987) Regional variation in Hodgkin's disease incidence by histologic subtype in the US. *Cancer*, **60**, 2841–2847

Glaser, S.L. (1990) Spatial clustering of Hodgkin's disease in the San Francisco Bay area. *Am. J. Epidemiol.*, **132**, S167–S177

Glaser, S.L., Lin, R.J., Stewart, S.L., Ambinder, R.F., Jarrett, R.F., Brousset, P., Pallesen, G., Gulley, M.L., Khan, G., O'Grady, J., Hummel, M., Preciado, M.V., Knecht, H., Chan, J.K.C. & Claviez, A. (1997) Epstein-Barr virus-associated Hodgkin's disease: Epidemiologic characteristics in international data. *Int. J. Cancer*, **70**, 375–382

Gledhill, S., Gallagher, A., Jones, D.B., Krajewski, A.S., Alexander, F.E., Klee, E., Wright, D.H., O'Brien, C., Onions, D.E. & Jarrett, R.F. (1991) Viral involvement in Hodgkin's disease: Detection of clonal type A Epstein-Barr virus genomes in tumour samples. *Br. J. Cancer*, **64**, 227–232

Glickman, J.N., Howe, J.G. & Steitz, J.A. (1988) Structural analyses of EBER1 and EBER2 ribonucleoprotein particles present in Epstein-Barr virus-infected cells. *J. Virol.*, **62**, 902–911

Goldberg, J.C. & Drut, R. (1986) Undifferentiated Burkitt type lymphoma in Argentina: 16 years later. *Med. Buenos Aires*, **46**, 646 (in Spanish)

Goldfeld, A.E., Liu, P., Liu, S., Flemington, E.K., Strominger, J.L. & Speck, S.H. (1995) Cyclo-sporin A and FK506 block induction of the Epstein-Barr virus lytic cycle by anti-immuno-globulin. *Virology*, **209**, 225–229

Goldman, J.M. & Aisenberg, A.C. (1970) Incidence of antibody to EB virus, herpes simplex, and cytomegalovirus in Hodgkin's diesease. *Cancer*, **26**, 327–331

Goldschmidts, W.L., Bhatia, K., Johnson, J.F., Akar, N., Gutiérrez, M.I., Shibata, D., Carolan, M., Levine, A. & Magrath, I.T. (1992) Epstein-Barr virus genotypes in AIDS-associated lymphomas are similar to those in endemic Burkitt's lymphomas. *Leukemia*, **6**, 875–878

Goldsmith, K., Bendell, L. & Frappier, L. (1993) Identification of EBNA1 amino acid sequences required for the interaction of the functional elements of the Epstein-Barr virus latent origin of DNA replication. *J. Virol.*, **67**, 3418–3426

Goma, L.K.H. (1965) The environmental background to cases of Burkitt's lymphoma syndrome in Uganda. *E. Afr. med. J.*, **42**, 62–66

Gong, M. & Kieff, E. (1990) Intracellular trafficking of two major Epstein-Barr virus glyco-proteins, gp350/220 and gp110. *J. Virol.*, **64**, 1507–1516

Gong, M., Ooka, T., Matsuo, T. & Kieff, E. (1987) Epstein-Barr virus glycoprotein homologous to herpes simplex virus gB. *J. Virol.*, **61**, 499–508

Goplen, A.K., Dunlop, O., Liestøl, K., Lingjaerde, O.C., Bruun, J.N. & Maehlen, J. (1997) The impact of primary central nervous system lymphoma in AIDS patients: A population-based autopsy study from Oslo. *J. Acquired Immune Defic. Synd. Hum. Retrovirol.*, **14**, 351–354

Gosselin, J., Flamand, L., D'Addario, M., Hiscott, J., Stefanescu, I., Ablashi D.V., Gallo R.C. & Menezes, J. (1992) Modulatory effects of Epstein-Barr, herpes simplex, and human herpes-6 viral infections and coinfections on cytokine synthesis. A comparative study. *J. Immunol.*, **149**, 181–187

Gotlieb-Stematsky, T., Vonsover, A., Ramot, B., Zaizov, R., Nordan, U., Aghai, E., Kende, G. & Modan, M. (1975) Antibodies to Epstein-Barr virus in patients with Hodgkin's disease and leukemia. *Cancer*, **36**, 1640–1645

Gotlieb-Stematsky, T., Ramot, B., Vonsover, A., Aghai, E., Kende, G., Ninio, M. & Modan, M. (1976) Antibodies to Epstein-Barr viral capsid and early antigens associated with Burkitt's lymphoma and lymphoblastic lymphosarcoma in Israel. *J. natl Cancer Inst.*, **56**, 721–723

Graham, F.L., Smiley, J., Russell, W.C. & Nairn, R. (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. gen. Virol.*, **36**, 59–72

Grässer, F.A., Murray, P.G., Kremmer, E., Klein, K., Remberger, K., Feiden, W., Reynolds, G., Niedobitek, G., Young, L.S. & Mueller Lantzsch, N. (1994) Monoclonal antibodies directed against the Epstein-Barr virus-encoded nuclear antigen 1 (EBNA1): Immunohistologic detection of EBNA1 in the malignant cells of Hodgkin's disease. *Blood*, **84**, 3792–3798

Gratama, J.W. & Ernberg, I. (1995) Molecular epidemiology of Epstein-Barr virus infection. *Adv. Cancer Res.*, **67**, 197–255

Gratama, J.W., Oosterveer, M.A.P., Zwaan, F.E., Lepoutre, J., Klein, G. & Ernberg, I. (1988) Eradication of Epstein-Barr virus by allogeneic bone marrow transplantation: Implications for sites of viral latency. *Proc. natl Acad. Sci. USA*, **85**, 8693–8696

Gratama, J.W., Oosterveer, M.A.P., Klein, G. & Ernberg, I. (1990) EBNA size polymorphism can be used to trace Epstein-Barr virus spread within families. *J. Virol.*, **64**, 4703–4708

Gratama, J.W., Lennette, E.T., Lönnqvist, B., Oosterveer, M.A.P., Klein, G., Ringdén, O. & Ernberg, I. (1992) Detection of multiple Epstein-Barr viral strains in allogeneic bone marrow transplant recipients. *J. med. Virol.*, **37**, 39–47

Gravell, M., Levine, P.H., McIntyre, R.F., Land, V.J. & Pagano, J.S. (1976) Epstein-Barr virus in an American patient with Burkitt's lymphoma: Detection of viral genome in tumor tissue and establishment of a tumor-derived cell line (NAB). *J. natl Cancer Inst.*, **56**, 701–704

Greenspan, J.S., Greenspan, D., Lennette, E.T., Abrams, D.I., Conant, M.A., Petersen, V. & Freese, U.K. (1985) Replication of Epstein-Barr virus within the epithelial cells of oral 'hairy' leukoplakia, an AIDS-associated lesion. *New Engl. J. Med.*, **313**, 1564–1571

Gregory, C.D., Kirchgens, C., Edwards, C.F., Young, L.S., Rowe, M., Forster, A., Rabbitts, T.H. & Rickinson, A.B. (1987a) Epstein-Barr virus-transformed human precursor B cell lines: Altered growth phenotype of lines with germ-line or rearranged but nonexpressed heavy chain genes. *Eur. J. Immunol.*, **17**, 1199–1207

Gregory, C.D., Tursz, T., Edwards, C.F., Tetaud, C,. Talbot, M., Caillou, B., Rickinson, A.B. & Lipinski, M. (1987b) Identification of a subset of normal B cells with a Burkitt's lymphoma (BL)-like phenotype. *J. Immunol.*, **139**, 313–318

Gregory, C.D., Murray, R.J., Edwards, C.F. & Rickinson, A.B. (1988) Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitt's lymphoma underlies tumor cell escape from virus-specific T cell surveillance. *J. exp. Med.*, **167**, 1811–1824

Gregory, C.D., Rowe, M. & Rickinson, A.B. (1990) Different Epstein-Barr virus–B cell interactions in phenotypically distinct clones of a Burkitt's lymphoma cell line. *J. gen. Virol.*, **71**, 1481–1495

Gregory, C.D., Dive, C., Henderson, S., Smith, C.A., Williams, G.T., Gordon, J. & Rickinson, A.B. (1991) Activation of Epstein-Barr virus latent genes protects human B cells from death by apoptosis. *Nature*, **349**, 612–614

Griffin, B.E. & Karran, L. (1984) Immortalization of monkey epithelial cells by specific fragments of Epstein-Barr virus DNA. *Nature*, **309**, 78–82

Grogan, E.A., Summers, W.P., Dowling, S., Shedd, D., Gradoville, L. & Miller, G. (1983) Two Epstein-Barr viral nuclear neoantigens distinguished by gene transfer, serology, and chromosome binding. *Proc. natl Acad. Sci. USA*, **80**, 7650–7653

Grossman, S.R., Johannsen, E., Tong, X., Yalamanchili, R. & Kieff, E. (1994) The Epstein-Barr virus nuclear antigen 2 transactivator is directed to response elements by the Jκ recombination signal binding protein. *Proc. natl Acad. Sci. USA*, **91**, 7568–7572

Groux, H., Cottrez, F., Montpellier, C., Quatannens, B., Coll, J., Stehelin, D. & Auriault, C. (1997) Isolation and characterization of transformed human T-cell lines infected by Epstein-Barr virus. *Blood*, **89**, 4521–4530

Gruffat, H. & Sergeant, A. (1994) Characterization of the DNA-binding site repertoire for the Epstein-Barr virus transcription factor R. *Nucleic Acids Res.*, **22**, 1172–1178

van Grunsven, W.M.J., Spaan, J.M. & Middeldorp, J.M. (1994) Localization and diagnostic application of immunodominant domains of the BFRF3-encoded Epstein-Barr virus capsid protein. *J. infect. Dis.*, **170**, 13–19

Gu, S.-Y. & Zeng, Y. (1978) Complement fixing antibody levels in sera from population groups in Kwangchow and in Peking. *Chin. J. ENT*, **1**, 23–25

Gu, S.-Y., Tang, W.P., Zeng, Y., Zhao, M.L., Zhao, E.W.P., Deng, W.H. & Li, K. (1983) An epithelial cell line established from poorly differentiated nasopharyngeal carcinoma. *Chin. J. Cancer*, **2**, 70–72

Gu, S.-Y., Huang, T.-M., Ruan, L., Miao, Y.-H., Lu, H., Chu, C.-M., Motz, M. & Wolf, H. (1995) First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. *Dev. biol. Stand.*, **84**, 171–177

Guarner, J., del Rio, C., Carr, D., Hendrix, L.E., Eley, J.W. & Unger, E.R. (1991) Non-Hodgkin's lymphomas in patients with human immunodeficiency virus infection. Presence of Epstein-Barr virus by in situ hybridization, clinical presentation, and follow-up. *Cancer*, **68**, 2460–2465

Guettier, C., Hamilton-Dutoit, S., Guillemain, R., Farge, D., Amrein, C., Vulser, C., Hofman, P., Carpentier, A. & Diebold, J. (1992) Primary gastrointestinal malignant lymphomas associated with Epstein-Barr virus after heart transplantation. *Histopathology*, **20**, 21–28

Guinee, D., Jr, Jaffe, E., Kingma, D., Fishback, N., Wallberg, K., Krishnan, J., Frizzera, G., Travis, W. & Koss, M. (1994) Pulmonary lymphomatoid granulomatosis. Evidence for a proliferation of Epstein-Barr virus infected B-lymphocytes with a prominent T-cell component and vasculitis. *Am J. surg. Pathol.*, **18**, 753-764

Gulley, M.L., Eagan, P.A., Quintanilla-Martinez, L., Picado, A.L., Smir, B.N., Childs, C., Dunn, C.D., Craig, F.E., Williams, J.W., Jr & Banks, P.M. (1994) Epstein-Barr virus DNA is abundant and monoclonal in the Reed-Sternberg cells of Hodgkin's disease: Association with mixed cellularity subtype and Hispanic American ethnicity. *Blood*, **83**, 1595-1602

Gulley, M.L., Amin, M.B., Nicholls, J.M., Banks, P.M., Ayala, A.G., Srigley, J.R., Eagan, P.A. & Ro, J.Y. (1995) Epstein-Barr virus is detected in undifferentiated nasopharyngeal carcinoma but not in lymphoepithelioma-like carcinoma of the urinary bladder. *Hum. Pathol.*, **26**, 1207-1214

Gulley, M.L., Pulitzer, D.R., Eagan, P.A. & Schneider, B.G. (1996) Epstein-Barr virus infection is an early event in gastric carcinogenesis and is independent of *bcl-2* expression and p53 accumulation. *Hum. Pathol.*, **27**, 20-27

Gunapala, D.E., Facer, C.A., Davidson, R. & Weir, W.R.C. (1990) In vitro analysis of Epstein-Barr virus: Host balance in patients with acute *Plasmodium falciparum* malaria. I. Defective T-cell control. *Parasitol. Res.*, **76**, 531-535

Gunvén, P., Klein, G., Klein, E., Norin, T. & Singh, S. (1980) Surface immunoglobulins on Burkitt's lymphoma biopsy cells from 91 patients. *Int. J. Cancer*, **25**, 711-719

Gupta, R.K. & Siber, G.R. (1995) Adjuvants for human vaccines — Current status, problems and future prospects. *Vaccine*, **13**, 1263-1276

Gussander, E. & Adams, A. (1984) Electron microscopic evidence for replication of circular Epstein-Barr virus genomes in latently infected Raji cells. *J. Virol.*, **52**, 549-556

Gutensohn, N.M. (1982) Social class and age at diagnosis of Hodgkin's disease: New epidemiologic evidence for the 'two-disease' hypothesis. *Cancer Treat. Rep.*, **66**, 689-695

Gutensohn, N. & Cole, P. (1977) Epidemiology of Hodgkin's disease in the young. *Int. J. Cancer*, **19**, 595-604

Gutensohn, N. & Cole, P. (1981) Childhood social environment and Hodgkin's disease. *New Engl. J. Med.*, **304**, 135-140

Gutensohn, N.M. & Shapiro, D.S. (1982) Social class risk factors among children with Hodgkin's disease. *Int. J. Cancer*, **30**, 433-435

Gutiérrez, M.I., Bhatia, K., Barriga, F., Diez, B., Muriel, F.S., de Andreas, M.L., Epelman, S., Risueño, C. & Magrath, I.T. (1992) Molecular epidemiology of Burkitt's lymphoma from South America: Differences in breakpoint location and Epstein-Barr virus association from tumors in other world regions. *Blood*, **79**, 3261-3266

Gutiérrez, M.I., Bhatia, K. & Magrath, I. (1993) Replicative viral DNA in Epstein-Barr virus associated Burkitt's lymphoma biopsies. *Leukemia Res.*, **17**, 285-289

Gutiérrez, M.I., Raj, A., Spangler, G., Sharma, A., Hussain, A., Judde, J.-G., Tsao, S.W., Yuen, P.W., Joab, I., Magrath, I.T. & Bhatia, K. (1997) Sequence variations in EBNA-1 may dictate restriction of tissue distribution of Epstein-Barr virus in normal and tumour cells. *J. gen. Virol.*, **78**, 1663-1670

Gutsch, D.E., Holley-Guthrie, E.A., Zhang, Q., Stein, B., Blanar, M.A., Baldwin, A.S. & Kenney, S.C. (1994) The bZIP transactivator of Epstein-Barr virus, BZLF1, functionally and physically interacts with the p65 subunit of NF-kappa B. *Mol. Cell Biol.*, **14**, 1939-1948

Haas, H., Mohr, U. & Krüger, F.W. (1973) Comparative studies with different doses of N-nitrosomorpholine, N-nitrosopiperidine, N-nitrosomethylurea, and dimethylnitrosamine in Syrian golden hamsters. *J. natl Cancer Inst.*, **51**, 1295–1301

Haddow, A.J. (1963) An improved map for the study of Burkitt's lymphoma syndrome in Africa. *E. Afr. med. J.*, **40**, 429–432

Haddy, T.B., Adde, M.A. & Magrath, I.T. (1991) CNS involvement in small non-cleaved cell lymphoma: Is CNS disease per se a poor prognostic sign? *J. clin. Oncol.*, **9**, 1973–1982

Halbert, S.P., Anken, M., Henle, W. & Golubjatnikov, R. (1982) Detection of infectious mononucleosis heterophil antibody by a rapid, standardized enzyme-linked immunosorbent assay procedure. *J. clin. Microbiol.*, **15**, 610–616

Hall, P.J., Levin, A.G., Entwistle, C.C., Knight, S.C., Wasunna, A., Kung'u, A. & Brubaker, G. (1982) HLA antigens in East African black patients with Burkitt's lymphoma or nasopharyngeal carcinoma and in controls: A pilot study. *Hum. Immunol.*, **5**, 91–105

Hallee, T.J., Evans, A.S., Niederman, J.C., Brooks, C.M. & Voegtly, J.H. (1974) Infectious mononucleosis at the United States military academy. A prospective study of a single class over four years. *Yale J. Biol. Med.*, **3**, 182–195

Halprin, J., Scott, A.L., Jacobson, L., Levine, P.H., Ho, J.H.C., Niederman, J.C., Hayward, D. & Milman, G. (1986) Enzyme-linked immunosorbent assay of antibodies to Epstein-Barr virus nuclear and early antigens in patients with infectious mononucleosis and nasopharyngeal carcinoma. *Am. Coll. Phys.*, **104**, 331–337

Haluska, F.G., Brufsky, A.M. & Canellos, G.P. (1994) The cellular biology of the Reed-Sternberg cell. *Blood*, **84**, 1005–1019

Hamilton-Dutoit, S.J. & Pallesen, G. (1992) A survey of Epstein-Barr virus gene expression in sporadic non-Hodgkin's lymphomas. Detection of Epstein-Barr virus in a subset of peripheral T-cell lymphomas. *Am. J. Pathol.*, **140**, 1315–1325

Hamilton-Dutoit, S.J. & Pallesen, G. (1994) Detection of Epstein-Barr virus small RNAs in routine paraffin sections using non-isotopic RNA/RNA in situ hybridization. *Histopathology*, **25**, 101–111

Hamilton-Dutoit, S.J., Therkildsen, M.H., Nielsen, N.H., Jensen, H., Hansen, J.P.H. & Pallesen, G. (1991a) Undifferentiated carcinoma of the salivary gland in Greenlandic Eskimos: Demonstration of Epstein-Barr virus DNA by in situ nucleic acid hybridization. *Hum. Pathol.*, **22**, 811–815

Hamilton-Dutoit, S.J., Pallesen, G., Franzmann, M.B., Karkov, J., Black, F., Skinhøj, P. & Pedersen, C. (1991b) AIDS-related lymphoma. Histopathology, immunophenotype, and association with Epstein-Barr virus as demonstrated by in situ nucleic acid hybridization. *Am. J. Pathol.*, **138**, 149–163

Hamilton-Dutoit, S.J., Rea, D., Raphael, M., Sandvej, K., Delecluse, H.J., Gisselbrecht, C., Marelle, L., van Krieken, J.H.J.M. & Pallesen, G. (1993a) Epstein-Barr virus-latent gene expression and tumor cell phenotype in acquired immunodeficiency syndrome-related non-Hodgkin's lymphoma. Correlation of lymphoma phenotype with three distinct patterns of viral latency. *Am. J. Pathol.*, **143**, 1072–1085

Hamilton-Dutoit, S.J., Raphael, M., Audouin, J., Diebold, J., Lisse, I., Pedersen, C., Oksenhendler, E., Marelle, L. & Pallesen, G. (1993b) In situ demonstration of Epstein-Barr virus small RNAs (EBER 1) in acquired immunodeficiency syndrome-related lymphomas: Correlation with tumor morphology and primary site. *Blood*, **82**, 619–624

Hammarskjöld, M.-L. & Simurda, M.C. (1992) Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-κ B activity. *J. Virol.*, **66**, 6496–6501

Hammerschmidt, W. & Sugden, B. (1988) Identification and characterization of *oriLyt*, a lytic origin of DNA replication of Epstein-Barr virus. *Cell*, **55**, 427–433

Hammerschmidt, W. & Sugden, B. (1989) Genetic analysis of immortalizing functions of Epstein-Barr virus in human B lymphocytes. *Nature*, **340**, 393–397

Hammerschmidt, W., Sugden, B. & Baichwal, V.R. (1989) The transforming domain alone of the latent membrane protein of Epstein-Barr virus is toxic to cells when expressed at high levels. *J. Virol.*, **63**, 2469–2475

Hampar, B., Tanaka, A., Nonoyama, M. & Derge, J.G. (1974) Replication of the resident repressed Epstein-Barr virus genome during the early S phase (S-1 period) of nonproducer Raji cells. *Proc. natl Acad. Sci. USA*, **71**, 631–633

Hansson, M., Falk, K. & Ernberg, I. (1983) Epstein-Barr virus transformation of human pre-B cells. *J. exp. Med.*, **158**, 616–622

Harabuchi, Y., Yamanaka, N., Kataura, A., Imai, S., Kinoshita, T., Mizuno, F. & Osato, T. (1990) Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. *Lancet*, **335**, 128–130

Harabuchi, Y., Imai, S., Wakashima, J., Hirao, M., Kataura, A., Osato, T. & Kon, S. (1996) Nasal T-cell lymphoma causally associated with Epstein-Barr virus. Clinicopathologic, phenotypic, and genotypic studies. *Cancer*, **77**, 2137-2149

Harada, S. & Kieff, E. (1997) Epstein-Barr virus nuclear protein LP stimulates EBNA-2 acidic domain-mediated transcriptional activation. *J. Virol.*, **71**, 6611–6618

Harn, H.-J., Chang, J.-Y., Wang, M.-W., Ho, L.-I., Lee, H.-S., Chiang, J.-H. & Lee, W.-H. (1995) Epstein-Barr virus-associated gastric adenocarcinoma in Taiwan. *Hum. Pathol.*, **26**, 267–271

Harrington, D.S., Weisenburger, D.D. & Purtilo, D.T. (1987) Malignant lymphoma in the X-linked lymphoproliferative syndrome. *Cancer*, **59**, 1419–1429

Harris, A., Young, B.D. & Griffin, B.E. (1985) Random association of Epstein-Barr virus genomes with host cell metaphase chromosomes in Burkitt's lymphoma-derived cell lines. *J. Virol.*, **56**, 328–332

Harris, N. L., Jaffe, E.S., Stein, H., Banks, P.M., Chan, J.K.C., Cleary, M. L., Delsol, G., De Wolf-Peeters, C., Falini, B., Gatter, K.C., Grogan, T.M., Isaacson, P.G., Knowles, D.M., Mason, D.Y., Muller-Hermelink, H.-K., Pileri, S.A., Piris, M.A., Ralfkiaer, E., & Warnke, R.A. (1994) A revised European–American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. *Blood*, **84**, 1361–1392

Hartge, P., Devesa, S.S. & Fraumeni, J.F., Jr (1994) Hodgkin's and non-Hodgkin's lymphomas. In: *Cancer Surveys*, Vol. 19/20, *Trends in Cancer Incidence and Mortality*, London, Imperial Cancer Research Fund, pp. 423–453

zur Hausen, H. (1976) Biochemical approaches to detection of Epstein-Barr virus in human tumors. *Cancer Res.*, **36**, 678–680

zur Hausen, H. & Schulte-Holthausen, H. (1970) Presence of EB virus nucleic acid homology in a 'virus-free' line of Burkitt tumour cells. *Nature*, **227**, 245–248

zur Hausen, H., Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P. & Santesson, L. (1970) EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature*, **228**, 1056–1058

Hawkins, E.P., Krischer, J.P., Smith, B.E., Hawkins, H.K. & Finegold, M.J. (1990) Nasopharyngeal carcinoma in children — A retrospective review and demonstration of Epstein-Barr viral genomes in tumor cell cytoplasm: A report of the Pediatric Oncology Group. *Hum. Pathol.*, **21**, 805–810

Hayes, R.B., Blair, A., Stewart, P.A., Herrick, R.F. & Mahar, H. (1990) Mortality of US embalmers and funeral directors. *Am. J. ind. Med.*, **18**, 641–652

Hearing, J.C. & Levine, A.J. (1985) The Epstein-Barr virus nuclear antigen (*Bam*HI K antigen) is a single-stranded DNA binding phosphoprotein. *Virology*, **145**, 105–116

Hedrick, J.A., Watry, D., Speiser, C., O'Donnell, P., Lambris, J.D. & Tsoukas, C.D. (1992) Interaction between Epstein-Barr virus and a T cell line (HSB-2) via a receptor phenotypically distinct from complement receptor type 2. *Eur. J. Immunol.*, **22**, 1123–1131

Heineman, T., Gong, M., Sample, J. & Kieff, E. (1988) Identification of the Epstein-Barr virus gp85 gene. *J. Virol.*, **62**, 1101–1107

Henderson, B.E. & Louie, E. (1978) Discussion of risk factors for nasopharyngeal carcinoma. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 251–260

Henderson, B.E., Dworsky, R., Menck, H., Alena, B., Henle, W., Henle, G., Teraski, P., Newell, G.R., Rawlings, W. & Kinnear, B.K. (1973) Case–control study of Hodgkin's disease. II. Herpesvirus group antibody titers and HL-A type. *J. natl Cancer Inst.*, **51**, 1443–1447

Henderson, B.E., Louie, E., Jing, J.S., Buell, P. & Gardner, M.B. (1976) Risk factors associated with nasopharyngeal carcinoma. *New Engl. J. Med.*, **295**, 1101–1106

Henderson, B.E., Dworsky, R., Pike, M.C., Baptista, J., Menck, H., Preston-Martin, S. & Mack, T. (1979) Risk factors for nodular sclerosis and other types of Hodgkin's disease. *Cancer Res.*, **39**, 4507–4511

Henderson, A., Ripley, S., Heller, M. & Kieff, E. (1983) Chromosome site for Epstein-Barr virus DNA in a Burkitt tumor cell line and in lymphocytes growth-transformed *in vitro*. *Proc. natl Acad. Sci. USA*, **80**, 1987–1991

Henderson, S., Rowe, M., Gregory, C., Croom-Carter, D., Wang, F., Longnecker, R., Kieff, E. & Rickinson, A. (1991) Induction of *bcl*-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell*, **65**, 1107–1115

Henderson, S., Huen, D., Rowe, M., Dawson, C., Johnson, G. & Rickinson, A. (1993) Epstein-Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2, protects human B cells from programmed cell death. *Proc. natl Acad. Sci. USA*, **90**, 8479–8483

Henkel, T., Ling, P.D., Hayward, S.D. & Peterson, M.G. (1994) Mediation of Epstein-Barr virus EBNA2 transactivation by recombination signal-binding protein J_κ. *Science*, **265**, 92–95

Henle, G. & Henle, W. (1966) Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriol.*, **91**, 1248–1256

Henle, G. & Henle, W. (1967) Immunofluorescence, interference, and complement fixation technics in the detection of herpes-type virus in Burkitt tumor cell lines. *Cancer Res.*, **27**, 2242–2246

Henle, G. & Henle, W. (1970) Observations on childhood infections with the Epstein-Barr virus. *J. infect. Dis.*, **121**, 303–310

Henle, W. & Henle, G. (1973a) Epstein-Barr virus and infectious mononucleosis. *New Engl. J. Med.*, **288**, 263–264

Henle, W. & Henle, G. (1973b) Epstein-Barr virus-related serology in Hodgkin's disease. *Natl Cancer Inst. Monogr.*, **36**, 79–84

Henle, W. & Henle, G. (1976a) The sero-epidemiology of Epstein-Barr virus. *Adv. Pathobiol.*, 5–17

Henle, G. & Henle, W. (1976b) Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. *Int. J. Cancer*, **17**, 1–7

Henle, W. & Henle, G. (1979) Seroepidemiology of the virus. In: Epstein, M.A. & Achong, B.G., eds, *The Epstein-Barr Virus*, Berlin, Springer-Verlag, pp. 61–78

Henle, W. & Henle, G. (1985) Epstein-Barr virus and human malignancies. *Adv. viral Oncol.*, **5**, 201–238

Henle, W., Diehl, V., Kohn, G., zur Hausen, H. & Henle, G. (1967) Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. *Science*, **157**, 1064–1065

Henle, G,. Henle, W., Clifford, P., Diehl, V., Kafuko, G.W., Kirya, B.G., Klein, G., Morrow, R.H., Munube, G.M.R., Pike, P., Tukei, P.M. & Ziegler, J.L. (1969) Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. *J. natl Cancer Inst.*, **43**, 1147–1157

Henle, W., Henle, G., Ho, H.-C., Burtin, P., Cachin, Y., Clifford, P., de Schryver, A., de Thé, G., Diehl, V. & Klein, G. (1970) Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms and control groups. *J. natl Cancer Inst.*, **44**, 225–231

Henle, G., Henle, W. & Klein, G. (1971a) Demonstration of two distinct components in the early antigen complex of Epstein-Barr virus-infected cells. *Int. J. Cancer*, **8**, 272–282

Henle, G., Henle, W., Klein, G., Gunven, P., Clifford, P., Morrow, R.H. & Ziegler, J.L. (1971b) Antibodies to early Epstein-Barr virus-induced antigens in Burkitt's lymphoma. *J. natl Cancer Inst.*, **46**, 861–871

Henle, W., Henle, G., Andersson, J., Ernberg, I., Klein, G., Horwitz, C.A., Marklund, G., Rymo, L., Wellinder, C. & Straus, S.E. (1987) Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proc. natl Acad. Sci. USA*, **84**, 570–574

Hennessy, K., Fennewald, S., Hummel, M., Cole, T. & Kieff, E. (1984) A membrane protein encoded by Epstein-Barr virus in latent growth-transforming infection. *Proc. natl Acad. Sci. USA*, **81**, 7207–7211

Hennessy, K., Fennewald, S. & Kieff, E. (1985) A third viral nuclear protein in lymphoblasts immortalized by Epstein-Barr virus. *Proc. natl Acad. Sci. USA*, **82**, 5944–5948

Hennessy, K., Wang, F., Bushman, E.W. & Kieff, E. (1986) Definitive identification of a member of the Epstein-Barr virus nuclear protein 3 family. *Proc. natl Acad. Sci. USA*, **83**, 5693–5697

Herbst, H. & Niedobitek, G. (1993) Epstein-Barr virus and Hodgkin's disease. *Int. J. clin. lab. Res.*, **23**, 13–16

Herbst, H., Tippelmann, G., Anagnostopoulos, I., Gerdes, J., Schwarting, R., Boehm, T., Pileri, S., Jones, D.B. & Stein, H. (1989) Immunoglobin and T-cell receptor gene rearrangements in Hodgkin's disease and Ki-1-positive anaplastic large cell lymphoma: Dissociation between phenotype and genotype. *Leukemia Res.*, **13**, 103–116

Herbst, H., Niedobitek, G., Kneba, M., Hummel, M., Finn, T., Anagnostopoulos, I., Bergholz, M., Krieger, G. & Stein, H. (1990) High incidence of Epstein-Barr virus genomes in Hodgkin's disease. *Am. J. Pathol.*, **137**, 13–18

Herbst, H., Dallenbach, F., Hummel, M., Niedobitek, G., Pileri, S., Müller-Lantzsch, N. & Stein, H. (1991a) Epstein-Barr virus latent membrane protein expression in Hodgkin and Reed-Sternberg cells. *Proc. natl Acad. Sci. USA*, **88**, 4766–4770

Herbst, H., Dallenbach, F., Hummel, M., Niedobitek, G., Finn, T., Young, L.S., Rowe, M., Müller-Lantzsch, N. & Stein, H. (1991b) Epstein-Barr virus DNA and latent gene products in Ki-1 (CD30)-positive anaplastic large cell lymphomas. *Blood*, **78**, 2666-2673

Herbst, H., Steinbrecher, E., Niedobitek, G., Young, L.S., Brooks, L., Müller-Lantzsch, N. & Stein, H. (1992) Distribution and phenotype of Epstein-Barr virus-harboring cells in Hodgkin's disease. *Blood*, **80**, 484–491

Herbst, H., Stein, H. & Niedobitek, G. (1993) Epstein-Barr virus and CD30⁺ malignant lymphomas. *Crit. Rev. Oncog. Hematol.*, **4**, 191–239

Herbst, H., Raff, T. & Stein, H. (1996a) Phenotypic modulation of Hodgkin and Reed-Sternberg cells by Epstein-Barr virus. *J. Pathol.*, **179**, 54–59

Herbst, H., Foss, H.-D., Samol, J., Araujo, I., Klotzbach, H., Krause, H., Agathanggelou, A., Niedobitek, G. & Stein, H. (1996b) Frequent expression of interleukin-10 by Epstein-Barr virus-harboring tumor cells of Hodgkin's disease. *Blood*, **87**, 2918–2929

Herndier, B.G., Sanchez, H.C., Chang, K.L., Chen, Y.-Y. & Weiss, L.M. (1993) High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of HIV-associated Hodgkin's disease. *Am. J. Pathol.*, **142**, 1073–1079

Heslop, H.E., Brenner, M.K. & Rooney, C.M. (1994) Donor T cells to treat EBV-associated lymphoma (Letter to the Editor). *New Engl. J. Med.*, **331**, 679–680

Heslop, H.E., Ng, C.Y.C., Li, C., Smith, C.A., Loftin, S.K., Krance, R.A., Brenner, M.K. & Rooney, C.M. (1996) Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nature Med.*, **2**, 551–555

Hesse, J., Levine, P.H., Ebbesen, P., Connelly, R.R. & Mordhorst, C.H. (1977) A case control study on immunity to two Epstein-Barr virus-associated antigens, and to herpes simplex virus and adenovirus in a population-based group of patients with Hodgkin's disease in Denmark, 1971–73. *Int. J. Cancer*, **19**, 49–58

Hessing, M., van Schijndel, H.B. & van Grunsven, W.M.J. (1992) Purification and quantification of recombinant Epstein-Barr viral glycoproteins gp350/220 from Chinese hamster ovary cells. *J. Chromatogr.*, **599**, 267–272

Hidayatalla, A., Malik, M.O.A., El Hadi, A.E., Osman, A.A. & Hutt, M.S.R. (1983) Studies on nasopharyngeal carcinoma in the Sudan. I. Epidemiology and aetiology. *Eur. J. Cancer clin. Oncol.*, **19**, 705–710

Hildesheim, A., West, S., De Veyra, E., De Guzman, M.F., Jurado, A., Jones, C., Imai, J. & Hinuma, Y. (1992) Herbal medicine use, Epstein-Barr virus, and risk of nasopharyngeal carcinoma. *Cancer Res.*, **52**, 3048–3051

Hilgers, F. & Hilgers, J. (1976) An immunofluorescence technique with counterstain on fixed cells for the detection of antibodies to human herpesviruses; antibody patterns in patients with Hodgkin's disease and nasopharyngeal carcinoma. *Intervirology*, **7**, 309–327

Hill, A.B., Lee, S.P., Haurum, J.S., Murray, N., Yao, Q.-Y., Rowe, M., Signoret, N., Rickinson, A.B. & McMichael, A.J. (1995) Class I major histocompatibility complex-restricted cytotoxic T lymphocytes specific for Epstein-Barr virus (EBV) nuclear antigen fail to lyse the EBV-transformed B lymphoblastoid cell lines against which they were raised. *J. exp. Med.*, **181**, 2221–2228

Hilton, D.A., Brown, L.J.R., Pringle, J.H. & Nandha, H. (1993) Absence of Epstein-Barr virus in carcinoma of the cervix. *Cancer*, **72**, 1946–1948

Hinuma, Y., Konn, M., Yamaguchi, J., Wudarski, D., Blakeslee, J. & Grace, J. (1967) Immunofluorescence and herpes type virus particles in the P3HR-1 Burkitt lymphoma gene. *J. Virol.*, **1**, 1045–1051

Hinuma, Y., Ohta-Hatano, R., Suto, T. & Numazaki, Y. (1969) High incidence of Japanese infants with antibody to a herpes-type virus associated with cultured Burkitt lymphoma cells. *Jpn. J. Microbiol.*, **13**, 309–311

Hirayama, T. & Ito, Y. (1981) A new view of the etiology of nasopharyngeal carcinoma. *Prev. Med.*, **10**, 614–622

Hirose, Y., Masaki, Y., Shimizu, S., Ogawa, Y., Takeshita, S, Fukutoku, M., Sugai, S. & Takiguchi, T. (1996) Association of Epstein-Barr virus with human immunodeficiency virus-negative T-cell lymphomas in Japan, in situ hybridization, polymerase chain reaction and immunohistochemical studies. *Leukemia*, **10**, 1673–1676

Hirshaut, Y., Cohen, M.H. & Stevens, D.A. (1973) Epstein-Barr-virus antibodies in American and African Burkitt's lymphoma. *Lancet*, **ii**, 114–116

Hirshaut, Y., Reagen, R.L., Perry, S., De Vita, V., Jr & Barile, M.F. (1974) The search for a viral agent in Hodgkin's disease. *Cancer*, **34**, 1080–1089

Hitt, M.M., Allday, M.J., Hara, T., Karran, L., Jones, M.D., Busson, P., Tursz, T., Ernberg, I. & Griffin, B.E. (1989) EBV gene expression in an NPC-related tumour. *EMBO J.*, **8**, 2639–2651

Ho, P.-T. (1959) *Studies on the Population of China, 1368–1953*, Cambridge, MA, Harvard University Press, pp. 166–168

Ho, H.C. (1967) Nasopharyngeal carcinoma in Hong Kong. In: Muir, C.S. & Shanmugaratnam, K., eds, *Cancer of the Nasopharynx* (UICC Monograph Series, Vol. 1), Copenhagen, Munksgaard, pp. 58–63

Ho, J.H.C. (1971) Genetic and environmental factors in nasopharyngeal carcinoma. In: Nakahara, W., Nishioka, K., Hirayama, T. & Ito, Y., eds, *Recent Advances in Human Tumor Virology and Immunology*, Tokyo, University of Tokyo Press, pp. 275–295

Ho, H.C. (1972) Current knowledge of the epidemiology of nasopharyngeal carcinoma — A review. In: Biggs, P.M., de Thé, G. & Payne, L.N., eds, *Oncogenesis and Herpesviruses* (IARC Scientific Publications No. 2), Lyon, IARC, pp. 357–366

Ho, H.C. (1976) Epidemiology of nasopharyngeal carcinoma. *Gann Monogr. Cancer Res.*, **18**, 49–61

Ho, J.H.C. (1978) An epidemiologic and clinical study of nasopharyngeal carcinoma. *Int. J. Radiat. Oncol. biol. Phys.*, **4**, 183–198

Ho, H.C., Ng, M.H., Kwan, H.C. & Chau, J.C.W. (1976) Epstein-Barr-virus-specific IgA and IgG serum antibodies in nasopharyngeal carcinoma. *Br. J. Cancer*, **34**, 655–660

Ho, M., Miller, G., Atchison, R.W., Breinig, M.K., Dummer, J.S., Andiman, W., Starzl, T.E., Eastman, R., Griffith, B.P., Hardesty, R.L., Bahnson, H.T., Hakala, T.R. & Rosenthal, J.T. (1985) Epstein-Barr virus infections and DNA hybridization studies in posttransplantation lymphoma and lymphoproliferative lesions: The role of primary infection. *J. infect. Dis.*, **152**, 876–886

Ho, Y.-S., Subhendu, C. & Hsu, S.-M. (1987) Induction of differentiation of African Burkitt's lymphoma cells by phorbol ester: Possible relation with early B cells. *Cancer Invest.*, **5**, 101–107

Ho, F.C.S., Srivastava, G., Loke, S.L., Fu, K.H., Leung, B.P.Y., Liang, R. & Choy, D. (1990) Presence of Epstein-Barr virus DNA in nasal lymphomas of B and 'T' cell type. *Hematol. Oncol.*, **8**, 271–281

Hochberg, F.H., Miller, G., Schooley, R.T., Hirsch, M.S., Feorino, P. & Henle, W. (1983) Central-nervous-system lymphoma related to Epstein-Barr virus. *New Engl. J. Med.*, **309**, 745-748

van Hoeven, K.H., Factor, S.M., Kress, Y. & Woodruff, J.M. (1993) Visceral myogenic tumors. A manifestation of HIV infection in children. *Am. J. surg. Pathol.*, **17**, 1176–1181

Holley-Guthrie, E.A., Quinlivan, E.B., Mar, E.-C. & Kenney, S. (1990) The Epstein-Barr virus (EBV) BMRF1 promoter for early antigen (EA-D) is regulated by the EBV transactivators, BRLF1 and BZLF1, in a cell-specific manner. *J. Virol.*, **64**, 3753–3759

Honess, R.W., Gompels, U.A., Barrell, B.G., Craxton, M., Cameron, K.R., Staden, R., Chang, Y.-N. & Hayward, G.S. (1989) Deviations from expected frequencies of CpG dinucleotides in herpesvirus DNAs may be diagnostic of differences in the states of their latent genomes. *J. gen. Virol.*, **70**, 837–855

Hoover, R., Mason, T.J., McKay, F.W. & Fraumeni, J.F., Jr (1975) Geographic patterns of cancer mortality in the United States. In: Fraumeni, J.F., ed., Jr, *Persons at High Risk of Cancer. An Approach to Cancer Etiology and Control*, New York, Academic Press, pp. 343–360

van der Horst, C.M., Lin, J.-C., Raab-Traub, N., Smith, M.C. & Pagano, J.S. (1987) Differential effects of acyclovir and 9-(1,3-dihydroxy-2-propoxymethyl)guanine on herpes simplex virus and Epstein-Barr virus in a dually infected human lymphoblastoid cell line. *J. Virol.*, **61**, 607–610

van der Horst, C., Joncas, J., Ahronheim, G., Gustafson, N., Stein, G., Gurwith, M., Fleisher, G., Sullivan, J., Sixbey, J., Roland, S., Fryer, J., Champney, K., Schooley, R., Sumaya, C. & Pagano, J.S. (1991) Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. *J. infect. Dis.*, **164**, 788–792

Hörtnagel, K., Mautner, J., Strobl, L.J., Wolf, D.A., Christoph, B., Geltinger, C. & Polack, A. (1995) The role of immunoglobulin κ elements in c-*myc* activation. *Oncogene*, **10**, 1393–1401

Horwitz, C.A., Henle, W., Henle, G. & Schmitz, H. (1975) Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. *Am. J. Med.*, **58**, 330–338

Horwitz, C.A., Henle, W., Henle, G., Rudnick, H. & Latts, E. (1985) Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. *J. infect. Dis.*, **151**, 1150–1153

Howe, J.G. & Shu, M.-D. (1988) Isolation and characterization of the genes of two small RNAs of Herpesvirus papio and their comparison with Epstein-Barr virus-encoded EBER RNAs. *J. Virol.*, **62**, 2790–2798

Howe, J.G. & Shu, M.-D. (1989) Epstein-Barr virus small RNA (EBER) genes: Unique transcription units that combine RNA polymerase II and III promoter elements. *Cell*, **57**, 825–834

Howe, J.G. & Steitz, J.A. (1986) Localization of Epstein-Barr virus-encoded small RNAs by in situ hybridization. *Proc. natl Acad. Sci. USA*, **83**, 9006–9010

Hsieh, J.J.-D. & Hayward, S.D. (1995) Masking of the CBF1/RBPJ$_\kappa$ transcriptional repression domain by Epstein-Barr virus EBNA2. *Science*, **268**, 560–563

Hsieh, J.J.-D., Henkel, T., Salmon, P., Robey, E., Peterson, M.G. & Hayward, S.D. (1996) Truncated mammalian Notch1 activates CBF1/RBPJk-repressed genes by a mechanism resembling that of Epstein-Barr virus EBNA2. *Mol. cell. Biol.*, **16**, 952–959

Hsu, H.C., Chen, C.C., Huang, G.T. & Lee, P.H. (1996) Clonal Epstein-Barr virus associated cholangiocarcinoma with lymphoepithelioma-like component. *Hum. Pathol.*, **27**, 848–850

Hu, E., Hufford, S., Lukes, R., Bernstein-Singer, M., Sobel, G., Gill, P., Pinter-Brown, L., Rarick, M., Rosen, P., Brynes, R., Nathwani, B., Feinstein, D. & Levine, A. (1988) Third-world Hodgkin's disease at Los Angeles County–University of Southern California Medical Center. *J. clin. Oncol.*, **6**, 1285–1292

Hu, L.-F., Zabarovsky, E.R., Chen, F., Cao, S.-L., Ernberg, I., Klein, G. & Winberg, G. (1991a) Isolation and sequencing of the Epstein-Barr virus BNLF-1 gene (LMP1) from a Chinese nasopharyngeal carcinoma. *J. gen. Virol.*, **72**, 2399–2409

Hu, L.-F., Minarovits, J., Cao, S.-L., Contreras-Salazar, B., Rymo, L., Falk, K., Klein, G. & Ernberg, I. (1991b) Variable expression of latent membrane protein in nasopharyngeal carcinoma can be related to methylation status of the Epstein-Barr virus BNLF-1 5'-flanking region. *J. Virol.*, **65**, 1558–1567

Hu, L.-F., Chen, F., Zheng, X., Ernberg, I., Cao, S.-L., Christensson, B., Klein, G. & Winberg, G. (1993) Clonability and tumorigenicity of human epithelial cells expressing the EBV encoded membrane protein LMP1. *Oncogene*, **8**, 1575–1583

Hu, L.-F., Chen, F., Zhen, Q.-F., Zhang, Y.-W., Luo, Y., Zheng, X., Winberg, G., Ernberg, I. & Klein, G. (1995) Differences in growth pattern and clinical course of EBV-LMP1 expressing and non-expressing nasopharyngeal carcinomas. *Eur. J. Cancer*, **31A**, 658–660

Huang, D.P., Ho, J.H.C., Henle, W. & Henle, G. (1974) Demonstration of Epstein-Barr virus-associated nuclear antigen in nasopharyngeal carcinoma cells from fresh biopsies. *Int. J. Cancer*, **14**, 580–588

Huang, D.P., Ho, J.H.C. & Ng, M.H. (1978a) Morphological transformation of nasopharyngeal epithelial cells in vitro by Epstein-Barr virus from B95-8 cells. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 359–368

Huang, D.P., Ho, H.C., Henle, W., Henle, G., Saw, D. & Lui, M. (1978b) Presence of EBNA in nasopharyngeal carcinoma and control patient tissues related to EBV serology. *Int. J. Cancer*, **22**, 266–274

Huang, D.P., Ho, J.H.C., Saw, D. & Teoh, T.B. (1978c) Carcinoma of the nasal and paranasal regions in rats fed Cantonese salted marine fish. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 315–328

Huang, D.P., Ho, J.H.C., Webb, K.S., Wood, B.J. & Gough, T.A. (1981) Volatile nitrosamines in salt-preserved fish before and after cooking. *Food Cosmet. Toxicol.*, **19**, 167–171

Huang, D.P., Ng, H.K., Ho, Y.H. & Chan, K.M. (1988) Epstein-Barr virus (EBV)-associated undifferentiated carcinoma of the parotid gland. *Histopathology*, **13**, 509–517

Huang, D.P., Lo, K.-W., Choi, P.H.K., Ng, A.Y.T., Tsao, S.-Y., Yiu, G.K.C. & Lee, J.C.K. (1991) Loss of heterozygosity on the short arm of chromosome 3 in nasopharyngeal carcinoma. *Cancer Genet. Cytogenet.*, **54**, 91–99

Huang, D.P., Lo, K.W., van Hasselt, C.A., Woo, J.K.S., Choi, P.H.K., Leung, S.-F., Cheung, S.-T., Cairns, P., Sidransky, D. & Lee, J.C.K. (1994) A region of homozygous deletion on chromosome 9p21–22 in primary nasopharyngeal carcinoma. *Cancer Res.*, **54**, 4003–4006

Hubscher, S.G., Williams, A., Davison, S.M., Young, L.S. & Niedobitek, G. (1994) Epstein-Barr virus in inflammatory diseases of the liver and liver allografts: An *in situ* hybridization study. *Hepatology*, **20**, 899–907

Hudewentz, J., Delius, H., Freese, U.K., Zimber, U. & Bornkamm, G.W. (1982) Two distant regions of the Epstein-Barr virus genome with sequence homologies have the same orientation and involve small tandem repeats. *EMBO J.*, **1**, 21–26

Hudson, G.S., Farrell, P.J. & Barrell, B.G. (1985) Two related but differentially expressed potential membrane proteins encoded by the *Eco*RI Dhet region of Epstein-Barr virus B95-8. *J. Virol.*, **53**, 528–535

Huen, D.S., Fox, A., Kumar, P. & Searle, P.F. (1993) Dilated heart failure in transgenic mice expressing the Epstein-Barr virus nuclear antigen-leader protein. *J. gen. Virol.*, **74**, 1381–1391

Huen, D.S., Henderson, S.A., Croom Carter, D. & Rowe, M. (1995) The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-κ B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. *Oncogene*, **10**, 549–560

Huh, J., Park, C., Juhng, S., Kim, C.E., Poppema, S. & Kim, C. (1996) A pathologic study of Hodgkin's disease in Korea and its association with the Epstein-Barr virus infection. *Cancer*, **77**, 949–955

Hummel, M. & Kieff, E. (1982a) Mapping of polypeptides encoded by the Epstein-Barr virus genome in productive infection. *Proc. natl Acad. Sci. USA*, **79**, 5698–5702

Hummel, M. & Kieff, E. (1982b) Epstein-Barr virus RNA. VIII. Viral RNA in permissively infected B95-8 cells. *J. Virol.*, **43**, 262–272

Hummel, M., Thorley-Lawson, D. & Kieff, E. (1984) An Epstein-Barr virus DNA fragment encodes messages for the two major envelope glycoproteins (gp350/300 and gp220/200). *J. Virol.*, **49**, 413–417

Hummel, M., Ziemann, K., Lammert, H., Pileri, S., Sabattini, E. & Stein, H. (1995a) Hodgkin's disease with monoclonal and polyclonal populations of Reed-Sternberg cells. *New Engl. J. Med.*, **333**, 901–906

Hummel, M., Anagnostopoulos, I., Korbjuhn, P. & Stein, H. (1995b) Epstein-Barr virus in B-cell non-Hodgkin's lymphomas: Unexpected infection patterns and different infection incidence in low-and high-grade types. *J. Pathol.*, **175**, 263–271

Hunter, T. (1991) Cooperation between oncogenes. *Cell*, **64**, 249–270

Hurley, E.A. & Thorley-Lawson, D.A. (1988) B Cell activation and the establishment of Epstein-Barr virus latency. *J. exp. Med.*, **168**, 2059–2075

Hurley, E.A., Agger, S., McNeil, J.A., Lawrence, J.B., Calendar, A., Lenoir, G. & Thorley-Lawson, D.A. (1991a) When Epstein-Barr virus persistently infects B-cell lines, it frequently integrates. *J. Virol.*, **65**, 1245–1254

Hurley, E.A., Klaman, L.D., Agger, S., Lawrence, J.B. & Thorley-Lawson, D.A. (1991b) The prototypical Epstein-Barr virus-transformed lymphoblastoid cell line IB4 is an unusual variant containing integrated but no episomal viral DNA. *J. Virol.*, **65**, 3958–3963

Hutt-Fletcher, L.M. (1987) Synergistic activation of cells by Epstein-Barr virus and B-cell growth factor. *J. Virol.*, **61**, 774–781

IARC (1986) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 38, *Tobacco Smoking*, Lyon, pp. 270–275

IARC (1990) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 50, *Some Pharmaceutical Drugs*, Lyon, pp. 77–114

IARC (1993) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 56, *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, Lyon, pp. 41–82

IARC (1995a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 62, *Wood Dusts and Formaldehyde*, Lyon, pp. 35–215

IARC (1995b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 62, *Wood Dusts and Formaldehyde*, Lyon, pp. 217–362

IARC (1996) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 67, *Human Immunodeficiency Viruses and Human T-cell Lymphotropic Viruses*, Lyon, pp. 31–259

Ifekwunigwe, A.E., Pulvertaft, R.J. & Williams, A.O. (1966) The cerebrospinal fluid in retinoblastoma. *Br. J. Cancer*, **20**, 250–255

Ilyas, M., Niedobitek, G., Agathanggelou, A., Barry, R.E., Read, A.E., Tierney, R., Young, L.S. & Rooney, N. (1995) Non-Hodgkin's lymphoma, coeliac disease, and Epstein-Barr virus: A study of 13 cases of enteropathy-associated T-and B-cell lymphoma. *J. Pathol.*, **177**, 115–122

Imai, S., Koizumi, S., Sugiura, M., Tokunaga, M., Uemura, Y., Yamamoto, N., Tanaka, S., Sato, E. & Osato, T. (1994a) Gastric carcinoma: Monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. *Proc. natl Acad. Sci. USA*, **91**, 9131–9135

Imai, S., Sugiura, M., Mizuno, F., Ohigashi, H., Koshimizu, K., Chiba, S. & Osato, T. (1994b) African Burkitt's lymphoma: A plant, *Euphorbia tirucalli*, reduces Epstein-Barr virus-specific cellular immunity. *Anticancer Res.*, **14**, 933–936

Imai, S., Sugiura, M., Oikawa, O., Koizumi, S., Hirao, M., Kimura, H., Hayashibara, H., Terai, N., Tsutsumi, H., Oda, T., Chiba, S. & Osato, T. (1996) Epstein-Barr virus (EBV)-carrying and -expressing T-cell lines established from severe chronic active EBV infection. *Blood*, **87**, 1446–1457

Imoto, S., Kameya, T., Sato, Y., Sato, B., Inoue, K. & Oya, M. (1995) Localization of virus in liver biopsy specimens of acute EBV hepatitis using non-isotopic in situ hybridization procedure: Presence of EBV in hepatocytes and lymphocytes. *Int. Hepatol. Commun.*, **3**, 53–56

Imreh, M.P., Zhang, Q.-J., de Campos-Lima, P.O., Imreh, S., Krausa, P., Browning, M., Klein, G. & Masucci, M.G. (1995) Mechanisms of allele-selective down regulation of HLA class I in Burkitt's lymphoma. *Int. J. Cancer*, **62**, 90–96

Inman, G.J. & Farrell, P.J. (1995) Epstein-Barr virus EBNA-LP and transcription regulation pro-
perties of pRB, p107 and p53 in transfection assays. *J. gen. Virol.*, **76**, 2141–2149

Isaacson, P.G., Schmid, C., Pan, L., Wotherspoon, A.C. & Wright, D.H. (1992) Epstein-Barr
virus latent membrane protein expression by Hodgkin and Reed-Sternberg-like cells in acute
infectious mononucleosis. *J. Pathol.*, **167**, 267–271

Ito, Y., Kawanishi, M., Harayama, T. & Takabayashi, S. (1981) Combined effect of the extracts
from *Croton tiglium*, *Euphorbia lathyris* or *Euphorbia tirucalli* and *n*-butyrate on
Epstein-Barr virus expression in human lymphoblastoid P3HR-1 and Raji cells. *Cancer Lett.*,
12, 175–180

Ito, Y., Ohigashi, H., Koshimizu, K. & Yi, Z. (1983) Epstein-Barr virus-activating principle in
the ether extracts of soils collected from under plants which contain active diterpene esters.
Cancer Lett., **19**, 113–117

Izumi, K.M., Kaye, K.M. & Kieff, E.D. (1997) The Epstein-Barr virus LMP1 amino acid
sequence that engages tumor necrosis factor receptor associated factors is critical for primary
B lymphocyte growth transformation. *Proc. natl Acad. Sci. USA*, **94**, 1447–1452

Jarrett, R.F., Gallagher, A., Jones, D.B., Alexander, F.E., Krajewski, A.S., Kelsey, A., Adams,
J., Angus, B., Gledhill, S., Wright, D.H., Cartwright, R.A. & Onions, D.E. (1991) Detection
of Epstein-Barr virus genomes in Hodgkin's disease: Relation to age. *J. clin. Pathol.*, **44**,
844–848

Jarrett, A.F., Armstrong A.A. & Alexander, E. (1996) Epidemiology of EBV and Hodgkin's
lymphoma. *Ann. Oncol.*, **7** (Suppl. 4), S5–S10

Jarriault, S., Brou, C., Logeat, F., Schroeter, E.H., Kopan, R. & Israel, A. (1995) Signalling
downstream of activated mammalian Notch. *Nature*, **377**, 355–358

Jeang, K.-T. & Hayward, S.D. (1983) Organization of the Epstein-Barr virus DNA molecule. III.
Location of the P3HR-1 deletion junction and characterization of the *Not*I repeat units that
form part of the template for an abundant 12-*O*-tetradecanoylphorbol-13-acetate-induced
mRNA transcript. *J. Virol.*, **48**, 135–148

Jeannel, D., Hubert, A., de Vathaire, F., Ellouz, R., Camoun, M., Ben Salem, M., Sancho-
Garnier, H. & de Thé, G. (1990) Diet, living conditions and nasopharyngeal carcinoma in
Tunisia — A case–control study. *Int. J. Cancer*, **46**, 421–425

Jeannel, D., Ghnassia, M., Hubert, A., Sancho-Garnier, H., Eschwège, F., Crognier, E. &
de Thé, G. (1993) Increased risk of nasopharyngeal carcinoma among males of French origin
born in Maghreb (North Africa). *Int. J. Cancer*, **54**, 536–539

Jerusalem, C. (1968) Relationship between malaria infection (*Plasmodium berghei*) and mali-
gnant lymphoma in mice. *Z. Tropenmed. Parasitol.*, **19**, 94–108

Jiang, W.-Q., Szekely, L., Wendel-Hansen, V., Ringertz, N., Klein, G. & Rosén, A. (1991) Co-
localization of the retinoblastoma protein and the Epstein-Barr virus-encoded nuclear antigen
EBNA-5. *Exp. Cell Res.*, **197**, 314–318

Jin, X.W. & Speck, S.H. (1992) Identification of critical *cis* elements involved in mediating
Epstein-Barr virus nuclear antigen 2-dependent activity of an enhancer located upstream of
the viral *Bam*HI C promoter. *J. Virol.*, **66**, 2846–2852

Jiwa, N.M., Kanavaros, P., De Bruin, P.C., van der Valk, P., Horstman, A., Vos, W., Mullink,
H., Walboomers, J.M.M. & Meijer, C.J.L.M. (1993a) Presence of Epstein-Barr virus harbou-
ring small and intermediate-sized cells in Hodgkin's disease. Is there a relationship with
Reed-Sternberg cells? *J. Pathol.*, **170**, 129–136

Jiwa, N.M., Kanavaros, P., van der Valk, P., Walboomers, J.M.M., Horstman, A., Vos, W., Mullink, H. & Meijer, C.J.L.M. (1993b) Expression of c-myc and bcl-2 oncogene products in Reed-Sternberg cells independent of presence of Epstein-Barr virus. *J. clin. Pathol.*, **46**, 211–217

Joab, I., Rowe, D.T., Bodescot, M., Nicolas, J.-C., Farrell, P.J. & Perricaudet, M. (1987) Mapping of the gene coding for Epstein-Barr virus-determined nuclear antigen EBNA3 and its transient overexpression in a human cell line by using an adenovirus expression vector. *J. Virol.*, **61**, 3340–3344

Jochner, N., Eick, D., Zimber-Strobl, U., Pawlita, M., Bornkamm, G.W. & Kempkes, B. (1996) Epstein-Barr virus nuclear antigen 2 is a transcriptional suppressor of the immunoglobulin μ gene: Implications for the expression of the translocated c-myc gene in Burkitt's lymphoma cells. *EMBO J.*, **15**, 375–382

Johannsen, E., Koh, E., Mosialos, G., Tong, X., Kieff, E. & Grossman, S.R. (1995) Epstein-Barr virus nuclear protein 2 transactivation of the latent membrane protein 1 promoter is mediated by J κ and PU.1. *J. Virol.*, **69**, 253–262

Johansson, B., Klein, G., Henle, W. & Henle, G. (1970) Epstein-Bar virus (EBV)-associated antibody patterns in malignant lymphoma and leukemia. I. Hodgkin's disease. *Int. J. Cancer*, **6**, 450–462

Johansson, B., Klein, G., Henle, W. & Henle, G. (1971) Epstein-Barr virus (EBV)-associated antibody patterns in malignant lymphoma and leukemia. II. Chronic lymphocytic leukemia and lymphocytic lymphoma. *Int. J. Cancer*, **8**, 475–486

Johnson, D.R., Wolfe, L.G., Levan, G., Klein, G., Ernberg, I. & Åman, P. (1983) Epstein-Barr virus (EBV) induced lymphoproliferative disease in cotton-topped marmoset. *Int. J. Cancer*, **31**, 91–97

Jonas, J.H., Rioux, E. & Robitaille, R. (1976) Multiple cases of lymphoepithelioma and Burkitt's lymphoma in a Canadian family. In: Clemmesen, J. & Yohn, D.S., eds, *Comparative Leukemia Research 1975*, Basel, Karger, pp. 224–226

Joncas, J.H., Rioux, E., Wastiaux, J.P., Leyritz, M., Robillard, L. & Menezes, J. (1976) Nasopharyngeal carcinoma and Burkitt's lymphoma in a Canadian family. I. HLA typing, EBV antibodies and serum immunoglobulins. *Can. med. Assoc. J.*, **115**, 858–860

Jones, J.F., Shurin, S., Abramowsky, C., Tubbs, R.R., Sciotto, C.G., Wahl, R., Sands, J., Gottman, D., Katz, B.Z. & Sklar, J. (1988) T-Cell lymphomas containing Epstein-Barr viral DNA in patients with chronic Epstein-Barr virus infections. *New Engl. J. Med.*, **318**, 733–741

Jones, K., Rivera, C., Sgadari, C., Franklin, J., Max, E.E., Bhatia, K. & Tosato, G. (1995) Infection of human endothelial cells with Epstein-Barr virus. *J. exp. Med.*, **182**, 1213–1221

Judson, S.C., Henle, W. & Henle, G. (1977) A cluster of Epstein-Barr-virus-associated American Burkitt's lymphoma. *New Engl. J. Med.*, **297**, 464–468

Kaaden, O.R. & Dietzschold, B. (1974) Alterations of the immunological specificity of plasma membranes from cells infected with Marek's disease and turkey herpes viruses. *J. gen. Virol.*, **25**, 1–10

Kachel, G., Bornkamm, G.-W., Hermanek, P., Kaduk, B. & Schricker, K.T. (1980) Burkitt's lymphoma of African type in Europe. *Dtsch. med. Wochenschr.*, **105**, 413–417 (in German)

Kadin, M.E., Muramoto, L. & Said, J. (1988) Expression of T-cell antigens on Reed-Sternberg cells in a subset of patients with nodular sclerosing and mixed cellularity Hodgkin's disease. *Am. J. Pathol.*, **130**, 345–353

Kafuko, G.W. & Burkitt, D.P. (1970) Burkitt's lymphoma and malaria. *Int. J. Cancer*, **6**, 1–9

Kafuko, G.W., Henderson, B.E., Kirya, B.G., Munube, G.M.R., Tukei, P.M., Day, N.E., Henle, G., Henle, W., Morrow, R.H., Pike, M.C., Smith, P.G. & Williams, E.H. (1972) Epstein-Barr virus antibody levels in children from the West Nile District of Uganda. *Lancet*, **i**, 706–709

Kallin, B., Dillner, J., Ernberg, I., Ehlin-Henriksson, B., Rosén, A., Henle, W., Henle, G. & Klein, G. (1986) Four virally determined nuclear antigens are expressed in Epstein-Barr virus-transformed cells. *Proc. natl Acad. Sci. USA*, **83**, 1499–1503

Kalter, S.S. (1994) Herpesviruses — Baboon and chimpanzees. In: Webster, R.G. & Granoff, A., eds, *Encyclopedia of Virology*, Vol. 2, London, Academic Press, pp. 609–614

Kalter, S.P., Riggs, S.A., Cabanillas, F., Butler, J.J., Hagemeister, F.B., Mansell, P.W., Newell, G.R., Velasquez, W.S., Salvador, P., Barlogie, B., Rios, A. & Hersh, E.M. (1985) Aggressive non-Hodgkin's lymphomas in immunocompromised homosexual males. *Blood*, **66**, 655–659

Kamel, O.W., van de Rijn, M., Weiss, L.M., Del Zoppo, G.J., Hench, P.K., Robbins, B.A., Montgomery, P.G., Warnke, R.A. & Dorfman, R.F. (1993) Reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *New Engl. J. Med.*, **328**, 1317–1321

Kamel, O.W., van de Rijn, M., LeBrun, D.P., Weiss, L.M., Warnke, R.A. & Dorfman, R.F. (1994) Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis: Frequency of Epstein-Barr virus and other features associated with immunosuppression. *Hum. Pathol.*, **25**, 638–643

Kanavaros, P., Jiwa, N.M., De Bruin, P.C., Van Der Valk, P., Noorduyn, L.A., Van Heerde, P., Gordijn, R., Horstman, A., Mullink, R., Willemze, R., Walboomers, J.M.M. & Meijer, C.J.L.M. (1992) High incidence of EBV genome in CD30-positive non-Hodgkin's lymphomas. *J. Pathol.*, **168**, 307–315

Kanavaros, P., Sakalidou, A., Tzardi, M., Darivianaki, K., Delides, G., Kazlaris, E. & Kalmanti, M. (1994) Frequent detection of Epstein-Barr virus (EBV), EBER transcripts and latent membrane protein-1 (LMP-1) in tumor cells in Hodgkin's disease arising in childhood. *Pathol. Res. Pract.*, **190**, 1026–1030

Kanavaros, P., Briere, J., Lescs, M.-C. & Gaulard, P. (1996) Epstein-Barr virus in non-Hodgkin's lymphomas of the upper respiratory tract: Association with sinonasal localization and expression of NK and/or T-cell antigens by tumour cells. *J. Pathol.*, **178**, 297–302

Kanzler, H., Kuppers, R., Hansmann, M.L. & Rajewsky, K. (1996) Hodgkin and Reed-Sternberg cells in Hodgkin's disease represent the outgrowth of a dominant tumor clone derived from (crippled) germinal center B cells. *J. exp. Med.*, **184**, 1495–1505

Kapadia, S.B., Roman, L.N., Kingma, D.W., Jaffe, E.S. & Frizzera, G. (1995) Hodgkin's disease of Waldeyer's ring. Clinical and histoimmunophenotypic findings and association with Epstein-Barr virus in 16 cases. *Am. J. surg. Pathol.*, **19**, 1431–1439

Kaplan, H.S. (1980) Hodgkin's disease: Unfolding concepts concerning its nature, management and prognosis. *Cancer*, **45**, 2439–2474

Karajannis, M.A., Hummel, M., Anagnostopoulos, I. & Stein, H. (1997) Strict lymphotropism of Epstein-Barr virus during acute infectious mononucleosis in nonimmunocompromised individuals. *Blood*, **89**, 2856–2862

Karran, L., Gao, Y., Smith, P.R. & Griffin, B.E. (1992) Expression of a family of complementary-strand transcripts in Epstein-Barr virus-infected cells. *Proc. natl Acad. Sci. USA*, **89**, 8058–8062

Karran, L., Jones, M., Morley, G., van Noorden, S., Smith, P., Lampert, I. & Griffin, B.E. (1995) Expression of a B-cell marker, CD24, on nasopharyngeal carcinoma cells. *Int. J. Cancer*, **60**, 562–566

Kaschka-Dierich, C., Adams, A., Lindahl, T., Bornkamm, G.W., Bjursell, G., Klein, G., Giovanella, B.C. & Singh, S. (1976) Intracellular forms of Epstein-Barr virus DNA in human tumor cells *in vivo*. *Nature*, **260**, 302–306

Kasl, S.V., Evans, A.S. & Niederman, J.C. (1979) Psychosocial risk factors in the development of infectious mononucleosis. *Psychosom. Med.*, **41**, 445–466

Katano, H., Morishita, Y., Cui, L.X., Watanabe, T., Hirai, K. & Mori, S. (1996) Expression of latent membrane protein 1 in clinically isolated cases and animal models of AIDS-associated non-Hodgkin's lymphomas. *Pathol. int.*, **46**, 568–574

Katzenstein, A.-L.A. & Peiper, S.C. (1990) Detection of Epstein-Barr virus genomes in lymphomatoid granulomatosis: Analysis of 29 cases by the polymerase chain reaction technique. *Mod. Pathol.*, **3**, 435–441

Katzin, W.E., Fishleder, A.J., Linden, M.D. & Tubbs, R.R. (1988) Immunoglobulin and T-cell receptor genes in thymomas: Genotypic evidence supporting the nonneoplastic nature of the lymphocytic component. *Hum. Pathol.*, **19**, 323–328

Kaufman Paterson, R.L., Kelleher, C., Amankonah, T.D., Streib, J.E., Xu, J.W., Jones, J.F. & Gelfand, E.W. (1995) Model of Epstein-Barr virus infection of human thymocytes: Expression of viral genome and impact on cellular receptor expression in the T-lymphoblastic cell line, HPB-ALL. *Blood*, **85**, 456–464

Kawachi, I., Pearce, N. & Fraser, J. (1989) A New Zealand cancer registry-based study of cancer in wood workers. *Cancer*, **64**, 2609–2613

Kawaguchi, H., Miyashita, T., Herbst, H., Niedobitek, G., Asada, M., Tsuchida, M., Hanada, R., Kinoshita, A., Sakurai, M., Kobayashi, N. & Mizutani, S. (1993) Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. *J. clin. Invest.*, **92**, 1444–1450

Kawamura, H., King, D.J., Jr & Anderson, D.P. (1969) A herpesvirus isolated from kidney cell culture of normal turkeys. *Avian Dis.*, **13**, 853–863

Kaye, K.M., Izumi, K.M. & Kieff, E. (1993) Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc. natl Acad. Sci. USA*, **90**, 9150–9154

Kaye, K.M., Izumi, K.M., Mosialos, G. & Kieff, E. (1995) The Epstein-Barr virus LMP1 cytoplasmic carboxy terminus is essential for B-lymphocyte transformation; fibroblast cocultivation complements a critical function within the terminal 155 residues. *J. Virol.*, **69**, 675–683

Kelleher, C.A., Kaufman Paterson, R., Dreyfus, D.H., Streib, J.E., Xu, J.W., Takase, K., Jones, J.F. & Gelfand, E.W. (1995) Epstein-Barr virus replicative gene transcription during *de novo* infection of human thymocytes: Simultaneous early expression of BZLF-1 and its repressor RAZ. *Virology*, **208**, 685–695

Kempkes, B., Pich, D., Zeidler, R., Sugden, B. & Hammerschmidt, W. (1995a) Immortalization of human B lymphocytes by a plasmid containing 71 kilobase pairs of Epstein-Barr virus DNA. *J. Virol.*, **69**, 231–238

Kempkes, B., Spitkovsky, D., Jansen-Dürr, P., Ellwart, J.W., Kremmer, E., Delecluse, H.-J., Rottenberger, C., Bornkamm, G.W. & Hammerschmidt, W. (1995b) B-Cell proliferation and induction of early G_1-regulating proteins by Epstein-Barr virus mutants conditional for EBNA2. *EMBO J.*, **14**, 88–96

Kempkes, B., Zimber-Strobl, U., Eissner, G., Pawlita, M., Falk, M., Hammerschmidt, W. & Bornkamm, G.W. (1996) Epstein-Barr virus nuclear antigen 2 (EBNA2)–oestrogen receptor fusion proteins complement the EBNA2-deficient Epstein-Barr virus strain P3HR1 in transformation of primary B cells but suppress growth of human B cell lymphoma lines. *J. gen. Virol.*, **77**, 227–237

Kenagy, D.N., Schlesinger, Y., Weck, K., Ritter, J.H., Gaudreault-Keener, M.M. & Storch, G.A. (1995) Epstein-Barr virus DNA in peripheral blood leukocytes of patients with post-transplant lymphoproliferative disease. *Transplantation*, **60**, 547–554

Kenzy, S.G. & Cho, B.R. (1969) Transmission of classical Marek's disease by affected and carrier birds. *Avian Dis.*, **13**, 211–214

Kerr, B.M., Lear, A.L., Rowe, M., Croom-Carter, D., Young, L.S., Rookes, S.M., Gallimore, P.H. & Rickinson, A.B. (1992) Three transcriptionally distinct forms of Epstein-Barr virus latency in somatic cell hybrids: Cell phenotype dependence of virus promoter usage. *Virology*, **187**, 189–201

Khan, G., Coates, P.J., Gupta, R.K., Kangro, H.O. & Slavin, G. (1992) Presence of Epstein-Barr virus in Hodgkin's disease is not exclusive to Reed–Sternberg cells. *Am. J. Pathol.*, **140**, 757–762

Khan, G., Norton, A.J. & Slavin, G. (1993) Epstein-Barr virus in Hodgkin disease. Relation to age and subtype. *Cancer*, **71**, 3124–3129

Khanim, F., Yao, Q.-Y., Niedobitek, G., Sihota, S., Rickinson, A.B. & Young, L.S. (1996) Analysis of Epstein-Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood*, **88**, 3491–3501

Khanna, R., Burrows, S.R., Kurilla, M.G., Jacob, C.A., Misko, I.S., Sculley, T.B., Kieff, E. & Moss, D.J. (1992) Localization of Epstein-Barr virus cytotoxic T cell epitopes using recombinant vaccinia: Implications for vaccine development. *J. exp. Med.*, **176**, 169–176

Khanna, R., Burrows, S.R., Steigerwald-Mullen, P.M., Thomson, S.A., Kurilla, M.G. & Moss, D.J. (1995) Isolation of cytotoxic T lymphocytes from healthy seropositive individuals specific for peptide epitopes from Epstein-Barr virus nuclear antigen 1: Implications for viral persistence and tumor surveillance. *Virology*, **214**, 633–637

Khanna, R., Burrows, S.R., Silins, S.L., Moss, D.J., Poulsen, L.M. & Burrows, J.M. (1996) Cytotoxic T-lymphocyte clones specific for an immunodominant epitope display discerning antagonistic response to naturally occurring Epstein-Barr virus variants. *J. Virol.*, **70**, 7306–7311

Khyatti, M., Patel, P.C., Stefanescu, I. & Menezes, J. (1991) Epstein-Barr virus (EBV) glyco-protein gp350 expressed on transfected cells resistant to natural killer cell activity serves as a target antigen for EBV-specific antibody-dependent cellular cytotoxicity. *J. Virol.*, **65**, 996–1001

Kieff, E. (1996) Epstein-Barr virus and its replication. In: Fields, B.N., Knipe, D.M., Howley, P., Chanock, R.M., Melnick, J.L., Month, T.P., Roizman, B. & Straus, S.E., eds, *Fields Virology*, Philadelphia, Lippincott–Raven, pp. 2343–2396

Kieff, E., Given, D., Powell, A.L., King, W., Dambaugh, T. & Raab-Traub, N. (1979) Epstein-Barr virus: Structure of the viral DNA and analysis of viral RNA in infected cells. *Biochim. biophys. Acta*, **560**, 355–373

Kikuta, H., Taguchi, Y., Tomizawa, K., Kojima, K., Kawamura, N., Ishizaka, A., Sakiyama, Y., Matsumoto, S., Imai, S., Kinoshita, T., Koizumi, S., Osato, T., Kobayashi, I., Hamada, I. & Hirai, K. (1988) Epstein-Barr virus genome-positive T lymphocytes in a boy with chronic active EBV infection associated with Kawasaki-like disease. *Nature*, **333**, 455–457

Kikuta, H., Sakiyama, Y., Matsumoto, S., Oh-Ishi, T., Nakano, T., Nagashima, T., Oka, T., Hironaka, T. & Hirai, K. (1993) Fatal Epstein-Barr virus-associated hemophagocytic syndrome. *Blood*, **82**, 3259–3264

Kim, O.-J. & Yates, J.L. (1993) Mutants of Epstein-Barr virus with a selective marker disrupting the TP gene transform B cells and replicate normally in culture. *J. Virol.*, **67**, 7634–7640

King, D.H. (1988) History, pharmacokinetics, and pharmacology of acyclovir. *J. Am. Acad. Dermatol.*, **18**, 176–179

King, H. & Haenszel, W. (1973) Cancer mortality among foreign- and native-born Chinese in the United States. *J. chron. Dis.*, **26**, 623–646

Kingma, D.W., Shad, A., Tsokos, M., Fest, T., Otsuki, T., Frekko, K., Werner, E., Werner, A., Magrath, I., Raffeld, M. & Jaffe, E.S. (1996) Epstein-Barr virus (EBV)-associated smooth-muscle tumor arising in a post-transplant patient treated successfully for two PT-EBV-associated large-cell lymphomas. Case report. *Am. J. surg. Pathol.*, **20**, 1511–1519

Kintner, C. & Sugden, B. (1981) Conservation and progressive methylation of Epstein-Barr viral DNA sequences in transformed cells. *J. Virol.*, **38**, 305–316

Kirchner, H., Tosato, G., Blaese, R.M., Broder, S. & Magrath, I.T. (1979) Polyclonal immuno-globulin secretion by human B lymphocytes exposed to Epstein-Barr virus *in vitro*. *J. Immunol.*, **122**, 1310–1313

Kitay, M.K. & Rowe, D.T. (1996a) Cell cycle stage-specific phosphorylation of the Epstein-Barr virus immortalization protein EBNA-LP. *J. Virol.*, **70**, 7885–7893

Kitay, M.K. & Rowe, D.T. (1996b) Protein–protein interactions between Epstein-Barr virus nuclear antigen-LP and cellular gene products binding of 70-kilodalton heat shock proteins. *Virology*, **220**, 91–99

Kitinya, J.N. & Lauren, P.A. (1982) Burkitt's lymphoma on Mount Kilimanjaro and in the inland regions of northern Tanzania. *E. Afr. med. J.*, **59**, 256–260

Klein, G. (1979) The relationship of the virus to nasopharyngeal carcinoma. In: Epstein, MA. & Achong, B.G., eds, *The Epstein-Barr Virus*, New York, Springer-Verlag, pp. 339–350

Klein, E., Klein, G., Nadkarni, J.S., Nadkarni, J.J., Wigzell, H.R. & Clifford, P. (1967) Surface IgM specificity on cells derived from a Burkitt's lymphoma. *Lancet*, **ii**, 1068–1070

Klein, G., Pearson, G., Henle, G., Henle, W., Goldstein, G. & Clifford, P. (1969) Relation between Epstein-Barr viral and cell membrane immunofluorescence in Burkitt tumor cells. III. Comparison of blocking of direct membrane immunofluorescence and anti-EBV reactivities of different sera. *J. exp. Med.*, **129**, 697–705

Klein, G., Geering, G., Old, L.J., Henle, G., Henle, W. & Clifford, P. (1970) Comparison of the anti-EBV titer and the EBV-associated membrane reactive and precipitating antibody levels in the sera of Burkitt lymphoma and nasopharyngeal carcinoma patients and controls. *Int. J. Cancer*, **5**, 185–194

Klein, G., Giovanella, B.C., Lindahl, T., Fialkow, P.J., Singh, S. & Stehlin, J.S. (1974) Direct evidence for the presence of Epstein-Barr virus DNA and nuclear antigen in malignant epithelial cells from patients with poorly differentiated carcinoma of the nasopharynx. *Proc. natl Acad. Sci. USA*, **71**, 4737–4741

Klein, E., Ernberg, I., Masucci, M.G., Szigeti, R., Wu, Y.T., Masucci, G. & Svedmyr, E. (1981) T-Cell response to B-cells and Epstein-Barr virus antigens in infectious mononucleosis. *Cancer Res.*, **41**, 4210–4215

Klein, U., Klein, G., Ehlin-Henriksson, B., Rajewsky, K. & Kuppers, R. (1995) Burkitt's lymphoma is a malignancy of mature B cells expressing somatically mutated V region genes. *Mol. Med.*, **1**, 495–505

Klein, S.C., Kube, D., Abts, H., Diehl, V. & Tesch, H. (1996) Promotion of IL8, IL10, TNF alpha and TNF beta production by EBV infection. *Leuk. Res.*, **20**, 633–636

von Knebel Doeberitz, M., Bornkamm, G.W. & zur Hausen, H. (1983) Establishment of spontaneously outgrowing lymphoblastoid cell lines with cyclosporin A. *Med. Microbiol. Immunol.*, **172**, 87–99

Knecht, H., Odermatt, B.F., Bachmann, E., Teixeira, S., Sahli, R., Hayoz, D., Heitz, P. & Bachmann, F. (1991) Frequent detection of Epstein-Barr virus DNA by the polymerase chain reaction in lymph node biopsies from patients with Hodgkin's disease without genomic evidence of B- or T-cell clonality. *Blood*, **78**, 760–767

Knecht, H., Bachmann, E., Brousset, P., Sandvej, K., Nadal, D., Bachmann, F., Odermatt, B.F., Delsol, G. & Pallesen, G. (1993) Deletions within the LMP1 oncogene of Epstein-Barr virus are clustered in Hodgkin's disease and identical to those observed in nasopharyngeal carcinoma. *Blood*, **82**, 2937–2942

Knecht, H., McQuain, C., Martin, J., Rothenberger, S., Drexler, H.G., Berger, C., Bachmann, E., Kittler, E.L., Odermatt, B.F. & Quesenberry, P.J. (1996) Expression of the LMP1 oncoprotein in the EBV negative Hodgkin's disease cell line L-428 is associated with Reed-Sternberg cell morphology. *Oncogene*, **13**, 947–953

Knowles, D.M. (1993) Biologic aspects of AIDS-associated non-Hodgkin's lymphoma. *Curr. Opin. Oncol.*, **5**, 845–851

Knowles, D.M. (1996) Etiology and pathogenesis of AIDS-related non-Hodgkin's lymphoma. *Hematol. Oncol. Clin. North Am.*, **10**, 1081–1109

Knowles, D.M., Cesarman, E., Chadburn, A., Frizzera, G., Chen, J., Rose, E.A. & Michler, R.E. (1995) Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. *Blood*, **85**, 552–565

Knox, P.G., Li, Q.-X., Rickinson, A.B. & Young, L.S. (1996) In vitro production of stable Epstein-Barr virus-positive epithelial cell clones which resemble the virus:cell interaction observed in nasopharyngeal carcinoma. *Virology*, **215**, 40–50

Knutson, J.C. (1990) The level of c-*fgr* RNA is increased by EBNA-2, an Epstein-Barr virus gene required for B-cell immortalization. *J. Virol.*, **64**, 2530–2536

Ko, Y.H. & Lee, J.D. (1994) EBV *in situ*-hybridization study for non-Hodgkin's lymphomas. *J. Korean med. Sci.*, **9**, 224–229

Kobashi, Y., Nakamura, S., Sasajima, Y., Koshikawa, T., Yatabe, Y., Kitoh, K., Mori, S., Ueda, R., Yamabe, H. & Suchi, T. (1996) Inconsistent association of Epstein-Barr virus with CD56 (NCAM)-positive angiocentric lymphoma occurring in sites other than the upper and lower respiratory tract. *Histopathology*, **28**, 111–120

Konoshima, T., Kozuka, M., Koyama, J., Okatani, T., Tagahara, K. & Tokuda, H. (1989) Studies on inhibitors of skin tumor promotion. VI. Inhibitory effects of quinones on Epstein-Barr virus activation. *J. nat. Prod.*, **52**, 987–995

Konoshima, T., Takasaki, M., Tatsumoto, T., Kozuka, M., Kasai, R., Tanaka, O., Nie, R.-L., Tokuda, H., Nishino, H. & Iwashima, A. (1994) Inhibitory effects of cucurbitane triterpenoids on Epstein-Barr virus activation and two-stage carcinogenesis of skin tumors. *Biol. pharm. Bull.*, **17**, 668–671

Konoshima, T., Takasaki, M., Kozuka, M., Nagao, T., Okabe, H., Irino, N., Nakasumi, T., Tokuda, H. & Nishino, H. (1995) Inhibitory effects of cucurbitane triterpenoids on Epstein-Barr virus activation and two-stage carcinogenesis of skin tumor. II. *Biol. pharm. Bull.*, **18**, 284–287

Konoshima, T., Takasaki, M., Tokuda, H., Masuda, K., Arai, Y., Shiojima, K. & Ageta, H. (1996) Anti-tumor-promoting activities of triterpenoids from ferns. I. *Biol. pharm. Bull.*, **19**, 962–965

Korbjuhn, P., Anagnostopoulos, I., Hummel, M., Tiemann, M., Dallenbach, F., Parwaresch, M.R. & Stein, H. (1993) Frequent latent Epstein-Barr virus infection of neoplastic T cells and bystander B cells in human immunodeficiency virus-negative European peripheral pleomorphic T-cell lymphomas. *Blood*, **82**, 217–223

Kotsianti, A., Costopoulos, J., Morgello, S. & Papadimitriou, C. (1996) Undifferentiated carcinoma of the parotid gland in a white patient: Detection of Epstein-Barr virus by in situ hybridization. *Hum. Pathol.*, **27**, 87–90

Kouzarides, T., Packham, G., Cook, A. & Farrell, P.J. (1991) The BZLF1 protein of EBV has a coiled coil dimerisation domain without a heptad leucine repeat but with homology to the C/EBP leucine zipper. *Oncogene*, **6**, 195–204

Krauer, K.G., Kienzle, N., Young, D.B. & Sculley, T.B. (1996) Epstein-Barr nuclear antigen-3 and -4 interact with RBP-2N, a major isoform of RBP-J$_\kappa$ in B lymphocytes. *Virology*, **226**, 346–353

Kripalani-Joshi, S. & Law, H.Y. (1994) Identification of integrated Epstein-Barr virus in nasopharyngeal carcinoma using pulse field gel electrophoresis. *Int. J. Cancer*, **56**, 187–192

Krogh-Jensen, M., d'Amore, F., Jensen, M.K., Christensen, B.E., Thorling, K., Pedersen, M., Johansen, P., Boesen, A.M. & Andersen, E. (1994) Incidence, clinicopathological features and outcome of primary central nervous system lymphomas. Population-based data from a Danish lymphoma registry. Danish Lymphoma Study Group, LYFO. *Ann. Oncol.*, **5**, 349–354

Krueger, J., Ieromnimon, V. & Dahr, W. (1981) Frequencies of HLA antigens in patients with NPC. In: Grundmann, E., Krueger, G.R.F. & Ablashi, D.V., eds, *Cancer Campaign*, Vol. 5, *Nasopharyngeal Carcinoma*, Stuttgart, Gustav Fischer Verlag, pp. 201–203

Kuhn-Hallek, I., Sage, D.R., Stein, L., Groelle, H. & Fingeroth, J.D. (1995) Expression of recombination activating genes (RAG-1 and RAG-2) in Epstein-Barr virus-bearing B cells. *Blood*, **85**, 1289–1299

Kumar, S. & Kumar, D. (1994) Lymphoepithelioma-like carcinoma of the breast. *Mod. Pathol.*, **7**, 129–131

Kumar, S., Kumar, D., Kingma, D.W. & Jaffe, E.S. (1993) Epstein-Barr virus-associated T-cell lymphoma in a renal transplant patient. *Am. J. surg. Pathol.*, **17**, 1046–1053

Kumari, P., Augustus, M., Naik, U., Ravi, V., Shenoy, A.M., Rao, C.R. & Kumar, R.V. (1995) Cytogenetic and viral studies in nasopharyngeal carcinomas in patients of Indian origin. *Indian J. Cancer*, **32**, 10–14

Küppers, R., Rajewsky, K., Zhao, M., Simons, G., Laumann, R., Fischer, R. & Hansmann, M.-L. (1994) Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *Proc. natl Acad. Sci. USA*, **91**, 10962–10966

Kvåle, G., Høiby, E.A. & Pedersen, E. (1979) Hodgkin's disease in patients with previous infectious mononucleosis. *Int. J. Cancer*, **23**, 593–597

Labrecque, L.G., Lampert, I., Kazembe, P., Philips, J. & Griffin, B.E. (1994) Correlation between cytopathological results and in situ hybridisation on needle aspiration biopsies of suspected African Burkitt's lymphomas. *Int. J. Cancer*, **59**, 591–596

Labrecque, L.G., Barnes, D.M., Fentiman, I.S. & Griffin, B.E. (1995) Epstein-Barr virus in epithelial cell tumors: A breast cancer study. *Cancer Res.*, **55**, 39–45

Laherty, C.D., Hu, H.M., Opipari, A.W., Wang, F. & Dixit, V.M. (1992) The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor κB. *J. biol. Chem.*, **267**, 24157–24160

Lai, H.M. (1988) On Chinese Americans: State of the art or challenge to the future? *Amerasia J.*, **14**, xi–xiii

Lam, K.M., Syed, N., Whittle, H. & Crawford, D.H. (1991) Circulating Epstein-Barr virus carrying B cells in acute malaria. *Lancet*, **337**, 876–878

Lamartine, J., Nichols, K.E., Yin, L., Krainer, M., Heitzmann, F., Bernard, A., Gaudi, S., Lenoir, G.M., Sullivan, J.L., Ikeda, J.E., Porta, G., Schlessinger, D., Romeo, G., Haber, D.A., Sylla, B.S. & Harkin, D.P. (1996) Physical map and cosmid contig encompassing a new interstitial deletion of the X-linked lymphoproliferative syndrome region. *Eur. J. hum. Genet.*, **4**, 342–351

Landers, R.J., O'Leary, J.J., Crowley, M., Healy, I., Annis, P., Burke, L., O'Brien, D., Hogan, J., Kealy, W.F., Lewis, F.A. & Doyle, C.T. (1993) Epstein-Barr virus in normal, pre-malignant, and malignant lesions of the uterine cervix. *J. clin. Pathol.*, **46**, 931–935

Landon, J.C., Ellis, L.B., Zeve, V.H. & Fabrizo, D.P. (1968) Herpes-type virus in cultured leukocytes from chimpanzees. *J. natl Cancer Inst.*, **40**, 181–192

Lange, B., Arbeter, A., Hewetson, J. & Henle, W. (1978) Longitudinal study of Epstein-Barr virus antibody titers and excretion in pediatric patients with Hodgkin's disease. *Int. J. Cancer*, **22**, 521–527

Langenhuysen, M.M.A.C., Cazemier, T., Houwen, B., Brouwers, T.M., Halie, M.R., The, T.H. & Nieweg, H.O. (1974) Antibodies to Epstein-Barr virus, cytomegalovirus, and Australia antigen in Hodgkin's disease. *Cancer*, **34**, 262–267

Lanier, A.P., Bender, T.R., Tschopp, C.F. & Dohan, P. (1979) Nasopharyngeal carcinoma in an Alaskan Eskimo family: Report of three cases. *J. natl Cancer Inst.*, **62**, 1121–1124

Lanier, A.P., Henle, W., Bender, T.R., Henle, G. & Talbot, M.L. (1980a) Epstein-Barr-virus-specific antibody titers in seven Alaskan natives before and after diagnosis of naso-pharyngeal carcinoma. *Int. J. Cancer*, **26**, 133–137

Lanier, A.P., Bender, T.R., Talbot, M.L., Wilmeth, S., Tschopp, C.F., Henle, W., Henle, G., Ritter, D. & Terasaki, P. (1980b) Nasopharyngeal carcinoma in Alaskan Eskimos, Indians, and Aleuts: a review of cases and study of Epstein-Barr virus, HLA, and environmental risk factors. *Cancer*, **46**, 2100–2106

Lanyi, A., Li, B.-F., Li, S.-B., Talmadge, C.B., Brichacek, B., Davis, J.R., Kozel, B.A., Trask, B., van den Engh, G., Uzvolgyi, E., Stanbridge, E.J., Nelson, D.L., Chinault, C., Heslop, H., Gross, T.G., Seemayer, T.A., Klein, G., Purtilo, D.T. & Sumegi, J. (1997) A yeast artificial chromosome (YAC) contig encompassing the critical region of the X-linked lymphoproliferative disease (XLP) locus. *Genomics*, **39**, 55–65

Lapin, B.A., Timanovskaya, V.V. & Yakovleva, L.A. (1985) Herpesvirus papio: A new repre-sentative in the group of the EBV-like B-lymphotropic herpesviruses of primates. *Hematol. Blood Transfus.*, **29**, 312–320

Larocca, D. & Clough, W. (1982) Hypomethylation of Epstein-Barr virus DNA in the non-producer B-cell line EBR. *J. Virol.*, **43**, 1129–1131

Larson, R.S., Scott, M.A., McCurley, T.L. & Vnencak-Jones, C.L. (1996) Microsatellite anal-ysis of posttransplant lymphoproliferative disorders: determination of donor/recipient origin and identification of putative lymphomagenic mechanism. *Cancer Res.*, **56**, 4378–4381

Lau, W.H., Kwan, H.C. & Ho, J.H.C. (1983) Serum IgA antibodies to the viral capsid antigen (VC) of Epstein-Barr virus as diagnostic markers in nasopharyngeal carcinoma. In: Prasad, U., Ablashi, D., Levine, P. & Pearson, G., eds, *Nasopharyngeal Carcinoma: Current Concepts*, Kuala Lumpur, University of Malaya Press

Lau, R., Middeldorp, J. & Farrell, P.J. (1993) Epstein-Barr virus gene expression in oral hairy leukoplakia. *Virology*, **195**, 463–474

Laufs, R. & Steinke, H. (1975) Vaccination of non-human primates against malignant lym-phoma. *Nature*, **253**, 71–72

Laux, G., Perricaudet, M. & Farrell, P.J. (1988a) A spliced Epstein-Barr virus gene expressed in immortalized lymphocytes is created by circularization of the linear viral genome. *EMBO J.*, **7**, 769–774

Laux, G., Freese, U.K., Fischer, R., Polack, A., Kofler, E. & Bornkamm, G.W. (1988b) TPA-inducible Epstein-Barr virus genes in Raji cells and their regulation. *Virology*, **162**, 503–507

Laux, G., Dugrillon, F., Eckert, C., Adam, B., Zimber-Strobl, U. & Bornkamm, G.W. (1994a) Identification and characterization of an Epstein-Barr virus nuclear antigen 2-responsive *cis* element in the bidirectional promoter region of latent membrane protein and terminal protein 2 genes. *J. Virol.*, **68**, 6947–6958

Laux, G., Adam, B., Strobl, L.J., & Moreau Gachelin, F. (1994b) The Spi-1/PU.1 and Spi-B ets family transcription factors and the recombination signal binding protein RBP-J kappa inter-act with an Epstein-Barr virus nuclear antigen 2 responsive cis-element. *EMBO J.*, **13**, 5624–5632

Lawrence, J.B., Villnave, C.A. & Singer, R.H. (1988) Sensitive, high-resolution chromatin and chromosome mapping *in situ*: Presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell*, **52**, 51–61

Lear, A.L., Rowe, M., Kurilla, M.G., Lee, S., Henderson, S., Kieff, E. & Rickinson, A.B. (1992) The Epstein-Barr virus (EBV) nuclear antigen 1 *Bam*HI F promoter is activated on entry of EBV-transformed B cells into the lytic cycle. *J. Virol.*, **66**, 7461–7468

Lee, M.-A. & Yates, J.L. (1992) BHRF1 of Epstein-Barr virus, which is homologous to human proto-oncogene *bcl2*, is not essential for transformation of B cells or for virus replication *in vitro. J. Virol.*, **66**, 1899–1906

Lee, Y.S., Tanaka, A., Lau, R.Y., Nonoyama, M. & Rabin, H. (1980) Comparative studies of Herpesvirus papio (baboon herpesvirus) DNA, and Epstein-Barr virus DNA. *J. gen. Virol.*, **51**, 245–253

Lee, Y.S., Nonoyama, M. & Rabin, H. (1981) Collinear relationships of Herpesvirus papio DNA to Epstein-Barr virus DNA. *Virology*, **110**, 248–252

Lee, H.P., Duffy, S.W., Day, N.E. & Shanmugaratnam, K. (1988) Recent trends in cancer incidence among Singapore Chinese. *Int. J. Cancer*, **42**, 159–166

Lee, S.-H., Su, I.-J., Chen, R.-L., Lin, K.-S., Lin, D.-T., Chuu, W.-M. & Lin, K.-S. (1991) A pathologic study of childhood lymphoma in Taiwan with special reference to peripheral T-cell lymphoma and the association with Epstein-Barr viral infection. *Cancer*, **68**, 1954-1962

Lee, H.P., Chia, K.S. & Shanmugaratnam, K. (1992) *Cancer Incidence in Singapore 1983–1987* (Singapore Cancer Registry Report No. 3), Singapore, Singapore Cancer Registry, p. 83

Lee, S.P., Thomas, W.A., Murray, R.J., Khanim, F., Kaur, S., Young, L.S., Rowe, M., Kurilla, M. & Rickinson, A.B. (1993a) HLA A2.1-restricted cytotoxic T cells recognizing a range of Epstein-Barr virus isolates through a defined epitope in latent membrane protein LMP2. *J. Virol.*, **67**, 7428–7435

Lee, S.P., Wallace, L.E., Mackett, M., Arrand, J.R., Searle, P.F., Rowe, M. & Rickinson, A.B. (1993b) MHC class II-restricted presentation of endogenously synthesized antigen: Epstein-Barr virus transformed B cell lines can present the viral glycoprotein gp340 by two distinct pathways. *Int. Immunol.*, **5**, 451–460

Lee, J.-H., Lee, S.-S., Park, J.-H., Kim, Y.-W. & Yang, M.-H. (1994a) Prevalence of EBV RNA in sinonasal and Waldeyer's ring lymphomas. *J. Korean med. Sci.*, **9**, 281–288

Lee, H.P, Gourley, L., Duffy, S.W., Esteve, J., Lee, J. & Day, N.E. (1994b) Preserved foods and nasopharyngeal carcinoma: A case–control study among Singapore Chinese. *Int. J. Cancer*, **59**, 585–590

Lee, S.P., Morgan, S., Skinner, J., Thomas, W.A., Jones, S.R., Sutton, J., Khanna, R., Whittle, H.C. & Rickinson, A.B. (1995a) Epstein-Barr virus isolates with the major HLA B35.01-restricted cytotoxic T lymphocyte epitope are prevalent in a highly B35.01-positive African population. *Eur. J. Immunol.*, **25**, 102–110

Lee, E.S., Locker, J., Nalesnik, M., Reyes, J., Jaffe, R., Alashari, M., Nour, B., Tzakis, A. & Dickman, P.S. (1995b) The association of Epstein-Barr virus with smooth-muscle tumors occurring after organ transplantation. *New Engl. J. Med.*, **332**, 19–25

Lee, S.S., Jang, J.J., Cho, K.J., Khang, S.K. & Kim, C.W. (1997) Epstein-Barr virus-associated primary gastrointestinal lymphoma in non-immunocompromised patients in Korea. *Histopathology*, **30**, 234–242

Lees, J.F., Arrand, J.E., Pepper, S.deV., Stewart, J.P., Mackett, M. & Arrand, J.R. (1993) The Epstein-Barr virus candidate vaccine antigen gp340/220 is highly conserved between virus types A and B. *Virology*, **195**, 578–586

Lehtinen, T., Lumio, J., Dillner, J., Hakama, M., Knekt, P., Lehtinen, M., Teppo, L. & Leinikki, P. (1993) Increased risk of malignant lymphoma indicated by elevated Epstein-Barr virus antibodies — A prospective study. *Cancer Causes Control*, **4**, 187–193

Leibold, W., Huldt, G., Flanagan, T.G., Andersson, M., Dalens, M., Wright, D.H., Voller, A. & Klein, G. (1976) Tumorigenicity of Epstein-Barr virus (EBV) transformed lymphoid line cells in autologus squirrel monkeys. *Int. J. Cancer*, **17**, 533–541

Lemon, S.M., Hutt, L.M., Shaw, J.E., Li, J.-L.H. & Pagano, J.S. (1977) Replication of EBV in epithelial cells during infectious mononucleosis. *Nature*, **268**, 268–270

Lemon, S.M., Hutt, L.M., Shaw, J.E., Li, J.-L.H. & Pagano, J.S. (1978) Replication of Epstein-Barr virus DNA in epithelial cells *in vivo*. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 739–744

Lennette, E.T., Ward, E., Henle, G. & Henle, W. (1982) Detection of antibodies to Epstein-Barr virus capsid antigen by immune adherence hemagglutination. *J. clin. Microbiol.*, **15**, 69–73

Lennette, E.T., Rymo, L., Yadav, M., Masucci, G., Merk, K., Timar, L. & Klein, G. (1993) Disease-related differences in antibody patterns against EBV-encoded nuclear antigens EBNA 1, EBNA 2 and EBNA 6. *Eur. J. Cancer*, **29A**, 1584–1589

Lennette, E.T., Winberg, G., Yadav, M., Enblad, G. & Klein, G. (1995) Antibodies to LMP2A/2B in EBV-carrying malignancies. *Eur. J. Cancer*, **31A**, 1875–1878

Lenoir, G.M. & Bornkamm, G.W. (1987) Burkitt's lymphoma, a human cancer model for the study of the multistep development of cancer: Proposal for a new scenario. *Adv. viral Oncol.*, **7**, 173–206

Lenoir, G. & de Thé, G. (1978) Epstein-Barr virus–epithelial cell interaction and its implication in the etiology of nasopharyngeal carcinoma. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 377–384

Lenoir, G.M., Preud'homme, J.L., Bernheim, A. & Berger, R. (1982) Correlation between immunoglobulin light chain expression and variant translocation in Burkitt's lymphoma. *Nature*, **298**, 474–476

Leoncini, L., Spina, D., Nyong'O, A., Abinya, O., Minacci, C., Disanto, A., De Luca, F., De Vivo, A., Sabattini, E., Poggi, S., Pileri, S. & Tosi, P. (1996) Neoplastic cells of Hodgkin's disease show differences in EBV expression between Kenya and Italy. *Int. J. Cancer*, **65**, 781–784

Le Riverend, E., Rengifo, E., Longchong, M., Ruíz, R., Tormo, B., García, J., Portero, R., Quintero, S., Valdés, M., Rodríguez, T., Rusánchez, N. & Gurtsevich, V. (1984) Burkitt's lymphoma in Cuba. I. Clinical and morphological features and EBV association. *Oncology*, **41**, 13–18

Lerner, M.R., Andrews, N.C., Miller, G. & Steitz, J.A. (1981) Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc. natl Acad. Sci. USA*, **78**, 805–809

Le Roux, A., Kerdiles, B., Walls, D., Dedieu, J.F. & Perricaudet, M. (1994) The Epstein-Barr virus determined nuclear antigens EBNA-3A, -3B, and -3C repress EBNA-2-mediated trans-activation of the viral terminal protein 1 gene promoter. Virology, 205, 596–602

Lespagnard, L., Cochaux, P., Larsimont, D., Degeyter, M., Velu, T. & Heimann, R. (1995) Absence of Epstein-Barr virus in medullary carcinoma of the breast as demonstrated by immunophenotyping, in situ hybridization and polymerase chain reaction. Am. J. clin. Pathol., 103, 449–452

Leung, S.Y., Chung, L.P., Yuen, S.T., Ho, C.M., Wong, M.P. & Chan, S.Y. (1995a) Lympho-epithelial carcinoma of the salivary gland: in situ detection of Epstein-Barr virus. J. clin. Pathol., 48, 1022–1027

Leung, S.Y., Yuen, S.T., Chung, L.P., Kwong, W.K., Wong, M.P. & Chan, S.Y. (1995b) Epstein-Barr virus is present in a wide histological spectrum of sinonasal carcinomas. Am. J. surg. Pathol., 19, 994–1001

Leung, S.Y., Chung, L.P., Ho, C.M., Yuen, S.T., Wong, M.P. & Kwong, W.K. (1996) An Epstein-Barr virus positive undifferentiated carcinoma in the lacrimal sac. Histopathology, 28, 71–75

Levine, P.H. & Connelly, R.R. (1985) Epidemiology of nasopharyngeal cancer. In: Wittes, R.E., ed., Head and Neck Cancer, New York, John Wiley & Sons, pp. 13–34

Levine, P.H., Ablashi, D.V., Berard, C.W., Carbone, P.P., Waggoner, D.E. & Malan, L. (1971) Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. Cancer, 27, 416–421

Levine, P.H., O'Conor, G.T. & Berard, C.W. (1972) Antibodies to Epstein-Barr virus (EBV) in American patients with Burkitt's lymphoma. Cancer, 30, 610–615

Levine, P.H., Leiseca, S.A., Hewetson, J.F., Traul, K.A., Andrese, A.P., Granlund, D.J., Fabrizio, P. & Stevens, D.A. (1980a) Infection of rhesus monkeys and chimpanzees with Epstein-Barr virus. Arch. Virol., 66, 341–351

Levine, P.H., Connelly, R.R. & Easton, J.M. (1980b) Demographic patterns for nasopharyngeal carcinoma in the United States. Int. J. Cancer, 26, 741–748

Levine, P.H., McKay, F.W. & Connelly, R.R. (1987) Patterns of nasopharyngeal cancer mortality in the United States. Int. J. Cancer, 39, 133–137

Levine, P.H., Pallesen, G., Ebbesen, P., Harris, N., Evans, A.S. & Mueller, N. (1994) Evaluation of Epstein-Barr virus antibody patterns and detection of viral markers in the biopsies of patients with Hodgkin's disease. Int. J. Cancer, 59, 48–50

Levitskaya, J., Coram, M., Levitsky, V., Imreh, S., Steigerwald-Mullen, P.M., Klein, G., Kurilla, M.G. & Masucci, M.G. (1995) Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. Nature, 375, 685–688

Lewin, N., Åman, P., Masucci, M.G., Klein, E., Klein, G., Öberg, B., Strander, H., Henle, W. & Henle, G. (1987) Characterization of EBV-carrying B-cell populations in healthy seropositive individuals with regard to density, release of transforming virus and spontaneous outgrowth. Int. J. Cancer, 39, 472–476

Lewin, N., Åman, P., Mellstedt, H., Zech, L. & Klein, G. (1988) Direct outgrowth of in vivo Epstein-Barr virus (EBV)-infected chronic lymphocytic leukemia (CLL) cells into permanent lines. Int. J. Cancer, 41, 892–895

Leyvraz, S., Henle, W., Chahinian, A.P., Perlmann, C., Klein, G., Gordon, R.E., Rosenblum, M. & Holland, J.F. (1985) Association of Epstein-Barr virus with thymic carcinoma. New Engl. J. Med., 312, 1296–1299

Li, C.-C., Yu, M.C. & Henderson, B.E. (1985) Some epidemiologic observations of naso-pharyngeal carcinoma in Guangdong, Peoples's Republic of China. *Natl Cancer Inst. Monogr.*, **69**, 49–52

Li, Q.-X., Young, L.S., Niedobitek, G., Dawson, C.W., Birkenbach, M., Wang, F. & Rickinson, A.B. (1992) Epstein-Barr virus infection and replication in a human epithelial cell system. *Nature*, **356**, 347–350

Li, S.L., Feichtinger, H., Kaaya, E., Migliorini, P., Putkonen, P., Biberfeld, G., Middeldorp, M., Biberfeld, P. & Ernberg, I. (1993) Expression of Epstein-Barr virus-related nuclear antigens and B-cell marker in lymphomas of SIV-immunosuppressed monkeys. *Int. J. Cancer*, **55**, 609–615

Li, S.L, Biberfeld, P. & Ernberg, I. (1994) DNA of lymphoma-associated herpesvirus (HVMF1) in SIV-infected monkeys (*Macaca fascicularis*) shows homologies to EBNA-1, 2 and 5 genes. *Int. J. Cancer.*, **59**, 287–295

Li, Q.-X., Turk, S.M. & Hutt-Fletcher, L.M. (1995a) The Epstein-Barr virus (EBV) BZLF2 gene product associates with the gH and gL homologs of EBV and carries an epitope critical to infection of B cells but not of epithelial cells. *J. Virol.*, **69**, 3987–3994

Li, D., Oda, K., Mikata, A. & Yumoto, N. (1995b) Epstein-Barr virus genomes in Hodgkin's disease and non-Hodgkin's lymphomas. *Pathol. int.*, **45**, 735–741

Li, S.N., Chang, Y.S. & Liu, S.T. (1996) Effect of a 10-amino acid deletion on the oncogenic activity of latent membrane protein 1 of Epstein-Barr virus. *Oncogene*, **12**, 2129–2135

Li, Q.-X., Spriggs, M.K., Kovats, S., Turk, S.M., Comeau, M.R., Nepom, B. & Hutt-Fletcher, L.M. (1997) Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. *J. Virol.*, **71**, 4657–4662

Libetta, C.M., Pringle, J.H., Angel, C.A., Craft, A.W., Malcolm, A.J. & Lauder, I. (1990) Demonstration of Epstein-Barr viral DNA in formalin-fixed, paraffin-embedded samples of Hodgkin's disease. *J. Pathol.*, **161**, 255–260

Lieberman, P.M. & Berk, A.J. (1990) In vitro transcriptional activation, dimerization, and DNA-binding specificity of the Epstein-Barr virus Zta protein. *J. Virol.*, **64**, 2560–2568

Liebowitz, D., Wang, D. & Kieff, E. (1986) Orientation and patching of the latent infection membrane protein encoded by Epstein-Barr virus. *J. Virol.*, **58**, 233–237

Liebowitz, D., Mannick, J., Takada, K. & Kieff, E. (1992) Phenotypes of Epstein-Barr virus LMP1 deletion mutants indicate transmembrane and amino-terminal cytoplasmic domains necessary for effects in B-lymphoma cells. *J. Virol.*, **66**, 4612–4616

Lijinsky, W. & Taylor, H.W. (1978) Relative carcinogenic effectiveness of derivatives of nitro-sodiethylamine in rats. *Cancer Res.*, **38**, 2391–2394

Lin, J.-C. & Machida, H. (1988) Comparison of two bromovinyl nucleoside analogs, 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil and *E*-5-(2-bromovinyl)-2′-deoxyuridine, with acyclovir in inhibition of Epstein-Barr virus replication. *Antimicrob. Agents Chemother.*, **32**, 1068–1072

Lin, J.-C. & Smith, M.C. (1984) Antagonistic action of retinoic acid against teleocidin and 12-*O*-tetradecanoyl-phorbol-13-acetate on activation of Epstein-Barr virus genomes. *Carcinogenesis*, **5**, 483–487

Lin, T.M., Chen, K.P., Lin, C.C., Hsu, M.M., Tu, S.M., Chiang, T.C., Jung, P.F. & Hirayama, T. (1973) Retrospective study on nasopharyngeal carcinoma. *J. natl Cancer Inst.*, **51**, 1403–1408

Lin, J.-C., Smith, M.C. & Pagano, J.S. (1982) Effects of 12-*O*-tetradecanoyl-phorbol-13-acetate on cell proliferation and Epstein-Barr virus DNA replication. *Virology*, **117**, 186–194

Lin, J.-C., De Clercq, E. & Pagano, J.S. (1991) Inhibitory effects of acyclic nucleoside phosphonate analogs, including (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, on Epstein-Barr virus replication. *Antimicrob. Agents Chemother.*, **35**, 2440–2443

Lin, J.-C., Reefschläger, J., Herrmann, G. & Pagano, J.S. (1992) Structure–activity relationship between (*E*)-5-(2-bromovinyl)- and 5-vinyl-1-β-D-arabinofuranosyluracil (BV-araU, V-araU) in inhibition of Epstein-Barr virus replication. *Antiviral Res.*, **17**, 43–52

Lin, J.-C., Lin, S.-C., De, B.K., Chan, W.P.-C. & Evatt, B.L. (1993a) Precision of genotyping of Epstein-Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBER): Predominance of type A virus associated with Hodgkin's disease. *Blood*, **81**, 3372–3381

Lin, C.-T., Chan, W.-Y., Chen, W., Huang, H.-M., Wu, H.-C., Hsu, M.-M., Chuang, S.-M. & Wang, C.-C. (1993b) Characterization of seven newly established nasopharyngeal carcinoma cell lines. *Lab. Invest.*, **68**, 716–727

Lin, C.-T., Dee, A.N., Chen, W. & Chan, W.-Y. (1994) Association of Epstein-Barr virus, human papilloma virus, and cytomegalovirus with nine nasopharyngeal carcinoma cell lines. *Lab. Invest.*, **71**, 731–736

Lindahl, T., Klein, G., Reedman, B.M., Johansson, B. & Singh, S. (1974) Relationship between Epstein-Barr virus (EBV) DNA and the EBV-determined nuclear antigen (EBNA) in Burkitt lymphoma biopsies and other lymphoproliferative malignancies. *Int. J. Cancer*, **13**, 764–772

Lindahl, T., Adams, A., Bjursell, G., Bornkamm, G.W., Kaschka Dierich, C. & Jehn, U. (1976) Covalently closed circular duplex DNA of Epstein-Barr virus in a human lymphoid cell line. *J. mol. Biol.*, **102**, 511–530

Lindhout, E., Lakeman, A., Mevissen, M.L. & de Groot, C. (1994) Functionally active Epstein-Barr virus-transformed follicular dendritic cell-like cell lines. *J. exp. Med.*, **179**, 1173–1184

Ling, P.D. & Hayward, S.D. (1995) Contribution of conserved amino acids in mediating the interaction between EBNA2 and CBF1/RBPJk. *J. Virol.*, **69**, 1944–1950

Ling, N.R., Hardie, D., Lowe, J., Johnson, G.D., Khan, M. & MacLennan, I.C. (1989) A phenotypic study of cells from Burkitt lymphoma and EBV-B-lymphoblastoid lines and their relationship to cells in normal lymphoid tissues. *Int. J. Cancer*, **43**, 112–118

Ling, P.D., Rawlins, D.R. & Hayward, S.D. (1993a) The Epstein-Barr virus immortalizing protein EBNA-2 is targeted to DNA by a cellular enhancer-binding protein. *Proc. natl Acad. Sci. USA*, **90**, 9237–9241

Ling, P.D., Ryon, J.J. & Hayward, S.D. (1993b) EBNA-2 of Herpesvirus papio diverges significantly from the type A and type B EBNA-2 proteins of Epstein-Barr virus but retains an efficient transactivation domain with a conversed hydrophobic motif. *J. Virol.*, **67**, 2990–3003

Lisi, A., Pozzi, D., Carloni, G., Da Villa, G., Iacovacci, S., Valli, M.B. & Grimaldi, S. (1995) Fusion of EBV with the surface of receptor-negative human hepatoma cell line Li7A permits virus penetration and infection. *Res. Virol.*, **146**, 295–300

Liu, Q., Ohshima, K., Masuda, Y. & Kikuchi, M. (1995) Detection of the Epstein-Barr virus in primary gastric lymphoma by in situ hybridization. *Pathol. int.*, **45**, 131-136

Liu, S., Liu, P., Borras, A., Chatila, T. & Speck, S.H. (1997) Cyclosporin A-sensitive induction of the Epstein-Barr virus lytic switch is mediated via a novel pathway involving a MEF2 family member. *EMBO J.*, **16**, 143–153

Lloyd-Still, J.D., Scott, J.P. & Crussi, F. (1986) The spectrum of Epstein-Barr virus hepatitis in children. *Pediatr. Pathol.*, **5**, 337–351

Lo, K.-W., Huang, D.P. & Lau, K.-M. (1995) p16 Gene alterations in nasopharyngeal carcinoma. *Cancer Res.*, **55**, 2039–2043

Locker, J. & Nalesnik, M. (1989) Molecular genetic analysis of lymphoid tumors arising after organ transplantation. *Am. J. Pathol.*, **135**, 977–987

Longnecker, R. & Kieff, E. (1990) A second Epstein-Barr virus membrane protein (LMP2) is expressed in latent infection and colocalizes with LMP1. *J. Virol.*, **64**, 2319–2326

Longnecker, R. & Miller, C.L. (1996) Regulation of Epstein-Barr virus latency by latent membrane protein 2. *Trends Microbiol.*, **4**, 38–42

Longnecker, R., Druker, B., Roberts, T.M. & Kieff, E. (1991) An Epstein-Barr virus protein associated with cell growth transformation interacts with a tyrosine kinase. *J. Virol.*, **65**, 3681–3692

Longnecker, R., Miller, C.L., Miao, X.-Q., Marchini, A. & Kieff, E. (1992) The only domain which distinguishes Epstein-Barr virus latent membrane protein 2A (LMP2A) from LMP2B is dispensable for lymphocyte infection and growth transformation *in vitro*; LMP2A is therefore nonessential. *J. Virol.*, **66**, 6461–6469

Longnecker, R., Miller, C.L., Tomkinson, B., Miao, X.-Q. & Kieff, E. (1993a) Deletion of DNA encoding the first five transmembrane domains of Epstein-Barr virus latent membrane proteins 2A and 2B. *J. Virol.*, **67**, 5068–5074

Longnecker, R., Miller, C.L., Miao, X.-Q., Tomkinson, B. & Kieff, E. (1993b) The last seven transmembrane and carboxy-terminal cytoplasmic domains of Epstein-Barr virus latent membrane protein 2 (LMP2) are dispensable for lymphocyte infection and growth transformation *in vitro*. *J. Virol.*, **67**, 2006–2013

Lopategui, J.R., Gaffey, M.J., Frierson, H.F., Jr, Chan, J.K.C., Mills, S.E., Chang, K.L., Chen, Y.-Y. & Weiss, L.M. (1994) Detection of Epstein-Barr viral RNA in sinonasal undifferentiated carcinoma from western and Asian patients. *Am. J. surg. Pathol.*, **18**, 391–398

Lopategui, J.R., Gaffey, M.J., Chan, J.K.C., Frierson, H.F., Jr, Sun, L.-H., Bellafiore, F.J., Chang, K.L. & Weiss, L.W. (1995) Infrequent association of Epstein-Barr virus with CD30-positive anaplastic large cell lymphomas from American and Asian patients. *Am. J. surg. Pathol.*, **19**, 42-49

López-Navidad, A., Domingo, P., López-Talavera, J.C., Rabella, N. & Verger, G. (1996) Epstein-Barr virus infection associated with interstitial nephritis and chronic fatigue. *Scand. J. infect. Dis.*, **28**, 185–187

Lowe, R.S., Keller, P.M., Keech, B.J., Davison, A.J., Whang, Y., Morgan, A.J., Kieff, E. & Ellis, R.W. (1987) Varicella-zoster virus as a live vector for the expression of foreign genes. *Proc. natl Acad. Sci. USA*, **84**, 3896–3900

Lu, S.-J., Day, N.E., Degos, L., Lepage, V., Wang, P.-C., Chan, S.-H., Simons, M., McKnight, B., Easton, D., Zeng, Y. & de Thé, G. (1990) Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region. *Nature*, **346**, 470–471

Lu, Q.-L., Elia, G., Lucas, S. & Thomas, J.A. (1993) *Bcl-2* proto-oncogene expression in Epstein-Barr-virus-associated nasopharyngeal carcinoma. *Int. J. Cancer*, **53**, 29–35

Lucas, K.G., Small, T.N., Heller, G., Dupont, B. & O'Reilly, R.J. (1996) The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. *Blood*, **87**, 2594–2603

Luka, J., Klein, G., Henle, W. & Henle, G. (1978) Detection of the EBV-determined nuclear antigen (EBNA) in Burkitt's lymphoma and nasopharyngeal carcinoma biopsies by the acid fixed nuclear binding (AFNB) technique. *Cancer Lett.*, **4**, 199–205

Luka, J., Kallin, B. & Klein, G. (1979) Induction of the Epstein-Barr virus (EBV) cycle in latently infected cells by *n*-butyrate. *Virology*, **94**, 228–231

Luka, J., Chase, R.C. & Pearson, G.R. (1984) A sensitive enzyme-linked immunosorbent assay (ELISA) against the major EBV-associated antigens. 1. Correlation between ELISA and immunofluorescence titres using purified antigens. *J. immunol. Meth.*, **67**, 145–156

Luka, J., Deeb, Z.E., Hartmann, D.P., Jenson, B. & Pearson, G.R. (1988) Detection of antigens associated with Epstein-Barr virus replication in extracts from biopsy specimens of naso-pharyngeal carcinomas. *J. natl Cancer Inst.*, **80**, 1164–1167

Lukes, R.J. & Butler, J.J. (1966) The pathology and nomenclature of Hodgkin's disease. *Cancer Res.*, **26**, 1063–1081

Lung, M.L. & Chang, G.C. (1992) Detection of distinct Epstein-Barr virus genotypes in NPC biopsies from southern Chinese and Caucasians. *Int. J. Cancer*, **52**, 34–37

Lung, M.L., Chan, K.H., Lam, W.P., Kou, S.K., Choy, D., Chan, C.W. & Ng, M.H. (1989) In situ detection of Epstein-Barr virus markers in nasopharyngeal carcinoma patients. *Onco-logy*, **46**, 310–317

Lung, M.L., Chang, R.S., Huang, M.L., Guo, H.Y., Choy, D., Sham, J., Tsao, S.Y., Cheng, P. & Ng, M.H. (1990) Epstein-Barr virus genotypes associated with nasopharyngeal carcinoma in southern China. *Virology*, **177**, 44–53

Lung, M.L., Lam, W.P., Chan, K.H., Li, S., Sham, J. & Choy, D. (1992) Direct detection of Epstein-Barr virus in peripheral blood and comparison of Epstein-Barr virus genotypes present in direct specimens and lymphoblastoid cell lines established from nasopharyngeal carcinoma patients and healthy carriers in Hong Kong. *Int. J. Cancer*, **52**, 174–177

Lüscher, B. & Eisenman, R.N. (1990) New light on myc and myb. Part I. Myc. *Genes Dev.*, **4**, 2025–2035

Mabuchi, K., Bross, D.S. & Kessler, I.I. (1985) Cigarette smoking and nasopharyngeal carci-noma. *Cancer*, **55**, 2874–2876

Mackett, M. & Arrand, J.R. (1985) Recombinant vaccinia virus induces neutralising antibodies in rabbits against Epstein-Barr virus membrane antigen gp340. *EMBO J.*, **4**, 3229–3234

Mackett, M., Conway, M.J., Arrand, J.R., Haddad, R.S. & Hutt-Fletcher, L.M. (1990) Charac-terization and expression of a glycoprotein encoded by the Epstein-Barr virus *Bam*HI I fragment. *J. Virol.*, **64**, 2545–2552

Mackett, M., Pepper, C.deV.S., Lees, J.F., Naylor, B.A., Wedderburn, N. & Arrand, J.R. (1996) Immunisation of common marmosets with vaccinia virus expressing Epstein-Barr virus (EBV) gp340 and challenge with EBV. *J. med. Virol.*, **50**, 263–271

MacMahon, B. (1957) Epidemiological evidence on the nature of Hodgkin's disease. *Cancer*, **10**, 1045–1054

MacMahon, B. (1960) The ethnic distribution of cancer mortality in New York City, 1955. *Acta Unio int. contra cancrum*, **16**, 1716–1724

MacMahon, B. (1966) Epidemiology of Hodgkin's disease. *Cancer Res.*, **26**, 1189–1200

MacMahon, E.M.E., Glass, J.D., Hayward, S.D., Mann, R.B., Becker, P.S., Charache, P., McArthur, J.C. & Ambinder, R.F. (1991) Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet*, **338**, 969-973

MacMillan, C., Kapadia, S.B., Finkelstein, S.D., Nalesnik, M.A. & Barnes, L. (1996) Lympho-epithelial carcinoma of the larynx and hypopharynx: Study of eight cases with relationship to Epstein-Barr virus and *p53* gene alterations, and review of the literature. *Hum. Pathol.*, **27**, 1172–1179

Madej, M., Conway, M.J., Morgan, A.J., Sweet, J., Wallace, L., Qualtière, L.F., Arrand, J.R. & Mackett, M. (1992) Purification and characterisation of Epstein-Barr virus gp340/220 produced by a bovine papillomavirus expression vector system. *Vaccine*, **10**, 777–782

Madisen, L. & Groudine, M. (1994) Identification of a locus control region in the immuno-globulin heavy-chain locus that deregulates c-*myc* expression in plasmacytoma and Burkitt's lymphoma cells. *Genes Dev.*, **8**, 2212–2226

Magrath, I.T. (1990) The pathogenesis of Burkitt's lymphoma. *Recent Adv. Cancer Res.*, **55**, 133–270

Magrath, I.T. (1991) African Burkitt's lymphoma. History, biology, clinical features, and treatment. *Am. J. pediatr. hematol. Oncol.*, **13**, 222–246

Magrath, I. (1997) Small noncleaved cell lymphomas. In: Magrath, I., ed., *The Non-Hodgkin's Lymphomas*, 2nd Ed., London, Edward Arnold (in press)

Magrath, I.T. & Ziegler, J.L. (1980) Bone marrow involvement in Burkitt's lymphoma and its relationship to acute B-cell leukemia. *Leukemia Res.*, **4**, 33–59

Magrath, I.T., Mugerwa, J., Bailey, I., Olweny, C. & Kiryabwire, Y. (1974) Intracerebral Burkitt's lymphoma: Pathology, clinical features and treatment. *Q. J. Med.*, **43**, 489–508

Magrath, I., Henle, W., Owor, R. & Olweny, C. (1975) Antibodies to Epstein-Barr virus anti-gens before and after the development of Burkitt's lymphoma in a patient treated for Hodgkin's disease. *New Engl. J. Med.*, **292**, 621–623

Magrath, I., Jain, V. & Bhatia, K. (1992) Epstein-Barr virus and Burkitt's lymphoma. *Semin. Cancer Biol.*, **3**, 285–295

Malcolm, S., Barton, P., Murphy, C., Fergusson-Smith, M.A., Bentley, D.L. & Rabbitts, T.H. (1982) Localization of human immunoglobulin kappa light chain variable region genes to the short arm of chromosome 2 by in situ hybridization. *Proc. natl Acad. Sci. USA*, **79**, 4957–4961

Mann, K.P., Staunton, D. & Thorley-Lawson, D.A. (1985) Epstein-Barr virus-encoded protein found in plasma membranes of transformed cells. *J. Virol.*, **55**, 710–720

Mann, R.B., Wu, T.-C., MacMahon, E.M.E., Ling, Y., Charache, P. & Ambinder, R.F. (1992) In situ localization of Epstein-Barr virus in thymic carcinoma. *Mod. Pathol.*, **5**, 363–366

Mannick, J.B., Cohen, J.I., Birkenbach, M., Marchini, A. & Kieff, E. (1991) The Epstein-Barr virus nuclear protein encoded by the leader of the EBNA RNAs is important in B-lym-phocyte transformation. *J. Virol.*, **65**, 6826–6837

Manolov, G. & Manolova, Y. (1972) Marker band in one chromosome 14 from Burkitt lymphomas. *Nature*, **237**, 33–34

Mar, E.-C., Chu, C.K. & Lin, J.-C. (1995) Some nucleoside analogs with anti-human immuno-deficiency virus activity inhibit replication of Epstein-Barr virus. *Antiviral Res.*, **28**, 1–11

Marchini, A., Tomkinson, B., Cohen, J.I. & Kieff, E. (1991) BHRF1, the Epstein-Barr virus gene with homology to *bcl2*, is dispensable for B-lymphocyte transformation and virus replication. *J. Virol.*, **65**, 5991–6000

Mark, W. & Sugden, B. (1982) Transformation of lymphocytes by Epstein-Barr virus requires only one-fourth of the viral genome. *Virology*, **122**, 431–443

Markin, R.S., Wood, R.P., Shaw, B.W., Jr, Brichacek, B. & Purtilo, D.T. (1990) Immunohistologic identification of Epstein-Barr virus-induced hepatitis reactivation after OKT-3 therapy following orthotopic liver transplant. *Am. J. Gastroenterol.*, **85**, 1014–1018

Marklund, G., Ernberg, I., Lundberg, C., Henle, W. & Henle, G. (1986) Differences in EBV-specific antibody patterns at onset of infectious mononucleosis. *Scand. J. infect. Dis.*, **18**, 25–32

Marschall, M., Schwarzmann, F., Leser, U., Oker, B., Alliger, P., Mairhofer, H. & Wolf, H. (1991) The BI'LF4 *trans*-activator of Epstein-Barr virus is modulated by type and differentiation of the host cell. *Virology*, **181**, 172–179

Martel-Renoir, D., Grunewald, V., Touitou, R., Schwaab, G. & Joab, I. (1995) Qualitative analysis of the expression of Epstein-Barr virus lytic genes in nasopharyngeal carcinoma biopsies. *J. gen. Virol.*, **76**, 1401–1408

Martin, J. & Sugden, B. (1991) Transformation by the oncogenic latent membrane protein correlates with its rapid turnover, membrane localization, and cytoskeletal association. *J. Virol.*, **65**, 3246–3258

Martin, J.M., Veis, D., Korsmeyer, S.J. & Sugden, B. (1993) Latent membrane protein of Epstein-Barr virus induces cellular phenotypes independently of expression of Bcl-2. *J. Virol.*, **67**, 5269–5278

Martínez-Leandro, E.P., Martorell, M., Alemany, P., Salvador, I. & Garcia-Guardiet, E. (1994) Lymphoepithelial-like carcinoma of the uterine cervix. Study of a case with in situ hybridization of the Epstein-Barr virus genome and the human papillomavirus genome. *Acta obstet. gynecol. scand.*, **73**, 589–592

Masih, A., Weisenburger, D., Duggan, M., Armitage, J., Bashir, R., Mitchell, D., Wickert, R. & Purtilo, D.T. (1991) Epstein-Barr viral genome in lymph nodes from patients with Hodgkin's disease may not be specific to Reed-Sternberg cells. *Am. J. Pathol.*, **139**, 37–43

Masood, R., Zhang, Y., Bond, M.W., Scadden, D.T., Moudgil, T., Law, R.E., Kaplan, M.H., Jung, B., Espina, B.M., Lunardi-Iskandar, Y., Levine, A.M. & Gill, P.S. (1995) Interleukin-10 is an autocrine growth factor for acquired immunodeficiency syndrome-related B-cell lymphoma. *Blood*, **85**, 3423–3430

Masucci, M.G. & Ernberg, I. (1994) Epstein-Barr virus: Adaptation to a life within the immune system. *Trends Microbiol.*, **2**, 125–130

Masucci, M.G., Bejarano, M.T., Masucci, G. & Klein, E. (1983) Large granular lymphocytes inhibit the in vitro growth of autologous Epstein-Barr virus-infected B cells. *Cell. Immunol.*, **76**, 311–321

Masucci, M.G., Torsteinsdottir, S., Colombani, J., Brautbar, C., Klein, E. & Klein, G. (1987) Down-regulation of class I HLA antigens and of the Epstein-Barr virus-encoded latent membrane protein in Burkitt lymphoma lines. *Proc. natl Acad. Sci. USA*, **84**, 4567–4571

Masucci, M.G., Contreras-Salazar, B., Ragnar, E., Falk, K., Minarovits, J., Ernberg, I. & Klein, G. (1989) 5-Azacytidine up regulates the expression of Epstein-Barr virus nuclear antigen 2 (EBNA-2) through EBNA-6 and latent membrane protein in the Burkitt's lymphoma line rael. *J. Virol.*, **63**, 3135–3141

Mathew, G.D., Qualtière, L.F., Neel, H.B., III & Pearson, G.R. (1981) IgA antibody, antibody-dependent cellular cytotoxicity and prognosis in patients with nasopharyngeal carcinoma. *Int. J. Cancer*, **27**, 175–180

Matsunou, H., Konishi, F., Hori, H., Ikeda, T., Sasaki, K., Hirose, Y. & Yamamichi, N. (1996) Characteristics of Epstein-Barr virus-associated gastric carcinoma with lymphoid stroma in Japan. *Cancer*, **77**, 1998–2004

Matsuo, T., Heller, M., Petti, L., O'Shiro, E. & Kieff, E. (1984) Persistence of the entire Epstein-Barr virus genome integrated into human lymphocyte DNA. *Science*, **226**, 1322–1325

Mauch, P.M., Kalish, L.A., Kadin, M., Coleman, C.N., Osteen, R. & Hellman, S. (1993) Patterns of presentation of Hodgkin's disease. Implications for etiology and pathogenesis. *Cancer*, **71**, 2062–2071

Mayer, H.B., Wanke, C.A., Williams, M., Crosson, A.W., Federman, M. & Hammer, S.M. (1996) Epstein-Barr virus-induced infectious mononucleosis complicated by acute renal failure: Case report and review. *Clin. infect. Dis.*, **22**, 1009–1018

McBride, O.W., Hieter, P.A., Hollis, G.F., Swan, D., Otey, M.C. & Leder, P. (1982) Chromosomal location of human kappa and lambda immunoglobulin light chain constant region genes. *J. exp. Med.*, **155**, 1480–1490

McCarthy, N.J., Hazlewood, S.A., Huen, D.S., Rickinson, A.B. & Williams, G.T. (1996) The Epstein-Barr virus gene BHRF1, a homologue of the cellular oncogene bcl-2, inhibits apoptosis induced by gamma radiation and chemotherapeutic drugs. *Adv. exp. Med. Biol.*, **406**, 83–97

McClain, K.L., Leach, C.T., Jenson, H.B., Joshi, V.V., Pollock, B.H., Parmley, R.T., DiCarlo, F.J., Chadwick, E.G. & Murphy, S.B. (1995) Association of Epstein-Barr virus with leiomyosarcomas in young people with AIDS. *New Engl. J. Med.*, **332**, 12–18

McGregor, I.A. (1970) Plasma immunoglobulin concentrations in an African (Gambian) community in relation to season, malaria and other infections and pregnancy. *Clin. exp. Immunol.*, **7**, 51–74

McGuire, L.J., Huang, D.P., Teoh, R., Arnold, M., Wong, K. & Lee, J.C.K. (1988) Epstein-Barr virus genome in thymoma and thymic lymphoid hyperplasia. *Am. J. Pathol.*, **131**, 385–390

Medeiros, L.J. & Greiner, T.C. (1995) Hodgkin's disease. *Cancer*, **75** (Suppl.), 357–369

Medeiros, L.J., Peiper, S.C., Elwood, L., Yano, T., Raffeld, M., & Jaffe, E.S. (1991) Angiocentric immunoproliferative lesions: A molecular analysis of eight cases. *Hum. Pathol.*, **22**, 1150–1157

Melbye, M., Coté, T.R., West, D., Kessler, L., Biggar, R.J. & the AIDS/Cancer Working Group (1996) Nasopharyngeal carcinoma: An EBV-associated tumour not significantly influenced by HIV-induced immunosuppression. *Br. J. Cancer*, **73**, 995–997

Mentzer, S.J., Longtine, J., Fingeroth, J., Reilly, J.J., DeCamp, M.M., O'Donnell, W., Swanson, S.J., Faller, D.V. & Sugarbaker, D.J. (1996) Immunoblastic lymphoma of donor origin in the allograft after lung transplantation. *Transplantation*, **61**, 1720–1725

Merk, K., Lennette, E., Holm, G., Johansson, B., Masucci, G., Grimfors, G., Klein, G. & Björkholm, M. (1995) Antibodies to Epstein-Barr virus in relation to clinical characteristics of untreated patients with Hodgkin's disease. *Cancer Res. Ther. Control*, **4**, 223–229

Mikaélian, I., Drouet, E., Marechal, V., Denoyel, G., Nicolas, J.-C. & Sergeant, A. (1993) The DNA-binding domain of two bZIP transcription factors, the Epstein-Barr virus switch gene product EB1 and Jun, is a bipartite nuclear targeting sequence. *J. Virol.*, **67**, 734–742

Miller, R.W. & Beebe, G.W. (1973) Infectious mononucleosis and the empirical risk of cancer. *J. natl Cancer Inst.*, **50**, 315–321

Miller, G. & Coope, D. (1974) Epstein-Barr viral nuclear antigen (EBNA) in tumor cell imprints of experimental lymphoma of marmosets. *Trans. Assoc. Am. Phys.*, **87**, 205–218

Miller, N. & Hutt-Fletcher, L.M. (1988) A monoclonal antibody to glycoprotein gp85 inhibits fusion but not attachment of Epstein-Barr virus. *J. Virol.*, **62**, 2366–2372

Miller, G., Shope, T., Lisco, H., Stitt, D. & Lipman, M. (1972) Epstein-Barr virus: Transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc. natl Acad. Sci. USA*, **69**, 383–387

Miller, G., Robinson, J., Heston, L. & Lipman, M. (1974) Differences between laboratory strains of Epstein-Barr virus based on immortalization, abortive infection, and interference. *Proc. natl Acad. Sci. USA*, **71**, 4006–4010

Miller, G., Shope, T., Coope, D., Waters, L., Pagano, J., Bornkamm, G.W. & Henlé, W. (1977) Lymphoma in cotton-top marmosets after inoculation with Epstein-Barr virus: Tumor incidence, histologic spectrum, antibody responses, demonstration of viral DNA, and characterization of viruses. *J. exp. Med.*, **145**, 948–967

Miller, G., Rabson, M. & Heston, L. (1984) Epstein-Barr virus with heterogeneous DNA disrupts latency. *J. Virol.*, **50**, 174–182

Miller, G., Grogan, E., Rowe, D., Rooney, C., Heston, L., Eastman, R., Andiman, W., Niederman, J., Lenoir, G., Henle, W., Henle, W., Sullivan, J., Schooley, R., Vossen, J., Strauss, S. & Issekutz, T. (1987) Selective lack of antibody to a component of EB nuclear antigen in patients with chronic active Epstein-Barr virus infection. *J. infect. Dis.*, **156**, 26–35

Miller, W.E., Edwards, R.H., Walling, D.M. & Raab-Traub, N. (1994a) Sequence variation in the Epstein-Barr latent virus membrane protein 1. *J. gen. Virol.*, **75**, 2729–2740

Miller, C.L., Lee, J.H., Kieff, E. & Longnecker, R. (1994b) An integral membrane protein (LMP2) blocks reactivation of Epstein-Barr virus from latency following surface immunoglobulin crosslinking. *Proc. natl Acad. Sci. USA*, **91**, 772–776

Miller, W.E., Earp, H.S. & Raab-Traub, N. (1995a) The Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor. *J. Virol.*, **69**, 4390–4398

Miller, C.L., Burkhardt, A.L., Lee, J.H., Stealey, B., Longnecker, R., Bolen, J.B. & Kieff, E. (1995b) Integral membrane protein 2 of Epstein-Barr virus regulates reactivation from latency through dominant negative effects on protein-tyrosine kinases. *Immunity*, **2**, 155–166

Miller, W.E., Mosialos, G., Kieff, E. & Raab-Traub, N. (1997) Epstein-Barr virus LMP1 induction of the epidermal growth factor receptor is mediated through a TRAF signaling pathway distinct from NF-κB activation. *J. Virol.*, **71**, 586–594

Min, K.-W., Holmquist, S., Peiper, S.C. & O'Leary, T.J. (1991) Poorly differentiated adeno-carcinoma with lymphoid stroma (lymphoepithelioma-like carcinomas) of the stomach. Report of three cases with Epstein-Barr virus genome demonstrated by the polymerase chain reaction. *Am. J. clin. Pathol.*, **96**, 219–227

Minarovits, J., Minarovits-Kormuta, S., Ehlin-Henriksson, B., Falk, K., Klein, G. & Ernberg, I. (1991) Host cell phenotype-dependent methylation patterns of Epstein-Barr virus DNA. *J. gen.Virol.*, **72**, 1591–1599

Minarovits, J., Hu, L.-F., Marcsek, Z., Minarovits-Kormuta, S., Klein, G. & Ernberg, I. (1992) RNA polymerase III-transcribed EBER 1 and 2 transcription units are expressed and hypo-methylated in the major Epstein-Barr virus-carrying cell types. *J. gen. Virol.*, **73**, 1687–1692

Minarovits, J., Hu, L.-F., Imai, S., Harabuchi, Y., Kataura, A., Minarovits-Kormuta, S., Osato, T. & Klein, G. (1994a) Clonality, expression and methylation patterns of the Epstein-Barr virus genomes in lethal midline granulomas classified as peripheral angiocentric T cell lym-phomas. *J. gen. Virol.*, **75**, 77–84

Minarovits, J., Hu, L.F., Minarovits-Kormuta, S., Klein, G. & Ernberg, I. (1994b) Sequence-specific methylation inhibits the activity of the Epstein-Barr virus LMP-1 and BCR2 enhancer–promoter regions. *Virology*, **200**, 661–667

Misko, I.S., Pope, J.H., Hütter, R., Soszynski, T.D. & Kane, R.G. (1984) HLA-DR-antigen-associated restriction of EBV-specific cytotoxic T-cell colonies. *Int. J. Cancer*, **33**, 239–243

Misko, I.S., Sculley, T.B., Schmidt, C., Moss, D.J., Soszynski, T. & Burman, K. (1991) Compo-site response of naive T cells to stimulation with the autologous lymphoblastoid cell line is mediated by CD4 cytotoxic T cell clones and includes an Epstein-Barr virus-specific com-ponent. *Cell. Immunol.*, **132**, 295–307

Miyashita, E.M., Yang, B., Lam, K.M.C., Crawford, D.H. & Thorley-Lawson, D.A. (1995) A novel form of Epstein-Barr virus latency in normal B cells *in vivo*. *Cell*, **80**, 593–601

Miyoshi, I. (1983) Japanese Burkitt's lymphoma: Clinicopathological review of 14 cases. *Jpn. J. clin. Oncol.*, **13**, 489–496

Miyoshi, I., Hiraki, S., Kubonishi, I., Matsuda, Y., Kishimoto, H., Nakayama, T., Tanaka, T., Masuji, H. & Kimura, I. (1977) Establishment of an Epstein-Barr virus-negative B-cell lym-phoma line from a Japanese Burkitt's lymphoma and its serial passage in hamsters. *Cancer*, **40**, 2999–3003

Miyoshi, I., Hiraki, S., Tsubota, T., Uno, J., Nakamura, K., Ota, T., Hikita, T., Hayashi, K., Kataoka, M., Tanaka, T., Kimura, I., Sairenji, T. & Hinuma, Y. (1978) Epstein-Barr virus-positive Japanese Burkitt lymphoma. *Gann*, **69**, 449–450

Mochanko, K., Fejes, M., Breazavscek, D.M., Suarez, A. & Bachmann, A.E. (1979) The relation between Epstein-Barr virus antibodies and clinical symptomatology and immunodeficiency in patients with Hodgkin's disease. *Cancer*, **44**, 2065–2070

Molineaux, L. & Gramiccia, G. (1980) *The Garki Project*, Geneva, World Health Organization

Montel, A.H., Morse, P.A. & Brahmi, Z. (1995) Upregulation of B7 molecules by the Epstein-Barr virus enhances susceptibility to lysis by a human NK-like cell line. *Cell Immunol.*, **160**, 104–114

Montone, K.T., Litzky, L.A., Wurster, A., Kaiser, L., Bavaria, J., Kotloff, R., Palevsky, H., Pietra, G.G. & Tomaszewski, J.E. (1996a) Analysis of Epstein-Barr virus-associated post-transplantation lymphoproliferative disorder after lung transplantation. *Surgery*, **119**, 544–551

Montone, K.T., Hodinka, R.L., Salhany, K.E., Lavi, E., Rostami, A. & Tomaszewski, J.E. (1996b) Identification of Epstein-Barr virus lytic activity in post-transplantation lympho-proliferative disease. *Mod. Pathol.*, **9**, 621–630

Moore, S.B., Pearson, G.R., Neel, H.B., III & Weiland, L.H. (1983) HLA and nasopharyngeal carcinoma in North American Caucasoids. *Tissue Antigens*, **22**, 72–75

Moore, M.D., DiScipio, R.G., Cooper, N.R. & Nemerow, G.R. (1989) Hydrodynamic, electron microscopic, and ligand-binding analysis of the Epstein-Barr virus/C3dg receptor (CR2). *J. biol. Chem.*, **264**, 20576–20582

Moore, K.W., Vieira, P., Fiorentino, D.F., Trounstine, M.L., Khan, T.A. & Mosmann, T.R. (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. *Science*, **248**, 1230–1234

Moorthy, R. & Thorley-Lawson, D.A. (1990) Processing of the Epstein-Barr virus-encoded latent membrane protein p63/LMP. *J. Virol.*, **64**, 829–837

Moorthy, R.K. & Thorley-Lawson, D.A. (1993a) All three domains of the Epstein-Barr virus-encoded latent membrane protein LMP-1 are required for transformation of rat-1 fibroblasts. *J. Virol.*, **67**, 1638–1646

Moorthy, R.K. & Thorley-Lawson, D.A. (1993b) Biochemical, genetic, and functional analyses of the phosphorylation sites on the Epstein-Barr virus-encoded oncogenic latent membrane protein LMP-1. *J. Virol.*, **67**, 2637–2645

Moran, C.A., Tuur, S., Angritt, P., Reid, A.H. & O'Leary, T.J. (1992) Epstein-Barr virus in Hodgkin's disease from patients with human immunodeficiency virus infection. *Mod. Pathol.*, **5**, 85–88

Morein, B., Sundquist, B., Höglund, S., Dalsgaard, K. & Osterhaus, A. (1984) Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. *Nature*, **308**, 457–460

Morein, B., Lövgren, K., Rönnberg, B., Sjölander, A. & Villacrés-Eriksson, M. (1995) Immuno-stimulating complexes. Clinical potential in vaccine development. *Clin. Immunother.*, **3**, 461–475

Morel, D., Merville, P., Le Bail, B., Berger, F., Saric, J. & Potaux, L. (1996) Epstein-Barr virus (EBV)-associated hepatic and splenic smooth muscle tumours after kidney transplantation. *Nephrol. Dial. Transplant.*, **11**, 1864–1866

Morgan, A.J., North, J.R. & Epstein, M.A. (1983) Purification and properties of the gp340 component of Epstein-Barr virus membrane antigen in an immunogenic form. *J. gen. Virol.*, **64**, 455–460

Morgan, A.J., Smith, A.R., Barker, R.N. & Epstein, M.A. (1984) A structural investigation of the Epstein-Barr (EB) virus membrane antigen glycoprotein, gp340. *J. gen. Virol.*, **65**, 397–404

Morgan, A.J., Finerty, S., Lovgren, K., Scullion, F.T. & Morein, B. (1988a) Prevention of Epstein-Barr (EB) virus-induced lymphoma in cottontop tamarins by vaccination with the EB virus envelope glycoprotein gp340 incorporated into immune-stimulating complexes. *J. gen. Virol.*, **69**, 2093–2096

Morgan, A.J., Mackett, M., Finerty, S., Arrand, J.R., Scullion, F.T. & Epstein, M.A. (1988b) Recombinant vaccinia virus expressing Epstein-Barr virus glycoprotein gp340 protects cottontop tamarins against EBV-induced malignant lymphomas. *J. med. Virol.*, **25**, 189–195

Morgan, A.J., Allison, A.C., Finerty, S., Scullion, F.T., Byars, N.E. & Epstein, M.A. (1989) Validation of a first-generation Epstein-Barr virus vaccine preparation suitable for human use. *J. med. Virol.*, **29**, 74–78

Morgan, S.M., Wilkinson, G.W.G., Floettmann, E., Blake, N. & Rickinson, A.B. (1996) A recombinant adenovirus expressing an Epstein-Barr virus (EBV) target antigen can selectively reactivate rare components of EBV cytotoxic T-lymphocyte memory *in vitro*. *J. Virol.*, **70**, 2394–2402

Morgello, S. (1992) Epstein-Barr and human immunodeficiency viruses in acquired immunodeficiency syndrome-related primary central nervous system lymphoma. *Am. J. Pathol.*, **141**, 441-450

Morgenlander, J.C. (1996) A syndrome of concurrent central and peripheral nervous system involvement due to Epstein-Barr virus infection. *Muscle Nerve*, **19**, 1037–1039

Mori, M., Watanabe, M., Tanaka, S., Mimori, K., Kuwano, H. & Sugimachi, K. (1994) Epstein-Barr virus-associated carcinomas of the esophagus and stomach. *Arch. Pathol. Lab. Med.*, **118**, 998–1001

Morrow, R.H., Jr (1985) Epidemiological evidence for the role of falciparum malaria in the pathogenesis of Burkitt's lymphoma. In: Lenoir, G., O'Conor, G. & Olweny, C.L.M., eds, *Burkitt's Lymphoma: A Human Cancer Model* (IARC Scientific Publications No. 60), Lyon, IARC, pp. 177–186

Morrow, R.H., Kisuule, A., Pike, M.C. & Smith, P.G. (1976) Burkitt's lymphoma in the Mengo districts of Uganda: Epidemiologic features and their relationship to malaria. *J. natl Cancer Inst.*, **56**, 479–483

Mosialos, G., Yamashiro, S., Baughman, R.W., Matsudaira, P., Vara, L., Matsumura, F., Kieff, E. & Birkenbach, M. (1994) Epstein-Barr virus infection induces expression in B lymphocytes of a novel gene encoding an evolutionarily conserved 55-kilodalton actin-bundling protein. *J. Virol.*, **68**, 7320–7328

Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArsdale, T., Ware, C. & Kieff, E. (1995) The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. *Cell*, **80**, 389–399

Mosier, D.E. (1996) Viral pathogenesis in hu-PBL-SCID mice. *Sem. Immunol.*, **8**, 255–262

Mosmann, T.R. & Coffman, R.L. (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol.*, **46**, 111–147

Moss, B. (1996) Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. *Proc. natl Acad. Sci. USA*, **93**, 11341–11348

Moss, D.J., Rickinson, A.B. & Pope, J.H. (1978) Long-term T-cell-mediated immunity to Epstein-Barr virus in man. I. Complete regression of virus-induced transformation in cultures of seropositive donor leukocytes. *Int. J. Cancer*, **22**, 662–668

Moss, D.J., Rickinson, A.B. & Pope, J.H. (1979) Long-term T-cell-mediated immunity to Epstein-Barr virus in man. III. Activation of cytotoxic T cells in virus-infected leukocyte cultures. *Int. J. Cancer*, **23**, 618–625

Moss, D.J., Burrows, S.R., Castelino, D.J., Kane, R.G., Pope, J.H., Rickinson, A.B., Alpers, M.P. & Heywood, P.F. (1983a) A comparison of Epstein-Barr virus-specific T-cell immunity in malaria-endemic and nonendemic regions of Papua New Guinea. *Int. J. Cancer*, **31**, 727–732

Moss, D.J., Chan, S.H., Burrows, S.R., Chew, T.S., Kane, R.G., Staples, J.A. & Kunaratnam, N. (1983b) Epstein-Barr virus specific T-cell response in nasopharyngeal carcinoma patients. *Int. J. Cancer*, **32**, 301–305

Moss, D.J., Bishop, C.J., Burrows, S.R. & Ryan, J.M. (1985) T Lymphocytes in infectious mononucleosis. I. T Cell death *in vitro*. *Clin. exp. Immunol.*, **60**, 61–69

Moss, D.J., Misko, I.S., Burrows, S.R., Burman, K., McCarthy, R. & Sculley, T.B. (1988) Cytotoxic T-cell clones discriminate between A- and B-type Epstein-Barr virus transformants. *Nature*, **331**, 719–721

Moss, D.J., Schmidt, C., Elliott, S., Suhrbier, A., Burrows, S. & Khanna, R. (1996) Strategies involved in developing an effective vaccine for EBV-associated diseases. *Adv. Cancer Res.*, **69**, 213–245

Motz, M., Deby, G., Jilg, W. & Wolf, H. (1986) Expression of the Epstein-Barr virus major membrane proteins in Chinese hamster ovary cells. *Gene*, **44**, 353–359

Mselati, J.C., Buriot, D. & Nezelof, C. (1983) Primary immunologic deficiences and cancer. Five anatomo-clinical case reports. *Arch. fr. Pediatr.*, **40**, 163–169

Mueller, N.E. (1987) The epidemiology of Hodgkin's disease. In: Selby, P. & McElwain, T.J., eds, *Hodgkin's Disease*, Oxford, Blackwell Scientific Publications, pp. 68–93

Mueller, N.E. (1996) Hodgkin's disease. In: Schottenfeld, D. & Fraumeni, J.F., Jr, eds, *Cancer Epidemiology and Prevention*, 2nd Ed., New York, Oxford University Press, pp. 893–919

Mueller, N.E. (1997) Epstein-Barr virus and Hodgkin's disease: An epidemiological paradox. *Epstein-Barr Virus Rep.*, **4**, 1–2

Mueller, N.E., Evans, A., Harris, N.L., Comstock, G.W., Jellum, E., Magnus, K., Orentreich, N., Polk, B.F. & Vogelman, J. (1989) Hodgkin's disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. *New Engl. J. Med.*, **320**, 689–695

Mueller, N.E., Mohar, A., Evans, A., Harris, N.L., Comstock, G.W., Jellum, E., Magnus, K., Orentreich, N., Polk, B.F. & Vogelman, J. (1991) Epstein-Barr virus antibody patterns preceding the diagnosis of non-Hodgkin's lymphoma. *Int. J. Cancer*, **49**, 387-393

Mueller, B.U., Butler, K.M., Higham, M.C., Husson, R.N., Montrella, K.A., Pizzo, P.A., Feuerstein, I.M. & Manjunath, K. (1992) Smooth muscle tumors in children with human immunodeficiency virus infection. *Pediatrics*, **90**, 460–463

Mueller-Lantzsch, N., Georg, B., Yamamoto, N. & zur Hausen, H. (1980) Epstein-Barr virus-induced proteins. III. Analysis of polypeptides from P3HR-1-EBV-superinfected NC37 cells by immunoprecipitation. *Virology*, **102**, 231–233

Muir, C.S. (1971) Nasopharyngeal carcinoma in non-Chinese populations with special reference to south-east Asia and Africa. *Int. J. Cancer*, **8**, 351–363

Muir, C.S. & Shanmugaratnam, K. (1967) *Cancer of the Nasopharynx* (UICC Monograph Series No. 1), New York, Medical Examination Publishing Co.

Muir, C., Waterhouse, J., Mack, T., Powell, J. & Whelan, S., eds (1987) *Cancer Incidence in Five Continents, Vol. V* (IARC Scientific Publications No. 88), Lyon, IARC

Muñoz, N., Davidson, R.J.L., Witthoff, B., Ericsson, J.E. & de Thé, G. (1978) Infectious mononucleosis and Hodgkin's disease. *Int. J. Cancer*, **22**, 10–13

Muñoz, A., Schrager, L.K., Bacellar, H., Speizer, I., Vermund, S.H., Detels, R., Saah, A.J., Kingsley, L.A., Seminara, D. & Phair, J.P. (1993) Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am. J. Epidemiol.*, **137**, 423–438

Murphy, F.A. (1989) The promise and challenge of vaccinia-vectored vaccines. *Res. Virol.*, **140**, 463–465

Murphy, J.K., Young, L.S., Bevan, I.S., Lewis, F.A., Dockey, D., Ironside, J.W., O'Brien, C.J. & Wells, M. (1990) Demonstration of Epstein-Barr virus in primary brain lymphoma by in situ DNA hybridization in paraffin wax embedded tissue. *J. clin Pathol.*, **43**, 220-223

Murray, R.J., Kurilla, M.G., Brooks, J.M., Thomas, W.A., Rowe, M., Kieff, E. & Rickinson, A.B. (1992a) Identification of target antigens for the human cytotoxic T-cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. *J. exp. Med.*, **176**, 157–168

Murray, P.G., Young, L.S., Rowe, M. & Crocker, J. (1992b) Immunohistochemical demonstration of the Epstein-Barr virus-encoded latent membrane protein in paraffin sections of Hodgkin's disease. *J. Pathol.*, **166**, 1–5

Murray, P.G., Niedobitek, G., Kremmer, E., Grässer, F., Reynolds, G.M., Cruchley, A., Williams, D.M., Müller-Lantzsch, N. & Young, L.S. (1996) In situ detection of the Epstein-Barr virus-encoded nuclear antigen 1 in oral hairy leukoplakia and virus-associated carcinomas. *J. Pathol.*, **178**, 44–47

Myers, J.L., Kurtin, P.J., Katzenstein, A.-L.A., Tazelaar, H.D., Colby, T.V., Strickler, J.G., Lloyd, R.V. & Isaacson, P.G. (1995) Lymphomatoid granulomatosis. Evidence of immunophenotypic diversity and relationship to Epstein-Barr virus infection. *Am. J. surg. Pathol.*, **19**, 1300-1312

Nagao, T., Ishida, Y., Sugano, I., Tajima, Y., Masuzaki, O., Hino, T., Konno, A., Kondo, Y. & Nagao, K. (1996) Epstein-Barr virus-associated undifferentiated carcinoma with lymphoid stroma of the salivary gland in Japanese patients: Comparison with benign lymphoepithelial lesion. *Cancer*, **78**, 695–703

Nakagomi, H., Dolcetti, R., Bejarano, M.T., Pisa, P., Kiessling, R. & Masucci, M.G. (1994) The Epstein-Barr virus latent membrane protein-1 (LMP1) induces interleukin-10 production in Burkitt lymphoma lines. *Int. J. Cancer*, **57**, 240–244

Nakamine, H., Okano, M., Taguchi, Y., Pirruccello, S.J., Davis, J.R., Beysel, K.W., Kleveland, K., Sanger, W.G., Fordyce, R.R. & Purtilo, D.T. (1991) Hematopathologic features of Epstein-Barr virus-induced human B-lymphoproliferation in mice with severe combined immunodeficiency. A model of lymphoproliferative disease in immunocompromised patients. *Lab. Invest.*, **65**, 389–399

Nakanishi, M., Kikuta, H., Tomizawa, K., Kojima, K., Ishizaka, A., Okano, M., Sakiyama, Y. & Matsumoto, S. (1993) Distinct clonotypic Epstein-Barr virus-induced fatal lymphoproliferative disorder in a patient with Wiskott-Aldrich syndrome. *Cancer*, **72**, 1376–1381

Nakhleh, R.E., Manivel, J.C., Copenhaver, C.M., Sung, J.H. & Strickler, J.G. (1991) In situ hybridization for the detection of Epstein-Barr virus in central nervous system lymphomas. *Cancer*, **67**, 444–448

Nalesnik, M.A. & Starzl, T.E. (1994) Epstein-Barr virus, infectious mononucleosis, and post-transplant lymphoproliferative disorders. *Transplant. Sci.*, **4**, 61–79

Nalesnik, M.A, Locker, J., Jaffe, R., Reyes, J., Cooper, M., Fung, J. & Starzl, T.E. (1992) Experience with posttransplant lymphoproliferative disorders in solid organ transplant recipients. *Clin. Transplant.*, **6**, 249–252

Nam, J.-M., McLaughlin, J.K. & Blot, W.J. (1992) Cigarette smoking, alcohol, and nasopharyngeal carcinoma: A case–control study among US whites. *J. natl Cancer Inst.*, **84**, 619–622

ten Napel, C.H.H., van Egten-Bijker, J., Halie, M.R., Langenhuysen, M.M.A.C. & The, T.H. (1980) Cytomegalovirus-specific immune response and the cellular immune status of malignant lymphoma. *J. clin. lab. Immunol.*, **4**, 145–151

Nash, A.A., Usherwood, E.J. & Stewart, J.P. (1996) Immunological features of murine gammaherpesvirus infection. *Sem. Virol.*, **7**, 125–130

Nasrin, N., Taiba, K., Hannan, N., Hannan, M. & al-Sedairy, S. (1994) A molecular study of EBV DNA and *p53* mutations in nasopharyngeal carcinoma of Saudi Arab patients. *Cancer Lett.*, **82**, 189–198

National Cancer Control Office, Nanjing Institute of Geography (1979) *Atlas of Cancer Mortality in the People's Republic of China*, Shanghai, China Map Press

National Cancer Control Office (1980) *Summary Data of the Cancer Mortality Survey in the People's Republic of China*, Beijing, Ministry of Public Health

Neri, A., Barriga, F., Inghirami, G., Knowles, D.M., Neequaye, J., Magrath, I.T. & Dalla-Favera, R. (1991) Epstein-Barr virus infection precedes clonal expansion in Burkitt's and acquired immunodeficiency syndrome-associated lymphoma. *Blood*, **77**, 1092-1095

Neubauer, R.H., Rabin, H., Strand, B.C., Nonoyama, M. & Nelson-Rees, W.A. (1979a) Establishment of a lymphoblastoid cell line and isolation of an Epstein-Barr virus-related virus of gorilla origin. *J. Virol.*, **31**, 845–848

Neubauer, R.H., Rabin, H., Strand, B.C., Lapin, B.A., Yakovleva, L.A. & Indzie, E. (1979b) Antibody responses to Herpesvirus papio antigens in baboons with lymphoma. *Int. J. Cancer*, **23**, 186–192

Nevo, S., Meyer, W. & Altman, M. (1971) Carcinoma of nasopharynx in twins. *Cancer*, **28**, 807–809

Nicholls, J.M., Pittaluga, S., Chung, L.P. & So, K.C. (1994) The association between carcinoma of the tonsil and Epstein-Barr virus — A study using radiolabelled in situ hybridization. *Pathology*, **26**, 94–98

Nicholls, J.M., Agathanggelou, A., Fung, K., Zeng, X. & Niedobitek, G. (1997) The association of squamous cell carcinomas of the nasopharynx with Epstein-Barr virus shows geographic variation reminiscent of Burkitt's lymphoma. *J. Pathol.* (in press)

Nicholson, A.G., Wotherspoon, A.C., Diss, T.C., Singh, N., Butcher, D.N., Pan, L.X., Isaacson, P.G. & Corrin, B. (1996) Lymphomatoid granulomatosis: Evidence that some cases represent Epstein-Barr virus-associated B-cell lymphoma. *Histopathology*, **29**, 317-324

Niederman, J.C., McCollum, R.W., Henle, G. & Henle, W. (1968) Infectious mononucleosis. *J. Am. med. Assoc.*, **203**, 139–143

Niedobitek, G., Finn, T., Herbst, H. & Stein, H. (1989a) Detection of viral genomes in the liver by in situ hybridization using ^{35}S-, bromodeoxyuridine-, and biotin-labeled probes. *Am. J. Pathol.*, **134**, 633–639

Niedobitek, G., Hamilton-Dutoit, S., Herbst, H., Finn, T., Vetner, M., Pallesen, G. & Stein, H. (1989b) Identification of Epstein-Barr virus-infected cells in tonsils of acute infectious mononucleosis by in situ hybridization. *Hum. Pathol.*, **20**, 796–799

Niedobitek, G., Young, L.S., Lau, R., Brooks, L., Greenspan, D., Greenspan, J.S. & Rickinson, A.B. (1991a) Epstein-Barr virus infection in oral hairy leukoplakia: Virus replication in the absence of a detectable latent phase. *J. gen. Virol.*, **72**, 3035–3046

Niedobitek, G., Hansmann, M.L., Herbst, H., Young, L.S., Dienemann, D., Hartmann, C.A., Finn, T., Pitteroff, S., Welt, A., Anagnostopoulos, I., Friedrich, R., Lobeck, H., Sam, C.K., Araujo, I., Rickinson, A.B. & Stein, H. (1991b) Epstein-Barr virus and carcinomas: Undifferentiated carcinomas but not squamous cell carcinomas of the nasopharynx are regularly associated with the virus. *J. Pathol.*, **165**, 17–24

Niedobitek, G., Herbst, H., Young, L.S., Brooks, L., Masucci, M.G., Crocker, J., Rickinson, A.B. & Stein, H. (1992a) Patterns of Epstein-Barr virus infection in non-neoplastic lymphoid tissue. *Blood*, **79**, 2520–2526

Niedobitek, G., Herbst, H., Young, L.S., Rowe, M., Dienemann, D., Germer, C. & Stein, H. (1992b) Epstein-Barr virus and carcinomas. Expression of the viral genome in an undifferentiated gastric carcinoma. *Diagn. mol. Pathol.*, **1**, 103–108

Niedobitek, G., Young, L.S., Sam, C.K., Brooks, L., Prasad, U. & Rickinson, A.B. (1992c) Expression of Epstein-Barr virus genes and of lymphocyte activation molecules in undifferentiated nasopharyngeal carcinomas. *Am. J. Pathol.*, **140**, 879–887

Niedobitek, G., Fahraeus, R., Herbst, H., Latza, U., Ferszt, A., Klein, G. & Stein, H. (1992d) The Epstein-Barr virus encoded membrane protein (LMP) induces phenotypic changes in epithelial cells. *Virchows Arch. B Cell Pathol.*, **62**, 55–59

Niedobitek, G., Rowlands, D.C., Young, L.S., Herbst, H., Williams, A., Hall, P., Padfield, J., Rooney, N. & Jones, E.L. (1993a) Overexpression of p53 in Hodgkin's disease: Lack of correlation with Epstein-Barr virus infection. *J. Pathol.*, **169**, 207–212

Niedobitek, G., Agathanggelou, A., Barber, P., Smallman, L.A., Jones, E.L. & Young, L.S. (1993b) p53 Overexpression and Epstein-Barr virus infection in undifferentiated and squamous cell nasopharyngeal carcinomas. *J. Pathol.*, **170**, 457–461

Niedobitek, G., Agathanoggelou, A., Finerty, S., Tierney, R., Watkins, P., Jones, E.L., Morgan, A., Young, L.S. & Rooney, N. (1994) Latent Epstein-Barr virus infection in cottontop tamarins: A possible model for Epstein-Barr virus infection in humans. *Am. J. Pathol.*, **145**, 969–978

Niedobitek, G., Agathanggelou, A., Rowe, M., Jones, E.L., Jones, D.B., Turyaguma, P., Oryema, J., Wright, D.H. & Young, L.S. (1995) Heterogeneous expression of Epstein-Barr virus latent proteins in endemic Burkitt's lymphoma. *Blood*, **86**, 659–665

Niedobitek, G., Kremmer, E., Herbst, H., Whitehead, L., Dawson, C.W., Niedobitek, E., von Ostau, C., Rooney, N., Grässer, F.A. & Young, L.S. (1997a) Immunohistochemical detection of the Epstein-Barr virus-encoded latent membrane protein 2A (LMP2A) in Hodgkin's disease and infectious mononucleosis. *Blood*, **90**, 1664–1672

Niedobitek, G., Agathanggelou, A., Herbst, H,. Whitehead, L., Wright, D.H. & Young, L.S. (1997b) Epstein-Barr virus (EBV) infection in infectious mononucleosis: virus latency, replication and phenotype of EBV-infected cells. *J. Pathol.*, **182**, 151–159

Niedobitek, G., Mutimer, D.J., Williams, A., Whitehead, L., Wilson, P., Rooney, N., Young, L.S. & Hübscher, S.G. (1997c) Epstein-Barr virus infection and malignant lymphomas in liver transplant recipients. *Int. J. Cancer* (in press)

Ning, J.-P., Yu, M.C., Wang, Q.-S. & Henderson, B.E. (1990) Consumption of salted fish and other risk factors for nasopharyngeal carcinoma (NPC) in Tianjin, a low-risk region for NPC in the People's Republic of China. *J. natl Cancer Inst.*, **82**, 291–296

Nkrumah, F.K. (1984) Changes in the presentation of Burkitt's lymphoma in Ghana over a 15-year period (1969–82). In: Williams, A.O., O'Conor, G.T., de Thé, G.B. & Johnson, C.A., eds, *Virus-associated Cancers in Africa* (IARC Scientific Publications No. 63), Lyon, IARC, pp. 665–674

Nkrumah, F.K. & Perkins, I.V. (1976) Sickle cell trait, hemoglobin C trait, and Burkitt's lymphoma. *Am. J. trop. Med. Hyg.*, **25**, 633–636

Nkrumah, F., Henle, W., Henle, G., Herberman, R., Perkins, V. & Depue, R. (1976) Burkitt's lymphoma: Its clinical course in relation to immunologic reactivities to Epstein-Barr virus and tumor-related antigens. *J. natl Cancer Inst.*, **57**, 1051–1056

Nolan, L.A. & Morgan, A.J. (1995) The Epstein-Barr virus open reading frame BDLF3 codes for a 100–150 kDa glycoprotein. *J. gen. Virol.*, **76**, 1381–1392

Nonkwelo, C.B. & Long, W.K. (1993) Regulation of Epstein-Barr virus *Bam*HI-H divergent promoter by DNA methylation. *Virology*, **197**, 205–215

Nonkwelo, C., Henson, E.B.D. & Sample, J. (1995) Characterization of the Epstein-Barr virus Fp promoter. *Virology*, **206**, 183–195

Nonkwelo, C., Skinner, J., Bell, A., Rickinson, A. & Sample, J. (1996) Transcription start sites downstream of the Epstein-Barr virus (EBV) Fp promoter in early-passage Burkitt lymphoma cells define a fourth promoter for expression of the EBV EBNA-1 protein. *J. Virol.*, **70**, 623–627

Nonkwelo, C., Ruf, I.K. & Sample, J. (1997) The Epstein-Barr virus EBNA-1 promoter Qp requires an initiator-like element. *J. Virol.*, **71**, 354–361

Nonoyama, M. & Pagano, J.S. (1971) Detection of Epstein-Barr viral genome in nonreproductive cells. *Nature new Biol.*, **233**, 103–106

Nonoyama, M. & Pagano, J.S. (1973) Homology between Epstein-Barr virus DNA and viral DNA from Burkitt's lymphoma and nasopharyngeal carcinoma determined by DNA–DNA reassociation kinetics. *Nature*, **242**, 44–47

Nonoyama, M., Huang, C.H., Pagano, J.S., Klein, G. & Singh, S. (1973) DNA of Epstein-Barr virus detected in tissue of Burkitt's lymphoma and nasopharyngeal carcinoma. *Proc. natl Acad. Sci. USA*, **70**, 3265–3268

North, J.R., Morgan, A.J. & Epstein, M.A. (1980) Observations on the EB virus envelope and virus-determined membrane antigen (MA) polypeptides. *Int. J. Cancer*, **26**, 231–240

North, J.R., Morgan, A.J., Thompson, J.L. & Epstein, M.A. (1982) Purified Epstein-Barr virus M_r 340,000 glycoprotein induces potent virus-neutralizing antibodies when incorporated in liposomes. *Proc. natl Acad. Sci. USA*, **79**, 7504–7508

Nuebling, C.M. & Mueller-Lantzsch, N. (1991) Identification of the gene product encoded by the *Pst*I repeats (IR4) of the Epstein-Barr virus genome. *Virology*, **185**, 519–523

Nuebling, C.M., Buck, M., Boos, H., von Deimling, A. & Mueller-Lantzsch, N. (1992) Expression of Epstein-Barr virus membrane antigen gp350/220 in *E. coli* and in insect cells. *Virology*, **191**, 443–447

Nutting, P.A., Freeman, W.L., Risser, D.R., Helgerson, S.D., Paisano, R., Hisnanick, J., Beaver, S.K., Peters, I., Carney, J.P. & Speers, M.A. (1993) Cancer incidence among American Indians and Alaska natives, 1980 through 1987. *Am. J. public Health*, **83**, 1589–1598

Oba, D.E. & Hutt-Fletcher, L.M. (1988) Induction of antibodies to the Epstein-Barr virus glycoprotein gp85 with a synthetic peptide corresponding to a sequence in the BXLF2 open reading frame. *J. Virol.*, **62**, 1108–1114

O'Conor, G. (1961) Malignant lymphoma in African children. Cancer. II. A pathological entity. *Cancer*, **14**, 270–283

O'Conor, G.T. & Davies, J.N.P. (1960) Malignant tumors in African children with special reference to malignant lymphomas. *J. Pediatr.*, **56**, 526–535

O'Conor, G., Rappaport, H. & Smith, E.B. (1965) Childhood lymphoma resembling Burkitt's tumor in the United States. *Cancer*, **18**, 411–417

Oda, K., Tamaru, J., Takenouchi, T., Mikata, A., Nunomura, M., Saitoh, N., Sarashina, H. & Nakajima, N. (1993) Association of Epstein-Barr virus with gastric carcinoma with lymphoid stroma. *Am. J. Pathol.*, **143**, 1063–1071

Odeku, E.L., Adeloye, A. & Osuntokun, B.O. (1973) The neurological picture of Burkitt's lymphoma in Ibadan. *Afr. J. med. Sci.*, **4**, 119–126

O'Grady, J., Stewart, S., Elton, R.A. & Krajewski, A.S. (1994) Epstein-Barr virus in Hodgkin's disease and site of origin of tumour. *Lancet*, **343**, 265–266

Ohno, S., Luka, J., Lindahl, T. & Klein, G. (1977) Identification of a purified complement-fixing antigen as the Epstein-Barr-virus determined nuclear antigen (EBNA) by its binding to metaphase chromosomes. *Proc. natl Acad. Sci. USA*, **74**, 1605–1609

Ohno, S., Luka, J., Falk, L.A. & Klein, G. (1978) Serological reactivities of human and baboon sera against EBNA and *Herpesvirus papio*-determined nuclear antigen (HUPNA). *Eur. J. Cancer*, **78**, 954–960

Ohshima, K., Kikuchi, M., Eguchi, F., Masuda, Y., Sumiyoshi, Y., Mohtai, H., Takeshita, M. & Kimura, N. (1990) Analysis of Epstein-Barr viral genomes in lymphoid malignancy using Southern blotting, polymerase chain reaction and in situ hybridization. *Virchows Arch. B*, **59**, 383–390

Ohshima, K., Kikuchi, M., Masuda, Y., Sumiyoshi, Y., Eguchi, F., Mohtai, H., Takeshita, M. & Kimura, N. (1991) Epstein-Barr viral genomes in carcinoma metastatic to lymph nodes. Association with nasopharyngeal carcinoma. *Acta. pathol. jpn.*, **41**, 437–443

Okan, I., Wang, Y., Chen, F., Hu, L.F., Imreh, S., Klein, G. & Wiman, K.G. (1995) The EBV-encoded LMP1 protein inhibits p53-triggered apoptosis but not growth arrest. *Oncogene*, **11**, 1027–1031

Okano, M., Mizuno, F., Osato, T., Takahashi, Y., Sakiyama, Y. & Matsumoto, S. (1984) Wiskott-Aldrich syndrome and Epstein-Barr virus-induced lymphoproliferation (Letter to the Editor). *Lancet*, **ii**, 933–934

Okano, M., Taguchi, Y., Nakamine, H., Pirruccello, S.J., Davis, J.R., Belisel, K.W., Kleveland, K.L., Sanger, W.G., Fordyce, R.R. & Purtilo, D.T. (1990) Characterization of Epstein-Barr virus-induced lymphoproliferation derived from human peripheral blood mononuclear cells transferred to severe combined immunodeficient mice. *Am. J. Pathol.*, **137**, 517–522

Okano, M., Kikuta, H., Abo, W., Koizumi, S., Aya, T., Yano, S., Takada, K., Mizuno, F. & Osato, T. (1992) Frequent association of Epstein-Barr virus in Japanese patients with Burkitt's lymphoma. *Jpn. J. clin. Oncol.*, **22**, 320–324

Okazaki, W., Purchase, H.G. & Burmester, B.R. (1970) Protection against Marek's disease by vaccination with a herpesvirus of turkeys. *Avian Dis.*, **14**, 413–429

Old, L.J., Boyse, E.A., Oettgen, H.P., de Harven, E., Geering, G., Williamson, B. & Clifford, P. (1966) Precipitating antibody in human serum to an antigen present in cultured Burkitt lymphoma cell. *Proc. natl Acad. Sci. USA*, **56**, 1699–1704

O'Leary, G. & Kennedy, S.M. (1995) Association of Epstein-Barr virus with sinonasal angiocentric T- cell lymphoma. *J. clin. Pathol.*, **48**, 946-949

Olisa, E.G., Kovi, J., Kennedy, J., Kish, M.H., Lanava, T.S. & Williams, A.O. (1976) Hodgkin's disease in American Negroes: Histologic classification of the disease in 143 untreated patients, and age distribution. *Am. J. clin. Pathol.*, **66**, 537–544

Olsen, J.H., Plough Jensen, S., Hink, M., Faurbo, K., Breum, N.O. & Møller-Jensen, O.M. (1984) Occupational formaldehyde exposure and increased nasal cancer risk in man. *Int. J. Cancer*, **34**, 639–644

Olurin, O. & Williams, A.O. (1972) Orbito-ocular tumors in Nigeria. *Cancer*, **30**, 580–587

Olweny, C.L.M., Atine, I., Kaddu-Mukasa, A., Owor, R., Andersson-Anvret, M., Klein, G., Henle, W. & de Thé, G. (1977) Epstein-Barr virus genome studies in Burkitt's and non-Burkitt's lymphomas in Uganda. *J. natl Cancer Inst.*, **58**, 1191–1196

Ono, K., Tanabe, S., Naito, M., Doi, T. & Kato, S. (1970) Antigen common to a herpes type virus from chickens with Marek's disease and EB virus from Burkitt's lymphoma cells. *Biken J.*, **13**, 213–217

Ooi, E.E., Ren, E.C. & Chan, S.H. (1997) Association between microsatellites within the human MHC and nasopharyngeal carcinoma. *Int. J. Cancer (Prev. Oncol.)*, **74**, 229–232

Opelz, G. & Henderson, R. (1993) Incidence of non-Hodgkin lymphoma in kidney and heart transplant recipients. *Lancet*, **342**, 1514–1516

Opstelten, D. & Osmond, D.G. (1983) Pre-B cells in the mouse bone marrow: Immunofluorescence stathmokinetic studies of the proliferation of cytoplasmic μ-chain-bearing cells in normal mice. *J. Immunol.*, **131**, 2635–2640

Orlowski, R. & Miller, G. (1991) Single-stranded structures are present within plasmids containing the Epstein-Barr virus latent origin of replication. *J. Virol.*, **65**, 677–686

Osato, T. & Imai, S. (1996) Epstein-Barr virus and gastric carcinoma. *Sem. Cancer Biol.*, **7**, 175–182

Osato, T., Mizuno, F., Imai, S., Aya, T., Koizumi, S., Kinoshita, T., Tokuda, H., Ito, Y., Hirai, N., Hirota, M., Ohigashi, H., Koshimizu, K., Kofi-Tsekpo, W.M., Were, J.B.O. & Mugambi, M. (1987) African Burkitt's lymphoma and an Epstein-Barr virus-enhancing plant Eurphorbia tirucalli (Letter to the Editor). *Lancet*, **i**, 1257–1258

Osato, T., Imai, S., Kinoshita, T., Aya, T., Sugiura, M., Koizumi, S. & Mizuno, F. (1990) Epstein Barr virus, Burkitt's lymphoma, and an African tumor promoter. *Adv. exp. Med. Biol.*, **278**, 147–150

Osmond, D.G., Priddle, S. & Rico-Vargas, S. (1990) Proliferation of B cell precursors in bone marrow of pristane-conditioned and malaria-infected mice. Implications for B cell oncogenesis. *Curr. Top. Microbiol. Immunol.*, **166**, 149–157

Osuntokun, B.O., Osuntokun, O., Adeloye, A. & Odeku, E.L. (1973) Primary neuro-ophthalmological presentation of Burkitt's lymphoma. *Afr. J. med. Sci.*, **4**, 111–117

Ott, G., Ott, M.M., Feller, A.C., Seidl, S. & Müller-Hermelink, H.K. (1992) Prevalence of Epstein-Barr virus DNA in different T-cell lymphoma entities in a European population. *Int. J. Cancer*, **51**, 562-567

Ott, G., Kirchner, T. & Müller-Hermelink, H.K. (1994) Monoclonal Epstein-Barr virus genomes but lack of EBV-related protein expression in different types of gastric carcinoma. *Histopathology*, **25**, 323–329

Oudejans, J.J., van den Brule, A.J., Jiwa, N.M., de Bruin, P.C., Ossenkoppele, G.J., van der Valk, P., Walboomers, J.M. & Meijer, C.J. (1995a) BHRF1, the Epstein-Barr virus (EBV) homologue of the bcl-2 protooncogene, is transcribed in EBV-associated B-cell lymphomas and in reactive lymphocytes. *Blood*, **86**, 1893–1902

Oudejans, J.J., Jiwa, M., van den Brule, A.J.C., Grässer, F.A., Horstman, A., Vos, W., Kluin, P.M., van der Valk, P., Walboomers, J.M.M. & Meijer, C.J.L.M. (1995b) Detection of heterogeneous Epstein-Barr virus gene expression patterns within individual post-transplantation lymphoproliferative disorders. *Am. J. Pathol.*, **147**, 923–933

Packham, G., Brimmell, M., Cook, D., Sinclair, A.J. & Farrell, P.J. (1993) Strain variation in Epstein-Barr virus immediate early genes. *Virology*, **192**, 541–550

Pagano, J.S., Huang, C.H. & Levine, P. (1973) Absence of Epstein-Barr viral DNA in American Burkitt's lymphoma. *New Engl. J. Med.*, **289**, 1395–1399

Pagano, J.S., Huang, C.-H., Klein, G., de Thé, G., Shanmugaratnam, K. & Yang, C.-S. (1975) . Homology of Epstein-Barr viral DNA in nasopharyngeal carcinomas from Kenya, Taiwan, Singapore and Tunisia. In: de Thé, G., Epstein, M.A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II* (IARC Scientific Publications No. 11), Lyon, IARC, pp. 179–190

Palazzo, J.P., Lundquist, K., Mitchell, D., Mittal, K.R., Hann, H.-W.L., Munoz, S., Moritz, M.J., Jacobs, J.M. & Martin, P. (1993) Rapid development of lymphoma following liver transplantation in a recipient with hepatitis B and primary hemochromatosis. *Am. J. Gastroenterol.*, **88**, 102–104

Pallesen, G., Hamilton-Dutoit, S.J., Rowe, M. & Young, L.S. (1991a) Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet*, **337**, 320–322

Pallesen, G., Hamilton-Dutoit, S.J., Rowe, M., Lisse, I., Ralfkiaer, E., Sandvej, K. & Young, L.S. (1991b) Expression of Epstein-Barr virus replicative proteins in AIDS-related non-Hodgkin's lymphoma cells. *J. Pathol.*, **165**, 289–299

Pallesen, G., Sandvej, K., Hamilton-Dutoit, S.J., Rowe, M. & Young, L.S. (1991c) Activation of Epstein-Barr virus replication in Hodgkin and Reed-Sternberg cells. *Blood*, **78**, 1162–1165

Pallesen, G., Hamilton-Dutoit, S.J. & Zhou, X. (1993) The association of Epstein-Barr virus (EBV) with T cell lymphoproliferations and Hodgkin's disease: Two new developments in the EBV field. *Adv. Cancer Res.*, **62**, 179–239

Pan, L., Diss, T.C., Peng, H., Lu, Q., Wotherspoon, A.C., Thomas, J.A. & Isaacson, P.G. (1993) Epstein-Barr virus (EBV) in enteropathy-associated T-cell lymphoma (EATL). *J. Pathol.*, **170**, 137–143

Papadopoulos, E.B., Ladanyi, M., Emanuel, D., Mackinnon, S., Boulad, F., Carabasi, M.H., Castro-Malaspina, H., Childs, B.H., Gillio, A.P., Small, T.N., Young, J.W., Kernan, N.A. & O'Reilly, R.J. (1994) Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *New Engl. J. Med.*, **330**, 1185–1191

Parker, G.A., Crook, T., Bain, M., Sara, E.A., Farrell, P.J. &. Allday, M.J. (1996) Epstein-Barr virus nuclear antigen (EBNA)3C is an immortalizing oncoprotein with similar properties to adenovirus E1A and papillomavirus E7. *Oncogene*, **13**, 2541–2549

Parkin, D.M., ed. (1986) *Cancer Occurrence in Developing Countries* (IARC Scientific Publications No. 75), Lyon, IARC

Parkin, D.M. & Iscovich, J. (1997) Risk of cancer in migrants and their descendants in Israel: II. Carcinomas and germ-cell tumours. *Int. J. Cancer*, **70**, 654–660

Parkin, D.M., Muir, C.S., Whelan, S.L., Gao, Y.-T., Ferlay, J. & Powell, J., eds (1992) *Cancer Incidence in Five Continents*, Vol. VI (IARC Scientific Publications No. 120), Lyon, IARC

Parkin, D.M., Whelan, S.L., Ferlay, J., Raymond, L. & Young, J., eds (1997) *Cancer Incidence in Five Continents, Vol. VII* (IARC Scientific Publications No. 143), Lyon, IARC

Pastore, C., Carbone, A., Gloghini, A., Volpe, G., Saglio, G. & Gaidano, G. (1996) Association of 6q deletions with AIDS-related diffuse large cell lymphoma. *Leukemia*, **10**, 1051–1053

Patarroyo, M., Blazar, B., Pearson, G., Klein, E. & Klein, G. (1980) Induction of the EBV cycle in B-lymphocyte-derived lines is accompanied by increased natural killer (NK) sensitivity and the expression of EBV-related antigen(s) detected by the ADCC reaction. *Int. J. Cancer*, **26**, 365–371

Pathmanathan, R. (1997) Pathology. In: Chong, V.F.H. & Tsao, S.Y., eds, *Nasopharyngeal Carcinoma*, Singapore, Armour Publishing, pp. 6–13

Pathmanathan, R., Prasad, U., Chandrika, G., Sadler, R., Flynn, K. & Raab-Traub, N. (1995a) Undifferentiated, nonkeratinizing, and squamous cell carcinoma of the nasopharynx. Variants of Epstein-Barr virus-infected neoplasia. *Am. J. Pathol.*, **146**, 1355–1367

Pathmanathan, R., Prasad, U., Sadler, R., Flynn, K. & Raab-Traub, N. (1995b) Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. *New Engl. J. Med.*, **333**, 693–698

Patton, D.F., Wilkowski, C.W., Hanson, C.A., Shapiro, R., Gajl-Peczalska, K.J., Filipovich, A.H. & McClain, K.L. (1990) Epstein-Barr virus — Determined clonality in posttransplant lymphoproliferative disease. *Transplantation*, **49**, 1080–1084

Paul, C.C., Keller, J.R., Armpriester, J.M. & Baumann, M.A. (1990) Epstein-Barr virus transformed B lymphocytes produce interleukin-5. *Blood*, **75**, 1400–1403

Payne, S., Kernohan, N.M. & Walker, F. (1995) Absence of in situ hybridization evidence for latent- or lytic-phase Epstein-Barr virus infection of preinvasive squamous lesions of the cervix. *J. Pathol.*, **176**, 221–226

Pearson, G., Dewey, F., Klein, G., Henle, G. & Henle, W. (1970) Relation between neutralization of Epstein-Barr virus and antibodies to cell-membrane antigens induced by the virus. *J. natl Cancer Inst.*, **45**, 989–995

Pearson, G.R., Johansson, B. & Klein, G. (1978a) Antibody-dependent cellular cytotoxicity against Epstein-Barr virus-associated antigens in African patients with nasopharyngeal carcinoma. *Int. J. Cancer*, **22**, 120–125

Pearson, G.R., Coates, H.L., Neel, H.B., Levine, P., Ablashi, D. & Easton, J. (1978b) Clinical evaluation of EBV serology in American patients with nasopharyngeal carcinoma. In: de Thé, G. & Ito Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 439–448

Pearson, G.R., Qualtiere, L.F., Klein, G., Norin, T. & Bal, I.S. (1979) Epstein-Barr virus-specific antibody-dependent cellular cytotoxicity in patients with Burkitt's lymphoma. *Int. J. Cancer*, **24**, 402–406

Pearson, G.R., Vroman, B., Chase, B., Sculley, T., Hummel, M. & Kieff, E. (1983a) Identification of polypeptide components of the Epstein-Barr virus early antigen complex with monoclonal antibodies. *J. Virol.*, **47**, 193–201

Pearson, G.R., Weiland, L.H., Neel, H.B., III, Taylor, W., Earle, J., Mulroney, S.E., Goepfert, H., Lanier, A., Talvot, M.L., Pilch, B., Goodman, M., Huang, A., Levine, P.H., Hyams, V., Moran, E., Henle, G. & Henle, W. (1983b) Application of Epstein-Barr virus (EBV) serology to the diagnosis of North American nasopharyngeal carcinoma. *Cancer*, **51**, 260–268

Pearson, G.R., Luka, J., Petti, L., Sample, J., Birkenbach, M., Braun, D. & Kieff, E. (1987) Identification of an Epstein-Barr virus early gene encoding a second component of the restricted early antigen complex. *Virology*, **160**, 151–161

Pedersen, C., Gerstoft, J., Lundgren, J.D., Skinhøj, P., Bottzauw, J., Geisler, C., Hamilton-Dutoit, J., Thorsen, S., Lisse, I., Ralfkiaer, E. & Pallesen, G. (1991) HIV-associated lymphoma: Histopathology and association with Epstein-Barr virus genome related to clinical, immunological and prognostic features. *Eur. J. Cancer*, **27**, 1416–1423

Peh, S.C., Sandvej, K. & Pallesen, G. (1995) Epstein-Barr virus (EBV) in Malaysian upper-aerodigestive-tract lymphoma: Incidence and sub-type. *Int. J. Cancer*, **61**, 327–332

Pelicci, P.G., Knowles, D.M., Magrath, I. & Dalla-Favera, R. (1986) Chromosomal breakpoints and structural alterations of the c-myc locus differ in endemic and sporadic forms of Burkitt lymphoma. *Proc. natl Acad. Sci. USA*, **83**, 2984–2988

Peng, M. & Lundgren, E. (1992) Transient expression of the Epstein-Barr virus *LMP1* gene in human primary B cells induces cellular activation and DNA synthesis. *Oncogene*, **7**, 1775–1782

Perlmann, C., Saemundsen, A.K. & Klein, G. (1982) A fraction of Epstein-Barr virus virion DNA is methylated in and around the *Eco*RI-J fragment. *Virology*, **123**, 217–221

Pesano, R.L. & Pagano, J.S. (1986) Herpesvirus papio contains a plasmid origin of replication that acts in *cis* interspecies with Epstein-Barr virus trans-activating function. *J. Virol.*, **60**, 1159–1162

Petti, L. & Kieff, E. (1988) A sixth Epstein-Barr virus nuclear protein (EBNA3B) is expressed in latently infected growth-transformed lymphocytes. *J. Virol.*, **62**, 2173–2178

Petti, L., Sample, J., Wang, F. & Kieff, E. (1988) A fifth Epstein-Barr virus nuclear protein (EBNA3C) is expressed in latently infected growth-transformed lymphocytes. *J. Virol.*, **62**, 1330–1338

Petti, L., Sample, C. & Kieff, E. (1990) Subnuclear localization and phosphorylation of Epstein-Barr virus latent infection nuclear proteins. *Virology*, **176**, 563–574

Pflugfelder, S.C., Crouse, C.A., Monroy, D., Yen, M., Rowe, M. & Atherton, S.S. (1993) Epstein-Barr virus and the lacrimal gland pathology of Sjögren's syndrome. *Am. J. Pathol.*, **143**, 49–64

Pfüller, R. & Hammerschmidt, W. (1996) Plasmid-like replicative intermediates of the Epstein-Barr virus lytic origin of DNA replication. *J. Virol.*, **70**, 3423–3431

Philip, T. (1985) Burkitt's lymphoma in Europe. In: Lenoir, G.M., O'Conor, G.T. & Olweny, C.L.M., eds, *Burkitt's Lymphoma: A Human Cancer Model* (IARC Scientific Publications No. 60), Lyon, IARC, pp. 107–118

Pike, M.C., Williams, E.H. & Wright, B. (1967) Burkitt's tumour in the West Nile District, Uganda, 1961–65. *Br. med. J.*, **ii**, 395–399

Pike, M.C., Morrow, R.H., Kisuule, A. & Mafigiri, J. (1970) Burkitt's lymphoma and sickle cell trait. *Br. J. prev. soc. Med.*, **24**, 39–41

Pisani, P., Parkin, D.M., Muñoz, N. & Ferlay, J. (1997) Cancer and infection: Estimates of the attributable fraction in 1990. *Cancer Epidemiol. Biomarkers Prev.*, **6**, 387–400

Pither, R.J., Zhang, C.X., Shiels, C., Tarlton, J., Finerty, S. & Morgan, A.J. (1992a) Mapping of B-cell epitopes on the polypeptide chain of the Epstein-Barr virus major envelope glyco-protein and candidate vaccine molecule gp340. *J. Virol.*, **66**, 1246–1251

Pither, R.J., Nolan, L., Tarlton, J., Walford, J. & Morgan A.J. (1992b) Distribution of epitopes within the amino acid sequence of the Epstein-Barr virus major envelope glycoprotein, gp340, recognized by hyperimmune rabbit sera. *J. gen. Virol.*, **73**, 1409–1415

Pittaluga, S., Loke, S.L., So, K.C., Cheung, K.N. & Ma, L. (1992) Clonal Epstein-Barr virus in lymphoepithelioma-like carcinoma of the stomach: demonstration of viral genome by in situ hybridization and Southern blot analysis. *Mod. Pathol.*, **5**, 661–664

Pittaluga, S., Wong, M.P., Chung, L.P. & Loke, S.-L. (1993) Clonal Epstein-Barr virus in lymphoepithelioma-like carcinoma of the lung. *Am. J. surg. Pathol.*, **17**, 678–682

Poirier, S., Ohshima, H., de Thé, G., Hubert, A., Bourgade, M.C. & Bartsch, H. (1987) Volatile nitrosamine levels in common foods from Tunisia, south China and Greenland, high-risk areas for nasopharyngeal carcinoma (NPC). *Int. J. Cancer*, **39**, 293–296

Poirier, S., Bouvier, G., Malaveille, C., Ohshima, H., Shao, Y.M., Hubert, A., Zeng, Y., de Thé, G. & Bartsch, H. (1989) Volatile nitrosamine levels and genotoxicity of food samples from high-risk areas for nasopharyngeal carcinoma before and after nitrosation. *Int. J. Cancer*, **44**, 1088–1094

Polack, A., Delius, H., Zimber, U. & Bornkamm, G.W. (1984a) Two deletions in the Epstein-Barr virus genome of the Burkitt lymphoma nonproducer line Raji. *Virology*, **133**, 146–157

Polack, A., Hartl, G., Zimber, U., Freese, U.-K., Laux, G., Takaki, K., Hohn, B., Gissman, L. & Bornkamm, G.W. (1984b) Complete set of overlapping cosmid clones of M-ABA virus derived from nasopharyngeal carcinoma and its similarity to other Epstein-Barr virus iso-lates. *Gene*, **27**, 279–288

Polack, A., Feederle, R., Klobeck, G. & Hörtnagel, K. (1993) Regulatory elements in the immu-noglobulin kappa locus induce c-*myc* activation and the promoter shift in Burkitt's lym-phoma cells. *EMBO J.*, **12**, 3913–3920

Polack, A., Hörtnagel, K., Pajic, A., Christoph, B., Baier, B., Falk, M., Mautner, J., Geltinger, C., Bornkamm, G.W. & Kempkes, B. (1996) c-*myc* Activation renders proliferation of Epstein-Barr virus (EBV)-transformed cells independent of EBV nuclear antigen 2 and latent membrane protein 1. *Proc. natl Acad. Sci. USA*, **93**, 10411–10416

Polito, P., Cilia, A.M., Gloghini, A., Cozzi, M., Perin, T., De Paoli, P., Gaidano, G. & Carbone, A. (1995) High frequency of EBV association with non-random abnormalities of the chro-mosome region 1q21-25 in AIDS-related Burkitt's lymphoma-derived cell lines. *Int. J. Cancer*, **61**, 370–374

Polvino-Bodnar, M., Kiso, J. & Schaffer, P.A. (1988) Mutational analysis of Epstein-Barr virus nuclear antigen 1 (EBNA 1). *Nucleic Acids Res.*, **16**, 3415–3435

Pomponi, R., Cariati, R., Zancai, P., De Paoli, P., Rizzo, S., Tedeschi, R.M., Pivetta, B., De Vita, S., Boiocchi, M. & Dolcetti, R. (1996) Retinoids irreversibly inhibit in vitro growth of Epstein-Barr virus-immortalized B lymphocytes. *Blood*, **88**, 3147–3159

Pope, J.H., Horne, M.K. & Scott, W. (1968) Transformation of foetal human leukocytes *in vitro* by filtrates of a human leukaemic cell line containing herpes-like virus. *Int. J. Cancer*, **3**, 857–866

Pope, J.H., Horne, J.M.K. & Wetters, E.J. (1969) Significance of a complement-fixing antigen associated with herpes-like virus and detected in the Raji cell line. *Nature*, **222**, 186–187

Poppema, S. & Visser, L. (1994) Epstein-Barr virus positivity in Hodgkin's disease does not correlate with an HLA A2-negative phenotype. *Cancer*, **73**, 3059–3063

Poppema, S., van Imhoff, G., Torensma, R. & Smit, J. (1985) Lymphadenopathy morphologically consistent with Hodgkin's disease associated with Epstein-Barr virus infection. *Am. J. clin. Pathol.*, **84**, 385–390

Poulsen, L.O., Christensen, J.H., Sørensen, B., Ebbesen, P., Pallesen, G. & Grunnet, N. (1991) Immunologic observations in close relatives of two sisters with mammary Burkitt's lymphoma. *Cancer*, **68**, 1031–1034

Pour, P., Kruger, F.W., Cardesa, A., Althoff, J. & Mohr, U. (1973) Carcinogenic effect of di-*n*-propylnitrosamine in Syrian golden hamsters. *J. natl Cancer Inst.*, **51**, 1019–1027

Preciado, M.V., De Matteo, E., Diez, B., Menárguez, J. & Grinstein, S. (1995) Presence of Epstein-Barr virus and strain type assignment in Argentine childhood Hodgkin's disease. *Blood*, **86**, 3922–3929

Prévot, S., Hamilton-Dutoit, S., Audouin, J., Walter, P., Pallesen, G. & Diebold, J.(1992) Analysis of African Burkitt's and high-grade B cell non-Burkitt's lymphoma for Epstein-Barr virus genomes using in situ hybridization. *Br. J. Haematol.*, **80**, 27–32

Prévot, S., Néris, J. & de Saint Maur, P.P. (1994) Detection of Epstein Barr virus in an hepatic leiomyomatous neoplasm in an adult human immunodeficiency virus 1-infected patient. *Virchows Arch.*, **425**, 321–325

Provisor, A.J., Iacuone, J.J., Chilcote, R.R., Neiburger, R.G., Crussi, F.G. & Baehner, R.L. (1975) Acquired agammaglobulinemia after a life-threatening illness with clinical and laboratory features of infectious mononucleosis in three related male children. *New Engl. J. Med.*, **293**, 62–65

Puglielli, M.T., Woisetschlaeger, M. & Speck, S.H. (1996) *ori*P is essential for EBNA gene promoter activity in Epstein-Barr virus-immortalized lymphoblastoid cell lines. *J. Virol.*, **70**, 5758–5768

Pulvertaft, R.J.V. (1965) A study of malignant tumours in Nigeria by short-term tissue culture. *J. clin. Pathol.*, **18**, 261

Purtilo, D.T. (1976) Pathogenesis and phenotypes of an X-linked recessive lymphoproliferative syndrome. *Lancet*, **ii**, 882–885

Purtilo, D.T. (1981) X-Linked lymphoproliferative syndrome. *Arch. Pathol. Lab. Med.*, **105**, 119–121

Purtilo, D.T. (1991) X-Linked lymphoproliferative disease (XLP) as a model of Epstein-Barr virus-induced immunopathology. *Semin. Immunopathol.*, **13**, 181–197

Purtilo, D.T. & Sakamoto, K. (1981) Epstein-Barr virus and human disease: Immune responses determine the clinical and pathologic expression. *Hum. Pathol.*, **12**, 677–679

Purtilo, D.T., Falk, K., Pirruccello, S.J., Nakamine, H., Kleveland, K., Davis, J.R., Okano, M., Taguchi, Y., Sanger, W.G. & Beisel K.W. (1991) SCID mouse model of Epstein-Barr virus induced lymphomagenesis of immunodeficient humans. *Int. J. Cancer*, **47**, 510–517

Qu, L. & Rowe, D.T. (1992) Epstein-Barr virus latent gene expression in uncultured peripheral blood lymphocytes. *J. Virol.*, **66**, 3715–3724

Qualtière, L.F. & Pearson, G.R. (1980) Radioimmune precipitation study comparing the Epstein-Barr virus-induced membrane antigens expressed on P₃HR-1 virus-superinfected Raji cells to those expressed on cells in a B-95 virus-transformed producer culture activated with tumor-promoting agent (TPA). *Virology*, **102**, 360–369

Qualtière, L.F., Decoteau, J.F. & Hassan Nasr-El-Din, M. (1987) Epitope mapping of the major Epstein-Barr virus outer envelope glycoprotein gp350/220. *J. gen. Virol.*, **68**, 535–543

Quintanilla-Martínez, L., Gamboa-Domínquez, A., Gamez-Ledesma, I., Angeles-Angeles, A. & Mohar, A. (1995) Association of Epstein-Barr virus latent membrane protein and Hodgkin's disease in Mexico. *Mod. Pathol.*, **8**, 675–679

Raab-Traub, N. (1992a) Epstein-Barr virus infection in nasopharyngeal carcinoma. *Infect. Agents Dis.*, **1**, 173–184

Raab-Traub, N. (1992b) Epstein-Barr virus and nasopharyngeal carcinoma. *Semin. Cancer Biol.*, **3**, 297–307

Raab-Traub, N. & Flynn, K. (1986) The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. *Cell*, **47**, 883–889

Raab-Traub, N., Dambaugh, T. & Kieff, E. (1980) DNA of Epstein-Barr virus VIII: B95-8, the previous prototype, is an unusual deletion derivative. *Cell*, **22**, 257–267

Raab-Traub, N., Hood, R., Yang, C.-S., Henry, B. & Pagano, J.S. (1983) Epstein-Barr virus transcription in nasopharyngeal carcinoma. *J. Virol.*, **48**, 580–590

Raab-Traub, N., Flynn, K., Pearson, G., Huang, A., Levine, P., Lanier, A. & Pagano, J. (1987) The differentiated form of nasopharyngeal carcinoma contains Epstein-Barr virus DNA. *Int. J. Cancer*, **39**, 25–29

Raab-Traub, N., Rajadurai, P., Flynn, K. & Lanier, A.P. (1991) Epstein-Barr virus infection in carcinoma of the salivary gland. *J. Virol.*, **65**, 7032–7036

Rabbitts, T.H., Forster, A., Hamlyn, P. & Baer, R. (1984) Effect of somatic mutation within translocated c-*myc* genes in Burkitt's lymphoma. *Nature*, **309**, 592–597

Rabin, H., Neubauer, R.H., Hopkins, R.F. & Levy, B.M. (1977) Characterization of lymphoid cell lines established from multiple Epstein-Barr virus (EBV)-induced lymphomas in a cotton-topped marmoset. *Int. J. Cancer*, **20**, 44–50

Rabkin, C.S., Hilgartner, M.W., Hedberg, K.W., Aledort, L.M., Hatzakis, A., Eichinger, S., Eyster, M.E., White, G.C., II, Kessler, C.M., Lederman, M.M., de Moerloose, P., Bray, G.L., Cohen, A.R., Andes, W.A., Manco-Johnson, M., Schramm, W., Kroner, B.L., Blattner, W.A. & Goedert, J.J. (1992) Incidence of lymphomas and other cancers in HIV-infected and HIV-uninfected patients with hemophilia. *J. Am. med. Assoc.*, **267**, 1090–1094

Raffeld, M., Yano, T., Hoang, A.T., Lewis, B., Clark, H.M., Otsuki, T. & Dang, C.V. (1995) Clustered mutations in the transcriptional activation domain of myc in 8q24 translocated lymphomas and their functional consequences. *Curr. Top. Microbiol. Immunol.*, **194**, 265–272

Ragona, G., Ernberg, I. & Klein, G. (1980) Induction and biological characterization of the EBV-virus (EBV) carried by the Jijoye lymphoma line. *Virology*, **101**, 553–557

Ragona, G., Sirianni, M.C., Soddu, S., Vercelli, B., Sebastiani, G., Piccoli, M. & Aiuti, F. (1986) Evidence for disregulation in the control of Epstein-Barr virus latency in patients with AIDS-related complex. *Clin. exp. Immunol.*, **66**, 17–24

Ragot, T., Finerty, S., Watkins, P.E., Perricaudet, M. & Morgan, A.J. (1993) Replication-defective recombinant adenovirus expressing the Epstein-Barr virus (EBV) envelope glycoprotein gp340/220 induces protective immunity against EBV-induced lymphomas in the cottontop tamarin. *J. gen. Virol.*, **74**, 501–507

Rajpert-de Meyts, E., Hørding, U., Nielsen, H.W. & Skakkebaek, N.E. (1994) Human papillomavirus and Epstein-Barr virus in the etiology of testicular germ cell tumours. *Acta pathol. microbiol. immunol. scand.*, **102**, 38–42

Randhawa, P.S., Markin, R.S., Starzl, T.E. & Demetris, A.J. (1990) Epstein-Barr virus-associated syndromes in immunosuppressed liver transplant recipients. Clinical profile and recognition on routine allograft biopsy. *Am. J. surg. Pathol.*, **14**, 538-547

Randhawa, P.S., Jaffe, R., Demetris, A.J., Nalesnik, M., Starzl, T.E., Chen, Y.-Y. & Weiss, L.M. (1992) Expression of Epstein-Barr virus-encoded small RNA (by the EBER-1 gene) in liver specimens from transplant recipients with post-transplantation lymphoproliferative disease. *New Engl. J. Med.*, **327**, 1710–1714

Randhawa, P.S., Magnone, M., Jordan, M., Shapiro, R., Demetris, A.J. & Nalesnik, M. (1996) Renal allograft involvement by Epstein-Barr virus associated post-transplant lymphoproliferative disease. *Am. J. surg. Pathol.*, **20**, 563–571

Rangan, S.R.S., Martin, L.N., Bozelka, B.E., Wang, N. & Gormus, B.J. (1986) Epstein-Barr virus-related herpesvirus from a rhesus monkey (*Macaca mulatta*) with malignant lymphoma. *Int. J. Cancer*, **38**, 425–432

Raphael, M.M., Audouin, J., Lamine, M., Delecluse, H.-J., Vuillaume, M., Lenoir, G.M., Gisselbrecht, C., Lennert, K., Diebold, J. & the French Study Group of Pathology for HIV-associated Tumors (1994) Immunophenotypic and genotypic analysis of acquired immunodeficiency syndrome-related non-Hodgkin's lymphomas. Correlation with histologic features in 36 cases. *Am. J. clin. Pathol.*, **101**, 773–782

Rasheed, S., Rongey, R.W., Bruszweski, J., Nelson-Rees, W.A., Rabin, H., Neubauer, R.H., Esra, G. & Gardner, M.B. (1997) Establishment of a cell line with associated Epstein-Barr-like virus from a leukemic orangutan. *Science*, **198**, 407–409

Rawlins, D.R., Milman, G., Hayward, S.D. & Hayward, G.S. (1985) Sequence-specific DNA binding of the Epstein-Barr virus nuclear antigen (EBNA-1) to clustered sites in the plasmid maintenance region. *Cell*, **42**, 859–868

Rea, D., Fourcade, C., Leblond, V., Rowe, M., Joab, I., Edelman, L., Bitker, M.O., Gandjbakhch, I., Suberbielle, C., Farcet, J.-P. & Raphael, M. (1994) Patterns of Epstein-Barr virus latent and replicative gene expression in Epstein-Barr virus B cell lymphoproliferative disorders after organ transplantation. *Transplantation*, **58**, 317–324

Reedman, B.M. & Klein, G. (1973) Cellular localization of an Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer*, **11**, 499–520

Reedman, B.M., Klein, G., Pope, J.H., Walters, M.K., Hilgers, J., Singh, S. & Johansson, B. (1974) Epstein-Barr virus-associated complement-fixing and nuclear antigens in Burkitt lymphoma biopsies. *Int. J. Cancer*, **13**, 755–763

Regaud, C. (1921) Discussion in Reverchon, L. & Coutard, H., Hypopharynx lymphoepithelioma treated by roentgen therapy. *Bull. Soc. fr. Otorhinolaryngol.*, **34**, 209 (in French)

Reisman, D. & Sugden, B. (1986) *trans*-Activation of an Epstein-Barr viral transcriptional enhancer by the Epstein-Barr viral nuclear antigen 1. *Mol. cell. Biol.*, **6**, 3838–3846

Reisman, D., Yates, J. & Sugden, B. (1985) A putative origin of replication of plasmids derived from Epstein-Barr virus is composed of two cis-acting components. Mol. cell. Biol., 5, 1822–1832

Requena, L., Sánchez Yus, E., Jiménez, E. & Roo, E. (1994) Lymphoepithelioma-like carcinoma of the skin: A light-microscopic and immunohistochemical study. J. cutaneous Pathol., 21, 541–548

Resnick, L., Herbst, J.S., Ablashi, D.V., Atherton, S., Frank, B., Rosen, L. & Horwitz, S.N. (1988) Regression of oral hairy leukoplakia after orally administered acyclovir therapy. J. Am. med. Assoc., 259, 384–388

Reth, M. (1989) Antigen receptor tail clue. Nature, 338, 383–384

Revoltella, R.P., Vigneti, E., Fruscalzo, A., Park, M., Ragona, G., Rocchi, G. & Calef, E. (1989) Epstein-Barr virus DNA sequences in precursor monocyte–macrophage cell lines established from the bone marrow of children with maturation defects of haematopoiesis. J. gen. Virol., 70, 1203–1215

Reynes, M., Aubert, J.P., Cohen, J.H.M., Audouin, J., Tricottet, V., Diebold, J. & Kazatchkine, M.D. (1985) Human follicular dendritic cells express CR1, CR2, and CR3 complement receptor antigens. J. Immunol., 135, 2687–2694

Reynolds, D.J., Banks, P.M. & Gulley, M.L. (1995) New characterization of infectious mononucleosis and a phenotypic comparison with Hodgkin's disease. Am. J. Pathol., 146, 379–388

Rhodes, G., Rumpold, H., Kurki, P., Patrick, K.M., Carson, D.A. & Vaughan, J.H. (1987) Autoantibodies in infectious mononucleosis have specificity for the glycine-alanine repeating region of the Epstein-Barr virus nuclear antigen. J. exp. Med., 165, 1026–1040

Rickinson, A.B. (1995) Immune intervention against virus-associated human cancers. Ann. Oncol., 6 (Suppl. 1), S69–S71

Rickinson, A.B. & Epstein, M.A. (1978) Sensitivity of the transforming and replicative functions of Epstein–Barr virus to inhibition by phosphonoacetate. J. gen. Virol., 40, 409–420

Rickinson, A.B. & Kieff, E. (1996) Epstein-Barr virus. In: Fields, B.N., Knipe, D.M. & Howley, P.M., eds, Fields Virology, 3rd Ed., Vol. 2, Philadelphia, Lippincott–Raven, pp. 2397–2476

Rickinson, A.B., Finerty, S. & Epstein, M.A. (1978) Inhibition by phosphonoacetate of the in vitro outgrowth of Epstein-Barr virus genome-containing cell lines from the blood of infectious mononucleosis patients. In: de Thé, G., Henle, W. & Rapp, F., eds, Oncogenesis and Herpesviruses III (IARC Scientific Publications No. 24), Lyon, IARC, pp. 721–728

Rickinson, A.B., Moss, D.J. & Pope, J.H. (1979) Long-term C-cell-mediated immunity to Epstein-Barr virus in man. II. Components necessary for regression in virus-infected leukocyte cultures. Int. J. Cancer, 23, 610–617

Rickinson, A.B., Moss, D.J., Pope, J.H. & Ahlberg, N. (1980a) Long-term T-cell-mediated immunity to Epstein-Barr virus in man. IV. Development of T-cell memory in convalescent infectious mononucleosis patients. Int. J. Cancer, 25, 59–65

Rickinson, A.B., Wallace, L.E. & Epstein, M.A. (1980b) HLA-restricted T-cell recognition of Epstein-Barr virus-infected B cells. Nature, 283, 865–867

Rickinson, A.B., Moss, D.J., Wallace, L.E., Rowe, M., Misko, I.S., Epstein, M.A. & Pope, J.H. (1981a) Long-term T-cell-mediated immunity to Epstein-Barr virus. Cancer Res., 41, 4216–4221

Rickinson, A.B., Moss, D.J., Allen, D.J., Wallace, L.E., Rowe, M. & Epstein, M.A. (1981b) Reactivation of Epstein-Barr virus-specific cytotoxic T cells by in vitro stimulation with the autologous lymphoblastoid cell line. *Int. J. Cancer*, **27**, 593–601

Rickinson, A.B., Rowe, M., Hart, I.J., Yao, Q.Y., Henderson, L.E., Rabin, H. & Epstein, M.A. (1984) T-Cell-mediated regression of 'spontaneous' and of Epstein-Barr virus-induced B-cell transformation *in vitro*: Studies with cyclosporin A. *Cell. Immunol.*, **87**, 646–658

Rickinson, A.B., Young, L.S. & Rowe, M. (1987) Influence of the Epstein-Barr virus nuclear antigen EBNA 2 on the growth phenotype of virus-transformed B cells. *J. Virol.*, **61**, 1310–1317

Rickinson, A.B., Murray, R.J., Brooks, J., Griffin, H., Moss, D.J. & Masucci, M.G. (1992) T Cell recognition of Epstein-Barr virus associated lymphomas. *Cancer Surv.*, **13**, 53–80

Ricksten, A., Kallin, B., Alexander, H., Dillner, J., Fåhraeus, R., Klein, G., Lerner, R. & Rymo, L. (1988) *Bam*HI E region of the Epstein-Barr virus genome encodes three transformation-associated nuclear proteins. *Proc. natl Acad. Sci. USA*, **85**, 995–999

Riddler, S.A., Breinig, M.C. & McKnight, J.L.C. (1994) Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood*, **84**, 972–984

Ries, L.A.G., Kosary, C.L., Hankey, B.F., Miller, B.A., Harras, A. & Edwards, B.K. (1997) *SEER Cancer Statistics Review, 1973–1994* (NIH Publ. No. 97-2789), Bethesda, National Cancer Institute

Rinaldo, C.R., Jr, Kingsley, L.A., Lyter, D.W., Rabin, B.S., Atchison, R.W., Bodner, A.J., Weiss, S.H. & Saxinger, W.C. (1986) Association of HTLV-III with Epstein-Barr virus infection and abnormalities of T lymphocytes in homosexual men. *J. infect. Dis.*, **154**, 556–561

Robertson, E. & Kieff, E. (1995) Reducing the complexity of the transforming Epstein-Barr virus genome to 64 kilobase pairs. *J. Virol.*, **69**, 983–993

Robertson, E.S., Tomkinson, B. & Kieff, E. (1994) An Epstein-Barr virus with a 58-kilobase-pair deletion that includes BARF0 transforms B lymphocytes *in vitro. J. Virol.*, **68**, 1449–1458

Robertson, E.S., Grossman, S., Johannsen, E., Miller, C., Lin, J., Tomkinson, B. & Kieff, E. (1995a) Epstein-Barr virus nuclear protein 3C modulates transcription through interaction with the sequence-specific DNA-binding protein Jκ. *J. Virol.*, **69**, 3108–3116

Robertson, K.D., Hayward, S.D., Ling, P.D., Samid, D. & Ambinder, R.F. (1995b) Transcriptional activation of the Epstein-Barr virus latency C promoter after 5-azacytidine treatment: Evidence that demethylation at a single CpG site is crucial. *Mol. cell. Biol.*, **15**, 6150–6159

Robertson, K.D., Barletta, J., Samid, D. & Ambinder, R.F. (1995c) Pharmacologic activation of expression of immunodominant viral antigens: A new strategy for the treatment of Epstein-Barr-virus-associated malignancies. *Curr. Top. Microbiol. Immunol.*, **194**, 145–154

Robertson, E.S., Lin, J. & Kieff, E. (1996) The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJκ. *J. Virol.*, **70**, 3068–3074

Robinson, J.E., Smith, D. & Niederman, J. (1981) Plasmacytic differentiation of circulating Epstein-Barr virus-infected B lymphocytes during acute infectious mononucleosis. *J. exp. Med.*, **153**, 235–244

Rocchi, G., Tosato, G., Papa, G. & Ragona, G. (1975) Antibodies to Epstein-Barr virus-associated nuclear antigen and to other viral and non-viral antigens in Hodgkin's disease. *Int. J. Cancer*, **16**, 323–328

Roeser, H.P., Pope, J.H., Biggs, J.S.G. & Cooke, R.A. (1977) Burkitt's lymphoma in Australia. *Med. J. Aust.*, **1**, 372–374

Rogers, R.P., Strominger, J.L. & Speck, S.H. (1992) Epstein-Barr virus in B lymphocytes: Viral gene expression and function in latency. *Adv. Cancer Res.*, **58**, 1–26

Roizman, B., Desrosiers, R.C., Fleckenstein, B., Lopez, C., Minson, A.C. & Studdert, M.J. (1992) The family Herpesviridae: An update. *Arch. Virol.*, **123**, 425–449

Rooney, C., Howe, J.G., Speck, S.H. & Miller, G. (1989) Influence of Burkitt's lymphoma and primary B cells on latent gene expression by the nonimmortalizing P3J-HR-1 strain of Epstein-Barr virus. *J. Virol.*, **63**, 1531–1539

Rooney, C.M., Smith, C.A., Ng, C.Y.C., Loftin, S., Li, C., Krance, R.A., Brenner, M.K. & Heslop, H.E. (1995) Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet*, **345**, 9–13

Rooney, C.M., Smith, C.A. & Heslop, H.E. (1997) Control of virus-induced lymphoproliferation: Epstein-Barr virus-induced lymphoproliferation and host immunity. *Mol. Med. Today*, **3**, 24–30

Rosdahl, N., Larsen, S.O. & Clemmesen, J. (1974) Hodgkin's disease in patients with previous infectious mononucleosis: 30 years' experience. *Br. med. J.*, **ii**, 253–256

Rosén, A., Gergely, P., Jondal, M., Klein, G. & Britton, S. (1977) Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes *in vitro*. *Nature*, **267**, 52–54

Rosenberg, S.A. (1982) National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Summary and description of a working formulation for clinical usage. *Cancer*, **49**, 2112-2135

Rothwell, R.I. (1979) Juvenile nasopharyngeal carcinoma in Sabah (Malaysia). *Clin. Oncol.*, **5**, 353–358

Rouah, E., Rogers, B.B., Wilson, D.R., Kirkpatrick, J.B. & Buffone, G.J. (1990) Demonstration of Epstein-Barr virus in primary central nervous system lymphomas by the polymerase chain reaction and in situ hybridization. *Hum. Pathol.*, **21**, 545-550

Roush, G.C., Walrath, J., Stayner, L.T., Kaplan, S.A., Flannery, J.T. & Blair, A. (1987) Nasopharyngeal cancer, sinonasal cancer, and occupations related to formaldehyde: A case–control study. *J. natl Cancer Inst.*, **79**, 1221–1224

Rousselet, G. & Tursz, T. (1992) CD23 and the Epstein-Barr virus. *Res. Immunol.*, **143**, 456–459

Rousset, F., Garcia, E., Defrance, T., Péronne, C., Vezzio, N., Hsu, D.-H., Kastelein, R., Moore, K.W. & Banchereau, J. (1992) Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. natl Acad. Sci. USA*, **89**, 1890–1893

Rowe, D.T., Rowe, M., Evan, G.J., Wallace, L.E., Farrell, P.J. & Rickinson, A.B. (1986) Restricted expression of EBV latent genes and T-lymphocyte-detected membrane antigen in Burkitt's lymphoma cells. *EMBO J.*, **5**, 2599–2607

Rowe, M., Rowe, D.T., Gregory, C.D.,Young, L.S., Farrell, P.J., Rupani, H. & Rickinson, A.B. (1987a) Differences in B cell growth phenotype reflect novel patterns of Epstein-Barr virus latent gene expression in Burkitt's lymphoma cells. *EMBO J.*, **6**, 2743–2751

Rowe, M., Evans, H.S., Young, L.S., Hennessy, K., Kieff, E. & Rickinson, A.B. (1987b) Monoclonal antibodies to the latent membrane protein of Epstein-Barr virus reveal heterogeneity of the protein and inducible expression in virus-transformed cells. *J. gen. Virol.*, **68**, 1575–1586

Rowe, M., Young, L.S., Cadwallader, K., Petti, L., Kieff, E. & Rickinson, A.B. (1989) Distinction between Epstein-Barr virus type A (EBNA 2A) and type B (EBNA 2B) isolates extends to the EBNA 3 family of nuclear proteins. *J. Virol.*, **63**, 1031–1039

Rowe, M., Lear, A.L., Croom-Carter, D., Davies, A.H. & Rickinson, A.B. (1992) Three pathways of Epstein-Barr virus gene activation from EBNA1-positive latency in B lymphocytes. *J. Virol.*, **66**, 122–131

Rowe, M., Peng-Pilon, M., Huen, D.S., Hardy, R., Croom-Carter, D., Lundgren, E. & Rickinson, A.B. (1994) Upregulation of *bcl*-2 by the Epstein-Barr virus latent membrane protein LMP1: A B-cell-specific response that is delayed relative to NF-κ B activation and to induction of cell surface markers. *J. Virol.*, **68**, 5602–5612

Rowe, M., Khanna, R., Jacob, C.A., Argaet, V., Kelly, A., Powis, S., Belich, M., Croom-Carter, D., Lee, S., Burrows, S.R., Trowsdale, J., Moss, D.J. & Rickinson, A.B. (1995) Restoration of endogenous antigen processing in Burkitt's lymphoma cells by Epstein-Barr virus latent membrane protein-1: Coordinate up-regulation of peptide transporters and HLA-class I antigen expression. *Eur. J. Immunol.*, **25**, 1374–1384

Rowlands, D.C., Ito, M., Mangham, D.C., Reynolds, G., Herbst, H., Hallissey, M.T., Fielding, J.W., Newbold, K.M., Jones, E.L., Young, L.S. & Niedobitek, G. (1993) Epstein-Barr virus and carcinomas: Rare association of the virus with gastric adenocarcinomas. *Br. J. Cancer*, **68**, 1014–1019

Ryon, J.J., Hayward, S.D., MacMahon, E.M.E., Mann, R.B., Ling, Y., Charache, P., Phelan, J.A., Miller, G. & Ambinder, R.F. (1993) In situ detection of lytic Epstein-Barr virus infection: Expression of the *Not*I early gene and viral interleukin-10 late gene in clinical specimens. *J. infect. Dis.*, **168**, 345–351

Sabourin, J.-C., Kanavaros, P., Briere, J., Lescs, M.-C., Petrella, T., Zafrani, E.S. & Gaulard, P. (1993) Epstein-Barr virus (EBV) genomes and EBV-encoded latent membrane protein (LMP) in pulmonary lymphomas occurring in nonimmunocompromised patients. *Am. J. surg. Pathol.*, **17**, 995–1002

Sadler, R.H. & Raab-Traub, N. (1995a) Structural analyses of the Epstein-Barr virus *Bam*HI A transcripts. *J. Virol.*, **69**, 1132–1141

Sadler, R.H. & Raab-Traub, N. (1995b) The Epstein-Barr virus 3.5-kilobase latent membrane protein 1 mRNA initiates from a TATA-less promoter within the first terminal repeat. *J. Virol.*, **69**, 4577–4581

Saemundsen, A.K., Kallin, B. & Klein, G. (1980) Effect of *n*-butyrate on cellular and viral DNA synthesis in cells latently infected with Epstein-Barr virus. *Virology*, **107**, 557–561

Saemundsen, A.K., Berkel, A.I., Henle, W., Henle, G., Anvret, M., Sanal, Ö, Ersoy, F., Çaglar, M. & Klein, G. (1981) Epstein-Barr-virus-carrying lymphoma in a patient with ataxia-telangiectasia. *Br. med. J.*, **282**, 425–427

Saemundsen, A.K., Albeck, H., Hansen, J.P.H., Nielsen, N.H., Anvret, M., Henle, W., Henle, G., Thomsen, K.A., Kristensen, H.K. & Klein, G. (1982) Epstein-Barr virus in nasopharyngeal and salivary gland carcinomas of Greenland Eskimoes. *Br. J. Cancer*, **46**, 721–728

Saiki, Y., Ohtani, H., Naito, Y., Miyazawa, M. & Nagura, H. (1996) Immunophenotypic characterization of Epstein-Barr virus-associated gastric carcinoma: Massive infiltration by proliferating CD8⁺ T-lymphocytes. *Lab. Invest.*, **75**, 67–76

Sakai, T. & Honjo, T. (1997) Transcriptional activity of EBNA2 through RBP-J. *Nippon Rinsho*, **55**, 293–298 (in Japanese)

Sakamoto, K., Freed, H.J. & Purtilo, D.T. (1980) Antibody responses to Epstein-Barr virus in families with the X-linked lymphoproliferative syndrome. *J. Immunol.*, **125**, 921–925

Salaman, M.H., Wedderburn, N. & Bruce-Chwatt, L.J. (1969) The immunodepressive effect of a murine plasmodium and its interaction with murine oncogenic viruses. *J. gen. Microbiol.*, **59**, 383–391

Salloum, E., Cooper, D.L., Howe, G., Lacy, J., Tallini, G., Crouch, J., Schultz, M. & Murren, J. (1996) Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic diseases. *J. clin. Oncol.*, **14**, 1943–1949

Sam, C.K., Prasad, U. & Pathmanathan, R. (1989) Serological markers in the diagnosis of histopathological types of nasopharyngeal carcinoma. *Eur. J. surg. Oncol.*, **15**, 357–360

Sam, C.K., Brooks, L.A., Niedobitek, G., Young, L.S., Prasad, U. & Rickinson, A.B. (1993) Analysis of Epstein-Barr virus infection in nasopharyngeal biopsies from a group at high risk of nasopharyngeal carcinoma. *Int. J. Cancer*, **53**, 957–962

Sample, J. & Kieff, E. (1990) Transcription of the Epstein-Barr virus genome during latency in growth-transformed lymphocytes. *J. Virol.*, **64**, 1667–1674

Sample, J., Hummel, M., Braun, D., Birkenbach, M. & Kieff, E. (1986) Nucleotide sequences of mRNAs encoding Epstein-Barr virus nuclear proteins: A probable transcriptional initiation site. *Proc. natl Acad. Sci. USA*, **83**, 5096–5100

Sample, J., Liebowitz, D. & Kieff, E. (1989) Two related Epstein-Barr virus membrane proteins are encoded by separate genes. *J. Virol.*, **63**, 933–937

Sample, J., Young, L., Martin, B., Chatman, T., Kieff, E. & Rickinson, A. (1990) Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J. Virol.*, **64**, 4084–4092

Sample, J., Henson, E.B.D. & Sample, C. (1992) The Epstein-Barr virus nuclear protein 1 promoter active in type I latency is autoregulated. *J. Virol.*, **66**, 4654–4661

Sample, J., Kieff, E.F. & Kieff, E.D. (1994) Epstein-Barr virus types 1 and 2 have nearly identical LMP-1 transforming genes. *J. gen. Virol.*, **75**, 2741–2746

Sandberg, M., Hammerschmidt, W. & Sugden, B. (1997) Characterization of LMP-1's association with TRAF1, TRAF2, and TRAF3. *J. Virol.*, **71**, 4649–4656

Sander, C.A., Medeiros, L.J., Weiss, L.M., Yano, T., Sneller, M.C. & Jaffe, E.S. (1992) Lymphoproliferative lesions in patients with common variable immunodeficiency syndrome. *Am. J. surg. Pathol.*, **16**, 1170–1182

Sandvej, K., Krenács, L., Hamilton-Dutoit, S.J., Rindum, J.L., Pindborg, J.J. & Pallesen, G. (1992) Epstein-Barr virus latent and replicative gene expression in oral hairy leukoplakia. *Histopathology*, **20**, 387–395

Santucci, M., Gallo, O., Calzolari, A. & Bondi, R. (1993) Detection of Epstein-Barr viral genome in tumor cells of Warthin's tumor of parotid gland. *Am. J. clin. Pathol.*, **100**, 662–665

Sato, H., Takimoto, T., Hatano, M., Pagano, J.S. & Raab-Traub, N. (1989) Epstein-Barr virus with transforming and early antigen-inducing ability originating from nasopharyngeal carcinoma: Mapping of the viral genome. *J. gen. Virol.*, **70**, 717–727

Savoie, A., Perpête, C., Carpentier, L., Joncas, J. & Alfieri, C. (1994) Direct correlation between the load of Epstein-Barr virus-infected lymphocytes in the peripheral blood of pediatric transplant patients and risk of lymphoproliferative disease. *Blood*, **83**, 2715–2722

Saw, D., Lau, W.H., Ho, J.H., Chan, J.K. & Ng, C.S. (1986) Malignant lymphoepithelial lesion of the salivary gland. *Hum. Pathol.*, **17**, 914–923

Sbih-Lammali, F., Djennaoui, D., Belaoui, H., Bouguermouh, A., Decaussin, G. & Ooka, T. (1996) Transcriptional expression of Epstein-Barr virus genes and proto-oncogenes in North African nasopharyngeal carcinoma. *J. med. Virol.*, **49**, 7–14

Schaefer, O., Hildes, J.A., Medd, L.M. & Cameron, D.G. (1975) The changing pattern of neoplastic disease in Canadian Eskimos. *Can. med. Assoc. J.*, **112**, 1399–1404

Schaefer, B.C., Strominger, J.L. & Speck, S.H. (1995) Redefining the Epstein-Barr virus-encoded nuclear antigen EBNA-1 gene promoter and transcription initiation site in group I Burkitt lymphoma cell lines. *Proc. natl Acad. Sci. USA*, **92**, 10565–10569

Schaefer, B.C., Strominger, J.L. & Speck, S.H. (1997) Host-cell-determined methylation of specific Epstein-Barr virus promoters regulates the choice between distinct viral latency programs. *Mol. cell. Biol.*, **17**, 364–377

Schendel, D.J., Reinhardt, C., Nelson, P.J., Maget, B., Pullen, L., Bornkamm, G.W. & Steinle, A. (1992) Cytotoxic T lymphocytes show HLA-C-restricted recognition of EBV-bearing cells and allorecognition of HLA class I molecules presenting self-peptides. *J. Immunol.*, **149**, 2406–2414

Schimke, R.N., Collins, D. & Cross, D. (1987) Nasopharyngeal carcinoma, aplastic anemia, and various malignancies in a family: Possible role of Epstein-Barr virus. *Am. J. med. Genet.*, **27**, 195–202

Schlager, S., Speck, S.H. & Woisetschläger, M. (1996) Transcription of the Epstein-Barr virus nuclear antigen 1 (EBNA1) gene occurs before induction of the BCR2 (Cp) EBNA gene promoter during the initial stages of infection in B cells. *J. Virol.*, **70**, 3561–3570

Schmauz, R. & Templeton, A.C. (1972) Nasopharyngeal carcinoma in Uganda. *Cancer*, **29**, 610–621

Schmid, C., Pan, L., Diss, T. & Isaacson, P.G. (1991) Expression of B-cell antigens by Hodgkin's and Reed-Sternberg cells. *Am. J. Pathol.*, **139**, 701–707

Schmincke, A. (1921) Lymphoepithelial tumours. *Beitr. path. Anat.*, **68**, 161–170 (in German)

Schneider, R.J., Safer, B., Munemitsu, S.M., Samuel, C.E. & Shenk, T. (1985) Adenovirus VA1 RNA prevents phosphorylation of the eukaryotic initiation factor 2α subunit subsequent to infection. *Proc. natl Acad. Sci. USA*, **82**, 4321–4325

de Schryver, A., Klein, G., Henle, G., Henle, W., Cameron, H.M., Santesson, L. & Clifford, P. (1972) EB-virus associated serology in malignant disease: Antibody levels to viral capsid antigens (VCA), membrane antigens (MA) and early antigens (EA) in patients with various neoplastic conditions. *Int. J. Cancer*, **9**, 353–364

de Schryver, A., Klein, G., Hewetson, J., Rocchi, G., Henle, W., Henle, G., Moss, D.J. & Pope, J.H. (1974) Comparison of EBV neutralization tests based on abortive infection or transformation of lymphoid cells and their relation to membrane reactive antibodies (anti-MA). *Int. J. Cancer*, **13**, 353–362

Schultz, L.D., Tanner, J., Hofmann, K.J., Emini, E.A., Condra, J.H., Jones, R.E., Kieff, E. & Ellis, R.W. (1987) Expression and secretion in yeast of a 400-kDa envelope glycoprotein derived from Epstein-Barr virus. *Gene*, **54**, 113–123

Schuster, V., Grimm, T., Kress, W., Seidenspinner, S., Belohradsky, B.H., Müller, P. & Kreth, H.W. (1995) X-Chromosomal-recessive lymphoproliferative disease (XLP): Molecular and genetic studies. *Klin.-Pädiatr.*, **207**, 271–276 (in German)

Schuster, V., Seidenspinner, S. & Kreth, H.W. (1996) Detection of a nuclear antigen 2 (EBNA2)-variant Epstein-Barr virus strain in two siblings with fatal lymphoproliferative disease. *J. med. Virol.*, **48**, 114–120

Schweisguth, F. & Posakony, J. (1992) Suppressor of hairless, the Drosophila homolog of the mouse recombination signal-binding protein gene, controls sensory organ cell fates. *Cell*, **69**, 1199–1212

Scott, J.K. & Smith, G.P. (1990) Searching for peptide ligands with an epitope library. *Science*, **249**, 386–390

Seeley, J., Svedmyr, E., Weiland, O., Klein, G., Moller, E., Eriksson, E., Andersson, K. & van der Waal, L. (1981) Epstein Barr virus selective T cells in infectious mononucleosis are not restricted to HLA-A and B antigens. *J. Immunol.*, **127**, 293–300

Seemayer, T.A., Gross, T.G., Maarten Egeler, R., Pirruccello, S.J., Davis, J.R., Kelly, C.M., Okano, M., Lanyi, A. & Sumegi, J. (1995) X-linked lymphoproliferative disease: Twenty-five years after the discovery. *Pediatr. Res.*, **38**, 471–478

Seigneurin, J.M., Lavoue, M.F., Genoulaz, O., Bornkamm, G.W. & Lenoir, G.M. (1987) Antibody response against the Epstein-Barr virus-coded nuclear antigen 2 (EBNA2) in different groups of individuals. *Int. J. Cancer*, **40**, 349–353

ten Seldam, R.E.J., Cooke, R. & Atkinson, L. (1966) Childhood lymphoma in the territories of Papua and New Guinea. *Cancer*, **19**, 437–446

Selves, J., Meggetto, F., Brousset, P., Voigt, J.-J., Pradère, B., Grasset, D., Icart, J., Mariamé, B., Knecht, H. & Delsol, G. (1996a) Inflammatory pseudotumor of the liver. Evidence for follicular dendritic reticulum cell proliferation associated with clonal Epstein-Barr virus. *Am. J. surg. Pathol.*, **20**, 747–753

Selves, J., Bibeau, F., Brousset, P., Meggetto, F., Mazerolles, C., Voigt, J.-J., Pradere, B., Chiotasso, P. & Delsol, G. (1996b) Epstein-Barr virus latent and replicative gene expression in gastric carcinoma. *Histopathology*, **28**, 121–127

Serafini-Cessi, F., Malagolini, N., Nanni, M., Dall'Olio, F., Campadelli-Fiume, G., Tanner, J. & Kieff, E. (1989) Characterization of N- and O-linked oligosaccharides of glycoprotein 350 from Epstein-Barr virus. *Virology*, **170**, 1–10

Serraino, D., Franceschi, S., Talamini, R., Barra, S., Negri, E., Carbone, A. & La Vecchia, C. (1991) Socioeconomic indicators, infectious diseases and Hodgkin's disease. *Int. J. Cancer*, **47**, 352–357

Se Thoe, S.Y., Wong, K.K., Pathmanathan, R., Sam, C.K., Cheng, H.M. & Prasad, U. (1993) Elevated secretory IgA antibodies to Epstein-Barr virus (EBV) and presence of EBV DNA and EBV receptors in patients with cervical carcinoma. *Gynecol. Oncol.*, **50**, 168–172

Shah, W.A., Ambinder, R.F., Hayward, G.S. & Hayward, S.D. (1992) Binding of EBNA-1 to DNA creates a protease-resistant domain that encompasses the DNA recognition and dimerization functions. *J. Virol.*, **66**, 3355–3362

Shanmugaratnam, K. & Sobin, L.H., eds (1991) *Histological Typing of Tumours of the Upper Respiratory Tract and Ear*, 2nd Ed., Berlin, Springer-Verlag

Shanmugaratnam, K., Tye, C.Y., Goh, E.H. & Chia, K.B. (1978) Etiological factors in nasopharyngeal carcinoma: A hospital-based, retrospective, case–control, questionnaire study. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 199–212

Shanmugaratnam, K., Chan, S.H., de Thé, G., Goh, J.E., Khor, T.H., Simons, M.J. & Tye, C.Y. (1979) Histopathology of nasopharyngeal carcinoma. Correlations with epidemiology, survival rates and other biological characteristics. *Cancer*, **44**, 1029–1044

Shao, Y.M., Poirier, S., Ohshima, H., Malaveille, C., Zeng, Y., de Thé, G. & Bartsch, H. (1988) Epstein-Barr virus activation in Raji cells by extracts of preserved food from high risk areas for nasopharyngeal carcinoma. *Carcinogenesis*, **9**, 1455–1457

Shapiro, R.S., McClain, K., Frizzera, G., Gajl-Peczalska, K.J., Kersey, J.H., Blazar, B.R., Arthur, D.C., Patton, D.F., Greenberg, J.S., Burke, B., Ramsay, N.K.C., McGlave, P. & Filipovich, A.H. (1988) Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. *Blood*, **71**, 1234–1243

Shaw, J.E. (1985) The circular intracellular form of Epstein-Barr virus DNA is amplified by the virus-associated DNA polymerase. *J. Virol.*, **53**, 1012–1015

Shek, T.W.H., Luk, I.S.C., Ng, I.O.L. & Lo, C.Y. (1996) Lymphoepithelioma-like carcinoma of the thyroid gland: Lack of evidence of association with Epstein-Barr virus. *Hum. Pathol.*, **27**, 851–853

Sheldon, P.J., Hemsted, E.H., Papamichail, M. & Holborow, E.J. (1973) Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet.*, **i**, 1153–1155

Shi, S.-R., Cote, R.J. & Taylor, C.R. (1997) Antigen retrieval immunohistochemistry: Past, present and future. *J. Histochem. Cytochem.*, **45**, 327–343

Shibata, D. & Weiss, L.M. (1992) Epstein-Barr virus-associated gastric adenocarcinoma. *Am. J. Pathol.*, **140**, 769–774

Shibata, D., Hansmann, M.-L., Weiss, L.M. & Nathwani, B.N. (1991a) Epstein-Barr virus infections and Hodgkin's disease: A study of fixed tissues using the polymerase chain reaction. *Hum. Pathol.*, **22**, 1262–1267

Shibata, D., Tokunaga, M., Uemura, Y., Sato, E., Tanaka, S. & Weiss, L.M. (1991b) Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration. Lymphoepithelioma-like carcinoma. *Am. J. Pathol.*, **139**, 469–474

Shibata, D., Weiss, L.M., Hernandez, A.M., Nathwani, B.N., Bernstein, L. & Levine, A.M. (1993) Epstein-Barr virus-associated non-Hodgkin's lymphoma in patients infected with the human immunodeficiency virus. *Blood*, **81**, 2102–2109

Shimizu, N., Tanabe-Tochikura, A., Kuroiwa, Y. & Takada, K. (1994) Isolation of Epstein-Barr virus (EBV)-negative cell clones from the EBV-positive Burkitt's lymphoma (BL) line Akata: Malignant phenotypes of BL cells are dependent on EBV. *J. Virol.*, **68**, 6069–6073

Shin, W.S., Kang, M.W., Kang, J.H., Choi, M.K., Ahn, B.M., Kim, J.K., Sun, H.S. & Min, K.-W. (1996) Epstein-Barr virus-associated gastric adenocarcinomas among Koreans. *Am. J. clin. Pathol.*, **105**, 174–181

Shiramizu, B., Barriga, F., Neequaye, J., Jafri, A., Dalla-Favera, R., Neri, A., Guttiérez, M., Levine, P. & Magrath, I. (1991) Patterns of chromosomal breakpoint locations in Burkitt's lymphoma: Relevance to geography and Epstein-Barr virus association. *Blood*, **77**, 1516–1526

Shiramizu, B., Herndier, B.G. & McGrath, M.S. (1994) Identification of a common clonal human immunodeficiency virus integration site in human immunodeficiency virus-associated lymphomas. *Cancer Res.*, **54**, 2069–2072

Shope, T.C. & Miller, G. (1975) EB virus: Malignant lymphoma in cottontop marmosets following inoculation and recovery of the virus from cells of an experimental tumor maintained in organ culture. In: Ito, Y. & Dutcher, R.M., eds, *Comparative Leukemia Research 1973, Leukemogenesis* (Bibliography in Haematology, No. 40), Tokyo, University of Tokyo Press, pp. 375–383

Shope, T., Dechairo, D. & Miller, G. (1973) Malignant lymphoma in cottontop marmosets after inoculation with Epstein-Barr virus. *Proc. natl Acad. Sci. USA*, **70**, 2487–2491

Shope, T.C., Khalifa, A.S., Smith, S.T. & Cushing, B. (1982) Epstein-Barr virus antibody in childhood Hodgkin's disease. *Am. J. Dis. Child.*, **136**, 701–703

Shore, H. (1961) O'Nyong-nyong fever: An epidemic virus disease in East Africa. III. Some clinical and epidemiological observations in the northern province of Uganda. *Trans. R. Soc. trop. Med. Hyg.*, **55**, 361–373

Silins, S.L. & Sculley, T.B.. (1994) Modulation of vimentin, the CD40 activation antigen and Burkitt's lymphoma antigen (CD77) by the Epstein-Barr virus nuclear antigen EBNA-4. *Virology*, **202**, 16–24

Simons, M.J. & Shanmugaratnam, K., eds (1982) *The Biology of Nasopharyngeal Carcinoma* (UICC Technical Report Series, Vol. 71), Geneva, International Union Against Cancer, pp. 46–54

Simons, M.J., Wee, G.B., Goh, E.H., Chan, S.H., Shanmugaratnam, K., Day, N.E. & de Thé, G. (1976) Immunogenetic aspects of nasopharyngeal carcinoma. IV. Increased risk in Chinese of nasopharyngeal carcinoma associated with a Chinese-related HLA profile (A2, Singapore 2). *J. natl Cancer Inst.*, **57**, 977–980

Simons, M.J., Wee, G.B., Singh, D., Dharmalingam, S., Yong, N.K., Chau, J.C.W., Ho, J.H.C., Day, N.E. & de Thé, G. (1977) Immunogenetic aspects of nasopharyngeal carcinoma (NPC). V. Confirmation of a Chinese-related HLA profile (A2, Singapore 2) associated with an increased risk in Chinese for NPC. *Natl Cancer Inst. Monogr.*, **47**, 147–151

Simons, M.J, Chan, S.H., Wee, G.B., Shanmugaratnam, K., Goh, E.H., Ho, J.H.C., Chau, J.C.W., Darmalingam, S., Prasad, U., Betuel, H., Day, N.E. & de Thé, G. (1978) Nasopharyngeal carcinoma and histocompatibility antigens. In: de Thé, G. & Ito Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 271–282

Simons, M.J., Chan, S.H. & Ou, B.X. (1980) Nasopharyngeal carcinoma (NPC), including analysis of HLA gene patterns in Chinese patients with cervical and hepatocellular carcinoma. In: Davis, W., Harrap, K.R., & Stathopoulos, G., eds, *Advances in Tumour Prevention, Detection and Characterization*, Vol. 5, *Human Cancer. Its Characterization and Treatment*, Amsterdam, Excerpta Medica, pp. 369–378

Sinclair, A.J. & Farrell, P.J. (1995) Host cell requirements for efficient infection of quiescent primary B lymphocytes by Epstein-Barr virus. *J. Virol.*, **69**, 5461–5468

Sinclair, A.J., Palmero, I., Peters, G. & Farrell, P.J. (1994) EBNA-2 and EBNA-LP cooperate to cause G_0 to G_1 transition during immortalization of resting human B lymphocytes by Epstein-Barr virus. *EMBO J.*, **13**, 3321–3328

Sinha, S.K., Todd, S.C., Hedrick, J.A., Speiser, C.L., Lambris, J.D. & Tsoukas, C.D. (1993) Characterization of the EBV/C3d receptor on the human Jurkat T cell line: Evidence for a novel transcript. *J. Immunol.*, **150**, 5311–5320

Sista, N.D., Barry, C., Sampson, K. & Pagano, J. (1995) Physical and functional interaction of the Epstein-Barr virus BZLF1 transactivator with the retinoic acid receptors RAR α and RXR α. *Nucleic Acids Res.*, **23**, 1729–1736

Sixbey, J.W. & Pagano, J.S. (1985) Epstein-Barr virus transformation of human B lymphocytes despite inhibition of viral polymerase. *J. Virol.*, **53**, 299–301

Sixbey, J.W. & Yao, Q.-Y. (1992) Immunoglobulin A — Induced shift of Epstein-Barr virus tissue tropism. *Science*, **255**, 1578–1580

Sixbey, J.W., Vesterinen, E.H., Nedrud, J.G., Raab-Traub, N., Walton, L.A. & Pagano, J.S. (1983) Replication of Epstein-Barr virus in human epithelial cells infected in vitro. *Nature*, **306**, 480–483

Sixbey, J.W., Nedrud, J.G., Raab-Traub, N., Hanes, R.A. & Pagano, J.S. (1984) Epstein-Barr virus replication in oropharyngeal epithelial cells. *New Engl. J. Med.*, **310**, 1225–1230

Sixbey, J.W., Lemon, S.M. & Pagano, J.S. (1986) A second site for Epstein-Barr virus shedding: The uterine cervix. *Lancet*, **ii**, 1122–1124

Sixbey, J.W., Davis, D.S., Young, L.S., Hutt-Fletcher, L., Tedder, T.F. & Rickinson, A.B. (1987) Human epithelial cell expression of an Epstein-Barr virus receptor. *J. gen. Virol.*, **68**, 805–811

Sixbey, J.W., Shirley, P., Chesney, P.J., Buntin, D.M. & Resnick, L. (1989) Detection of a second widespread strain of Epstein-Barr virus. *Lancet.*, **ii**, 761–765

Sjöblom, A., Nerstedt, A., Jansson, A. & Rymo, L. (1995a) Domains of the Epstein-Barr virus nuclear antigen 2 (EBNA2) involved in the transactivation of the latent membrane protein 1 and the EBNA Cp promoters. *J. gen. Virol.*, **76**, 2669–2678

Sjöblom, A., Jansson, A., Yang, W., Laín, S., Nilsson, T. & Rymo, L. (1995b) PU box-binding transcription factors and a POU domain protein cooperate in the Epstein-Barr virus (EBV) nuclear antigen 2-induced transactivation of the EBV latent membrane protein 1 promoter. *J. gen. Virol.*, **76**, 2679–2692

Skare, J., Farley, J., Strominger, J.L., Fresen, K.O., Cho, M.S. & zur Hausen, H. (1985) Transformation by Epstein-Barr virus requires DNA sequences in the region of BamHI fragments Y and H. *J. Virol.*, **55**, 286–297

Skare, J.C., Milunsky, A., Byron, K.S. & Sullivan, J.L. (1987) Mapping the X-linked lymphoproliferative syndrome. *Proc. natl Acad. Sci. USA*, **84**, 2015–2018

Skare, J., Wu, B.-L., Madan, S., Pulijaal, V., Purtilo, D., Haber, D., Nelson, D., Sylla, B., Grierson, H., Nitowsky, H., Glaser, J., Wissink, J., White, B., Holden, J., Housman, D., Lenoir, G., Wyandt, H. & Milunsky, A. (1993) Characterization of three overlapping deletions causing X-linked lymphoproliferative disease. *Genomics*, **16**, 254–255

Slivnick, D.J., Ellis, T.M., Nawrocki, J.F. & Fisher, R.I. (1990) The impact of Hodgkin's disease on the immune system. *Semin. Oncol.*, **17**, 673–682

Smith, E.C. & Elmes, B.G.T. (1934) Malignant disease in natives of Nigeria. *Ann. trop. Med. Parasitol.*, **28**, 461–512

Smith, P.R. & Griffin, B.E. (1991) Differential expression of Epstein Barr viral transcripts for two proteins (TP1 and LMP) in lymphocyte and epithelial cells. *Nucleic Acids Res.*, **19**, 2435–2440

Smith, P.R. & Griffin, B.E. (1992) Transcription of Epstein-Barr virus gene EBNA-1 from different promoters in nasopharyngeal carcinoma and B-lymphoblastoid cells. *J. Virol.*, **66**, 706–714

Smith, P.R., Gao, Y., Karran, L. Jones, M.D., Snudden D. & Griffin, B.E. (1993) Complex nature of the major viral polyadenylated transcripts in Epstein-Barr virus-associated tumors. *J. Virol.*, **67**, 3217–3225

Snudden, D.K., Hearing, J., Smith, P.R., Grässer, F.A. & Griffin, B.E. (1994) EBNA-1, the major nuclear antigen of Epstein-Barr virus, resembles 'RGG' RNA binding proteins. *EMBO J.*, **13**, 4840–4847

Snudden, D.K., Smith, P.R., Lai, D., Ng, M.H. & Griffin, B.E. (1995) Alterations in the structure of the EBV nuclear antigen, EBNA1, in epithelial cell tumors. *Oncogene*, **10**, 1545–1552

Sobrinho-Simões, M.A. & Areias, M.A. (1978) Relative high frequency of childhood Hodgkin's disease in the north of Portugal. *Cancer*, **42**, 1952–1956

Sohier, R. & de Thé, G. (1971) Complement fixation with a soluble antigen: Large differences in activity between the sera of Burkitt's lymphoma, rhinopharyngeal cancers and infectious mononucleosis. *C.R. Acad. Sci. Hebd. Séances Acad. Sci.*, **273**, 121–124 (in French)

Sohier, R & de Thé, G. (1972) Evolution of complement-fixing antibody titers with the development of Burkitt's lymphoma. *Int. J. Cancer*, **9**, 524–528

Spencer, C.A. & Groudine, M. (1991) Control of c-*myc* regulation in normal and neoplastic cells. *Adv. Cancer Res.*, **56**, 1–48

Spruck, C.H., III, Tsai, Y.C., Huang, D.P., Yang, A.S., Rideout, W.M., III, Gonzalez-Zulueta, M., Choi, P., Lo, K.-W., Yu, M.C. & Jones, P.A. (1992) Absence of p53 gene mutations in primary nasopharyngeal carcinomas. *Cancer Res.*, **52**, 4787–4790

Sriamporn, S., Vatanasapt, V., Pisani, P., Yongchaiyudha, S. & Rungpitarangsri, V. (1992) Environmental risk factors for nasopharyngeal carcinoma: A case–control study in northeastern Thailand. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 345–348

Srinivas, S.K. & Sixbey, J.W. (1995) Epstein-Barr virus induction of recombinase-activating genes RAG1 and RAG2. *J.Virol.*, **69**, 8155–8158

Sriskandan, S., Labrecque, L.G. & Schofield, J. (1996) Diffuse pneumonia associated with infectious mononucleosis: Detection of Epstein-Barr virus in lung tissue by in situ hybridization. *Clin. infect. Dis.*, **22**, 578–579

Staal, S.P., Ambinder, R., Beschorner, W.E., Hayward, G.S. & Mann, R. (1989) A survey of Epstein-Barr virus DNA in lymphoid tissue. Frequent detection in Hodgkin's disease. *Am. J. clin. Pathol.*, **91**, 1–5

Stanley, R. (1997) Clinical presentation and diagnosis. In: Chong, V.F.H. & Tsao, S.Y., eds, *Nasopharyngeal Carcinoma*, Singapore, Armour Publishing (in press)

Stansfeld, A., Diebold, J., Kapanci, Y., Kelenyi, G., Lennert, K., Mioduszewska, O., Noel, H., Rilke, F., Sundstrom, C., van Unnik, J. & Wright, D. (1988) Updated Kiel classification for lymphomas. *Lancet*, **i**, 292–293

Starzl, T.E., Nalesnik, M.A., Porter, K.A., Ho, M., Iwatsuki, S., Griffith, B.P., Rosenthal, J.T., Hakala, T.R., Shaw, B.W., Jr, Hardesty, R.L., Atchinson, R.W., Jaffe, R. & Bahnson, H.T. (1984) Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. *Lancet*, i, 583–587

Stayner, L.T., Elliott, L, Blade, L, Keenlyside, R. & Halperin, W. (1988) A retrospective cohort mortality study of workers exposed to formaldehyde in the garment industry. *Am. J. ind. Med.*, **13**, 667–681

Steinitz, R., Parkin, D.M., Young, J.L., Bieber, C.A. & Katz, L. (1989) *Cancer Incidence in Jewish Migrants to Israel, 1961–1981* (IARC Scientific Publications No. 98), Lyon, IARC

Sternås, L., Eliasson, L., Lerner, R. & Klein, G. (1986) Quantitation of Epstein-Barr virus (EBV)-determined nuclear antigen (EBNA) by a two-site enzyme immunoassay, in parallel with EBV-DNA. *J. immunol. Meth.*, **89**, 151–158

Steven, N.M., Leese, A.M., Annels, N.E., Lee, S.P. & Rickinson, A.B. (1996) Epitope focusing in the primary cytotoxic T cell response to Epstein-Barr virus and its relationship to T cell memory. *J. exp. Med.*, **184**, 1801–1813

Steven, N.M., Annels, N.E., Kumar, A., Leese, A.M., Kurilla, M.G. & Rickinson, A.B. (1997) Immediate early and late lytic cycle proteins are frequent targets of the Epstein-Barr virus-induced cytotoxic T cell response. *J. exp. Med.*, **185**, 1605–1617

Stevens, D.A., Kottaridis, S.D. & Luginbuhl, R.E. (1971) Investigation of antigenic relationship of Marek's disease, herpes virus and EB virus (herpes-type virus). *J. comp. Pathol.*, **81**, 137–140

Stevens, D.A., O'Conor, G.T., Levine, P.H. & Rosen, R.B. (1972) Acute leukemia with 'Burkitt's lymphoma cells' and Burkitt's lymphoma. Simultaneous onset in American siblings; description of a new entity. *Ann. intern. Med.*, **76**, 967–973

Stewart, J.P. & Arrand, J.R. (1993) Expression of the Epstein-Barr virus latent membrane protein in nasopharyngeal carcinoma biopsy specimens. *Hum. Pathol.*, **24**, 239–242

Stinson, W.D. (1940) Epidermoid carcinoma of the nasopharynx occurring in two young brothers. *Ann. Otolaryngol.*, **49**, 536–539

Stolzenberg, M.C., Debouze, S., Ng, M., Sham, J., Choy, D., Bouguermouh, A., Chan, K.H. & Ooka, T. (1996) Purified recombinant deoxyribonuclease in serological diagnosis of nasopharyngeal carcinoma. *Int. J. Cancer*, **66**, 337–341

Strang, G. & Rickinson, A.B. (1987a) In vitro expansion of Epstein-Barr virus-specific HLA-restricted cytotoxic T cells direct from the blood of infectious mononucleosis patients. *Immunology*, **62**, 647–654

Strang, G. & Rickinson, A.B. (1987b) Multiple HLA class I-dependent cytotoxicities constitute the 'non-HLA-restricted' response in infectious mononucleosis. *Eur. J. Immunol.*, **17**, 1007–1013

Straus, S.E., Tosato, G., Armstrong, G., Lawley, T., Preble, O.T., Henle, W., Davey, R., Pearson, G., Epstein, J., Brus, I. & Blaese, R.M. (1985) Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. *Ann. intern. Med.*, **102**, 7–16

Su, I.-J., Lin, K.-H., Chen, C.-J., Tien, H.-F., Hsieh, H.-C., Lin, D.-T. & Chen, J.-Y. (1990) Epstein-Barr virus-associated peripheral T-cell lymphoma of activated CD8 phenotype. *Cancer*, **66**, 2557–2562

Su, I.-J., Chen, R.-L., Lin, D.-T., Lin, K.-S. & Chen, C.-C. (1994) Epstein-Barr virus (EBV) infects T lymphocytes in childhood EBV-associated hemophagocytic syndrome in Taiwan. *Am. J. Pathol.*, **144**, 1219–1225

Subar, M., Neri, A., Inghirami, G., Knowles, D.M. & Dalla-Favera, R. (1988) Frequent c-*myc* oncogene activation and infrequent presence of Epstein-Barr virus genome in AIDS-associated lymphoma. *Blood*, **72**, 667-671

Sugano, H., Sakamoto, G., Sawaki, S. & Hirayama, T. (1978) Histopathological types of naso-pharyngeal carcinoma in a low-risk area: Japan. In: de Thé, G. & Ito Y., eds, *Naso-pharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 27–39

Sugden, B. & Warren, N. (1989) A promoter of Epstein-Barr virus that can function during latent infection can be transactivated by EBNA-1, a viral protein required for viral DNA replication during latent infection. *J. Virol.*, **63**, 2644–2649

Sugden, B., Phelps, M. & Domoradzki, J. (1979) Epstein-Barr virus DNA is amplified in trans-formed lymphocytes. *J. Virol.*, **31**, 590–595

Sugiura, M., Imai, S., Tokunaga, M., Koizumi, S., Uchizawa, M., Okamoto, K. & Osato, T. (1996) Transcriptional analysis of Epstein-Barr virus gene expression in EBV-positive gastric carcinoma: Unique viral latency in the tumour cells. *Br. J. Cancer*, **74**, 625–631

Sullivan, J.L. & Woda, B.A. (1989) X-Linked lymphoproliferative syndrome. *Immunodeficiency Rev.*, **1**, 325–347

Sun, Y., Hegameyer, G., Cheng, Y.J., Hildesheim, A., Chen, J.Y., Chen, I.H., Cao, Y., Yao, K.T. & Colburn, N.H. (1992) An infrequent point mutation of the p53 gene in human naso-pharyngeal carcinoma. *Proc. natl Acad. Sci. USA*, **89**, 6516–6520

Sun, Y., Hegameyer, G. & Colburn, N.H. (1993) Nasopharyngeal carcinoma shows no detectable retinoblastoma susceptibility gene alterations. *Oncogene*, **8**, 791–795

Sun, Y., Hildesheim, A., Lanier, A.E., Cao, Y., Yao, K.T., Raab-Traub, N. & Yang, C.S. (1995) No point mutation but decreased expression of the p16/MTS1 tumor suppressor gene in nasopharyngeal carcinomas. *Oncogene*, **10**, 785–788

Sundar, S.K., Levine, P.H., Ablashi, D.V., Leiseca, S.A., Armstrong, G.R., Cicmane, J.L., Parker, G.A. & Nonoyama, M. (1981) Epstein-Barr virus-induced malignant lymphoma in a white-lipped marmoset. *Int. J. Cancer*, **27**, 107–111

Sung, N.S., Kenney, S., Gutsch, D. & Pagano, J.S. (1991) EBNA-2 transactivates a lymphoid-specific enhancer in the *Bam*HI C promoter of Epstein-Barr virus. *J. Virol.*, **65**, 2164–2169

Sung, N.S., Wilson, J., Davenport, M., Sista, N.D. & Pagano, J.S. (1994) Reciprocal regulation of the Epstein-Barr virus *Bam*HI-F promoter by EBNA-1 and an E2F transcription factor. *Mol. cell. Biol.*, **14**, 7144–7152

Sunil-Chandra, N.P., Efstathiou, S. & Nash, A.A. (1992) Murine gammaherpesvirus 68 esta-blishes a latent infection in mouse B lymphocytes *in vivo*. *J. gen. Virol.*, **73**, 3275–3279

Sutkowski, N., Palkama, T., Ciurli, C., Sekaly, R.-P., Thorley-Lawson, D.A. & Huber, B.T. (1996a) An Epstein-Barr virus-associated superantigen. *J. exp. Med.*, **184**, 971–980

Sutkowski, N., Thorley-Lawson, D.A. & Huber, B.T. (1996b) The interplay of herpesviruses in AIDS: Superantigen sharing. *Trends Microbiol.*, **4**, 89–91

Svedmyr, E. & Jondal, M. (1975) Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients with infectious mononucleosis. *Proc. natl Acad. Sci. USA*, **72**, 1622–1626

Svedmyr, E., Ernberg, I., Seeley, J., Weiland, O., Masucci, G., Tsukuda, K., Szigeti, R., Masucci, M.G., Blomgren, H., Berthold, W., Henle, W. & Klein, G. (1984) Virologic, immunologic, and clinical observations on a patient during the incubation, acute, and convalescent phases of infectious mononucleosis. *Clin. Immunol. Immunopathol.*, **30**, 437–450

Swaminathan, S., Tomkinson, B. & Kieff, E. (1991) Recombinant Epstein-Barr virus with small RNA (EBER) genes deleted transforms lymphocytes and replicates *in vitro*. *Proc. natl Acad. Sci. USA*, **88**, 1546–1550

Swaminathan, S., Hesselton, R., Sullivan, J. & Kieff, E. (1993) Epstein-Barr virus recombinants with specifically mutated BCRF1 genes. *J. Virol.*, **67**, 7406–7413

Swerdlow, S.H. (1992) Post-transplant lymphoproliferative disorders: A morphologic, phenotypic and genotypic spectrum of disease. *Histopathology*, **20**, 373–385

Swinnen, L.J. (1996) Lymphoproliferative disorders following organ transplantation: Clinical syndromes and treatment options. *Epstein-Barr Virus Rep.*, **3**, 141–145

Sylla, B.S., Wang, Q, Hayoz, D., Lathrop, G.M. & Lenoir, G.M. (1989) Multipoint linkage mapping of the Xq25-q26 region in a family affected by the X-linked lymphoproliferative syndrome. *Clin. Genet.*, **36**, 459–462

Szekely, L., Selivanova, G., Magnusson, K.P., Klein, G. & Wiman, K.G. (1993) EBNA-5, an Epstein-Barr virus-encoded nuclear antigen, binds to the retinoblastoma and p53 proteins. *Proc. natl Acad. Sci. USA*, **90**, 5455–5459

Szekely, L., Pokrovskaja, K., Jiang, W.-Q., de Thé, H., Ringertz, N. & Klein, G. (1996) The Epstein-Barr virus-encoded nuclear antigen EBNA-5 accumulates in PML-containing bodies. *J. Virol.*, **70**, 2562–2568

Takada, K. (1984) Cross-linking of cell surface immunoglobulins induces Epstein-Barr virus in Burkitt lymphoma lines. *Int. J. Cancer*, **33**, 27–32

Takada, K. & Ono, Y. (1989) Synchronous and sequential activation of latently infected Epstein-Barr virus genomes. *J. Virol.*, **63**, 445–449

Takada, K., Shimizu, N., Tanabe-Tochikura, A. & Kuroiwa, Y. (1995) Pathogenic role of Epstein-Barr virus in human cancer. *Intervirology*, **38**, 214–220

Takahashi, H., Takeshita, T., Morein, B., Putney, S., Germain, R.N. & Berzofsky, J.A. (1990) Induction of CD8⁺ cytotoxic T cells by immunization with purified HIV-1 envelope protein in iscoms. *Nature*, **344**, 873–875

Takeshita, M., Akamatsu, M., Ohshima, K., Suzumiya, J., Kikuchi, M., Kimura, N., Uike, N. & Okamura, T. (1996) Angiocentric immunoproliferative lesions of the lymph node. *Am J. clin. Pathol.*, **106**, 69-77

Takimoto, T., Ogura, H., Sato, H., Umeda, R. & Hatano, M. (1985) Isolation of transforming and early antigen-inducing EBV from nasopharyngeal carcinoma hybrid cells (NPC-KT). *J. natl Cancer Inst.*, **74**, 57–60

Takimoto, T., Tanaka, S., Ishikawa, S. & Umeda, R. (1989) The human nasopharynx as a reservoir for Epstein-Barr virus. *Auris Nasus Larynx*, **16**, 109–115

Tamaru, J., Hummel, M., Marafioti, T., Kalvelage, B., Leoncini, L., Minacci, C., Tosi, P., Wright, D. & Stein, H. (1995) Burkitt's lymphomas express VH genes with a moderate number of antigen-selected somatic mutations. *Am. J. Pathol.*, **147**, 1398–1407

Tamura, S., Yamazaki, A., Kunimoto, M., Takemura, K., Tabata, T., Hinuma, Y. & Yoshie, O. (1992) Impaired long-term T cell immunity to Epstein-Barr virus in patients with nasopharyngeal carcinoma. *Jpn. J. Cancer Res.*, **83**, 445–449

Tamura, S., Kunimoto, M., Tabata, T. & Yoshie, O. (1993) Genotypic analysis of Epstein-Barr virus associated with nasopharyngeal carcinoma of Japanese patients. *Jpn. J. Cancer Res.*, **84**, 246–249

Tanaka, T., Komatsubara, S., Miyoshi, I., Hiraki, S., Tada, S., Kubonishi, I., Hujii, M. & Sezaki, T. (1976) Non-African Burkitt's lymphoma in a young woman. *Acta pathol. jpn.*, **26**, 619–628

Tannenbaum, S.R., Bishop, W., Yu, M.C. & Henderson, B.E. (1985) Attempts to isolate N-nitroso compounds from Chinese-style salted fish. *Natl Cancer Inst. Monogr.*, **69**, 209–211

Tanner, J.E. & Tosato, G. (1992) Regulation of B-cell growth and immunoglobulin gene transcription by interleukin-6. *Blood*, **79**, 452–459

Tanner, J., Weis, J., Fearon, D., Whang, Y. & Kieff, E. (1987) Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping and endocytosis. *Cell*, **50**, 203–213

Tanner, J., Whang, Y., Sample, J., Sears, A. & Kieff, E. (1988) Soluble gp350/220 and deletion mutant glycoproteins block Epstein-Barr virus adsorption to lymphocytes. *J. Virol.*, **62**, 4452–4464

Tanner, J.E., Alfieri, C., Chatila, T.A. & Diaz-Mitoma, F. (1996) Induction of interleukin-6 after stimulation of human B-cell CD21 by Epstein-Barr virus glycoproteins gp350 and gp220. *J. Virol.*, **70**, 570–575

Tao, Q., Srivastava, G., Chan, A.C.L., Chung, L.P., Loke, S.L. & Ho, F.C.S. (1995) Evidence for lytic infection by Epstein-Barr virus in mucosal lymphocytes instead of nasopharyngeal epithelial cells in normal individuals. *J. med. Virol.*, **45**, 71–77

Tao, Q., Srivastava, G., Dickens, P. & Ho, F.C. (1996) Detection of Epstein-Barr virus-infected mucosal lymphocytes in nasal polyps. *Am. J. Pathol.*, **149**, 1111–1118

Tartaglia, J., Perkus, M.E., Taylor, J., Norton, E.K., Audonnet, J-C., Cox, W.I., Davis, S.W., Van der Hoeven, J., Meignier, B., Riviere, M., Languet, B. & Paoletti, E. (1992) NYVAC: A highly attenuated strain of vaccinia virus. *Virology*, **188**, 217–232

Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. natl Acad. Sci. USA*, **79**, 7837–7841

Taylor, N., Flemington, E., Kolman, J.L., Baumann, R.P., Speck, S.H. & Miller, G. (1991) ZEBRA and a Fos-GCN4 chimeric protein differ in their DNA-binding specificities for sites in the Epstein-Barr virus BZLF1 promoter. *J. Virol.*, **65**, 4033–4041

Taylor, J., Weinberg, R., Tartaglia, J., Richardson, C., Alkhatib, G., Briedis, D., Appel, M., Norton, E. & Paoletti, E. (1992) Nonreplicating viral vectors as potential vaccines: Recombinant canarypox virus expressing measles virus fusion (F) and hemagglutinin (HA) glycoproteins. *Virology*, **187**, 321–328

Tefuarani, N., Vince, J.D., Murthy, D.P., Sengupta, S.K. & White, J.C. (1988) Childhood malignant tumours in Papua New Guinea. *Ann. trop. Paediatr.*, **8**, 201–206

Telford, E.A.R., Studdert, M.J., Agius, C.T., Watson, M.S., Aird, H.C. & Davison, A.J. (1993) Equine herpesvirus 2 and 5 are γ-herpesviruses. *Virology*, **195**, 492–499

Teoh, R., McGuire, L., Wong, K. & Chin, D. (1989) Increased incidence of thymoma in Chinese myasthenia gravis: Possible relationship with Epstein-Barr virus. *Acta neurol. scand.*, **80**, 221–225

de Thé, G. (1977) Is Burkitt's lymphoma related to perinatal infection by Epstein-Barr virus? *Lancet*, **i**, 335–337

de Thé, G. & Ito, Y., eds (1978) *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC

de Thé, G., Ho, H.C., Kwan, H.C., Desgranges, C. & Favre, M.C. (1970) Nasopharyngeal carcinoma (NPC). I. Types of cultures derived from tumour biopsies and non-tumorous tissues of Chinese patients with special reference to lymphoblastoid transformation. *Int. J. Cancer*, **6**, 189–206

de Thé, G., Ablashi, D.V., Liabeuf, A. & Mourali, N. (1973) Nasopharyngeal carcinoma (NPC). VI. Presence of an EBV nuclear antigen in fresh tumour biopsies. Preliminary results. *Biomedicine*, **19**, 349–352

de Thé, G., Ho, J.H.C., Ablashi, D.V., Day, N.E., Macario, A.J.L., Martin-Berthelon, M.C., Pearson, G. & Sohier, R. (1975) Nasopharyngeal carcinoma. IX. Antibodies to EBNA and correlation with response to other EBV antigens in Chinese patients. *Int. J. Cancer*, **16**, 713–721

de Thé, G., Geser, A. & Day, N.E.. (1978a) Epidemiological evidence for a causal relationship between Epstein-Barr virus and Burkitt's lymphoma from a Ugandan prospective study. *Nature*, **274**, 756–761

de Thé, G., Lavoué, M.-F. & Muenz, L. (1978b) Differences in EBV antibody titres of patients with nasopharyngeal carcinoma originating from high, intermediate and low incidence areas. In: de Thé, G. & Ito, Y., eds, *Nasopharyngeal Carcinoma: Etiology and Control* (IARC Scientific Publications No. 20), Lyon, IARC, pp. 471–481

Theodorakis, P., D'Sa-Eipper, C., Subramanian, T. & Chinnadurai, G. (1996) Unmasking of a proliferation-restraining activity of the anti-apoptosis protein EBV BHRF1. *Oncogene*, **12**, 1707–1713

Thijs, A. (1957) Remarks on malignant tumours among natives of the Belgian Congo and Ruanda-Urundi: 2536 cases. *Ann. Soc. belge Med. trop.*, **37**, 483–514 (in French)

Thomas, J.A., Hotchin, N.A., Allday, M.J., Amlot, P., Rose, M., Yacoub, M. & Crawford, D.H. (1990) Immunohistology of Epstein-Barr virus-associated antigens in B cell disorders from immunocompromised individuals. *Transplantation*, **49**, 944–953

Thomas, J.A., Felix, D.H., Wray, D., Southam, J.C., Cubie, H.A. & Crawford, D.H. (1991a) Epstein-Barr virus gene expression and epithelial cell differentiation in oral hairy leukoplakia. *Am. J. Pathol.*, **139**, 1369–1380

Thomas, J.A., Allday, M.J. & Crawford, D.H. (1991b) Epstein-Barr virus-associated lymphoproliferative disorders in immunocompromised individuals. *Adv. Cancer Res.*, **57**, 329–380

Thomason, R.W., Craig, F.E., Banks, P.M., Sears, D.L., Myerson, G.E. & Gulley, M.L. (1996) Epstein-Barr virus and lymphoproliferation in methotrexate-treated rheumatoid arthritis. *Mod. Pathol.*, **9**, 261–266

Thorley-Lawson, D.A. (1980) The suppression of Epstein-Barr virus infection *in vitro* occurs after infection but before transformation of the cell. *J. Immunol.*, **124**, 745–751

Thorley-Lawson, D.A. (1981) The transformation of adult but not newborn human lymphocytes by Epstein Barr virus and phytohemagglutinin is inhibited by interferon: The early suppression by T cells of Epstein Barr infection is mediated by interferon. *J. Immunol.*, **126**, 829–833

Thorley-Lawson, D.A. & Geilinger, K. (1980) Monoclonal antibodies against the major glyco-protein (gp350/220) of Epstein-Barr virus neutralize infectivity. *Proc. natl Acad. Sci. USA*, **77**, 5307–5311

Thorley-Lawson, D.A. & Poodry, C.A. (1982) Identification and isolation of the main compo-nent (gp350-gp220) of Epstein-Barr virus responsible for generating neutralizing antibodies *in vivo*. *J. Virol.*, **43**, 730–736

Tierney, R.J., Steven, N., Young, L.S. & Rickinson, A.B. (1994) Epstein-Barr virus latency in blood mononuclear cells: Analysis of viral gene transcription during primary infection and in the carrier state. *J. Virol.*, **68**, 7374–7385

Timens, W., Boes, A., Vos, H. & Poppema, S. (1991) Tissue distribution of the C3d/EBV-receptor: CD21 monoclonal antibodies reactive with a variety of epithelial cells, medullary thymocytes, and peripheral T-cells. *Histochemistry*, **95**, 605–611

Timmons, C.F., Dawson, D.B., Richards, C.S., Andrews, W.S. & Katz, J.A. (1995) Epstein-Barr virus-associated leiomyosarcomas in liver transplantation recipients. Origin from either donor or recipient tissue. *Cancer*, **76**, 1481–1489

Tirelli, U., Crivellari, D., Galligioni, E., Veronesi, A., Trovò, M.G., Tumolo, S., Grigoletto, E., Volpe, R. & Carbone, A. (1984) Malignant lymphoma, undifferentiated, Burkitt's type in north-east Italy. *Clin. Oncol.*, **10**, 129–140

Tirelli, U., Errante, D., Dolcetti, R., Gloghini, A., Serraino, D., Vaccher, E., Franceschi, S., Boiocchi, M. & Carbone, A. (1995) Hodgkin's disease and human immunodeficiency virus infection: Clinicopathologic and virologic features of 114 patients from the Italian Coope-rative Group on AIDS and Tumors. *J. clin. Oncol.*, **13**, 1758–1767

Tischendorf, P., Shramek, G.J., Balagtas, R.C., Deinhardt, F., Knospe, W.H., Noble, G.R. & Maynard, J.E. (1970) Development and persistence of immunity to Epstein-Barr virus in man. *J. infect. Dis.*, **122**, 401–409

Toczyski, D.P. & Steitz, J.A. (1991) EAP, a highly conserved cellular protein associated with Epstein-Barr virus small RNAs (EBERs). *EMBO J.*, **10**, 459–466

Tokunaga, M., Land, C.E., Uemura, Y., Tokudome, T., Tanaka, S. & Sato, E. (1993) Epstein-Barr virus in gastric carcinoma. *Am. J. Pathol.*, **143**, 1250–1254

Tomita, Y., Ohsawa, M., Kanno, H., Hashimoto, M., Ohnishi, A., Nakanishi, H. & Aozasa, K. (1996) Epstein-Barr virus in Hodgkin's disease patients in Japan. *Cancer*, **77**, 186–192

Tomkinson, B. & Kieff, E. (1992a) Use of second-site homologous recombination to demons-trate that Epstein-Barr virus nuclear protein 3B is not important for lymphocyte infection or growth transformation *in vitro*. *J. Virol.*, **66**, 2893–2903

Tomkinson, B. & Kieff, E. (1992b) Second-site homologous recombination in Epstein-Barr virus: Insertion of type 1 EBNA 3 genes in place of type 2 has no effect on in vitro infection. *J. Virol.*, **66**, 780–789

Tomkinson, B., Robertson, E. & Kieff, E. (1993) Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. *J. Virol.*, **67**, 2014–2025

Tong, X., Yalamanchili, R., Harada, S. & Kieff, E. (1994) The EBNA-2 arginine-glycine domain is critical but not essential for B-lymphocyte growth transformation; the rest of region 3 lacks essential interactive domains. *J. Virol.*, **68**, 6188–6197

Tong, X., Wang, F., Thut, C.J. & Kieff, E. (1995) The Epstein-Barr virus nuclear protein 2 acidic domain can interact with TFIIB, TAF40, and RPA70 but not with TATA-binding protein. *J. Virol.*, **69**, 585–588

Top, F.H., Jr, Büscher, E.L., Bancroft, W.H. & Russell, P.K. (1971a) Immunization with live types 7 and 4 adenovirus vaccines. II. Antibody response and protective effect against acute respiratory disease due to adenovirus type 7. *J. infect. Dis.*, **124**, 155–160

Top, F.H., Jr, Grossman, R.A., Bartelloni, P.J., Segal, H.E., Dudding, B.A., Russell, P.K., & Büscher, E.L. (1971b) Immunization with live types 7 and 4 adenovirus vaccines. I. Safety, infectivity, antigenicity, and potency of adenovirus type 7 vaccine in humans. *J. infect. Dis.*, **124**, 148–154

Törnell, J., Farzad, S., Espander-Jansson, A.F., Mateika, G., Isaksson, O. & Rymo, L. (1996) Expression of Epstein-Barr nuclear antigen 2 in kidney tubule cell induces tumor in transgenic mice. *Oncogene*, **12**, 1521–1528

Torsteinsdottir, S., Andersson, M.L., Avila-Carino, J., Ehlin-Henriksson, B., Masucci, M.G., Klein, G. & Klein, E. (1989) Reversion of tumorigenicity and decreased agarose clonability after EBV conversion of an IgH/myc translocation-carrying BL line. *Int. J. Cancer*, **43**, 273–278

Tosato, G., Magrath, I., Koski, I., Dooley, N. & Blaese, M. (1979) Activation of suppressor T cells during Epstein-Barr-virus-induced infectious mononucleosis. *New Engl. J. Med.*, **301**, 1133–1137

Touitou, R., Cochet, C. & Joab, I. (1996) Transcriptional analysis of the Epstein-Barr virus interleukin-10 homologue during the lytic cycle. *J. gen. Virol.*, **77**, 1163–1168

Trivedi, P., Hu, L.F., Chen, F., Christensson, B., Masucci, M.G., Klein, G. & Winberg, G. (1994) Epstein-Barr virus (EBV)-encoded membrane protein LMP1 from a nasopharyngeal carcinoma is non-immunogenic in a murine model system, in contrast to a B cell-derived homologue. *Eur. J. Cancer*, **30A**, 84–88

Trivedi, P., Zhang, Q.-J., Chen, F., Minarovits, J., Ekman, M., Biberfeld, P., Klein, G. & Winberg, G. (1995) Parallel existence of Epstein-Barr virus (EBV) positive and negative cells in a sporadic case of Burkitt lymphoma. *Oncogene*, **11**, 505–510

Trumper, P.A., Epstein, M.A., Giovanella, B.C. & Finerty, S. (1977) Isolation of infectious EB virus from the epithelial tumour cells of nasopharyngeal carcinoma. *Int. J. Cancer*, **20**, 655–662

Tsai, C.-N., Liu, S.-T. & Chang, Y.-S. (1995) Identification of a novel promoter located within the *Bam* HI Q region of the Epstein-Barr virus genome for the EBNA 1 gene. *DNA cell. Biol.*, **14**, 767–776

Tsai, C.-C., Chen, C.-L. & Hsu, H.-C. (1996a) Expression of Epstein-Barr virus in carcinomas of major salivary glands: A strong association with lymphoepithelioma-like carcinoma. *Hum. Pathol.*, **27**, 258–262

Tsai, S.-T., Jin, Y.-T. & Su, I.-J. (1996b) Expression of EBER1 in primary and metastatic nasopharyngeal carcinoma tissues using in situ hybridization: A correlation with WHO histologic subtypes. *Cancer*, **77**, 231–236

Tsang, S.-F., Wang, F., Izumi, K.M. & Kieff, E. (1991) Delineation of the *cis*-acting element mediating EBNA-2 transactivation of latent infection membrane protein expression. *J. Virol.*, **65**, 6765–6771

Tsang, W.Y.W., Chan, J.K.C., Yip, T.T.C., Ng, C.S., Wong, K.F., Poon., Y.F. & Ma, V.W.S. (1994) In situ localization of Epstein-Barr virus encoded RNA in non-nasal/nasopharyngeal CD56-positive and CD56-negative T-cell lymphomas. *Hum. Pathol.*, **25**, 758-765

Tun, T., Hamaguchi, Y., Matsunami, N., Furukawa, T., Honjo, T. & Kawaichi, M. (1994) Recognition sequence of a highly conserved DNA binding protein RBB-J kappa. *Nucleic Acids Res.*, **22**, 965-971

Turner, A.M., Berdoukas, V.A., Tobias, V.H., Ziegler, J.B., Toogood, I.R., Mulley, J.C., Skare, J. & Purtilo, D.T. (1992) Report on the X-linked lymphoproliferative disease in an Australian family. *J. Paediatr. Child Health*, **28**, 184-189

Tyan, Y.-S., Liu, S.-T., Ong, W.-R., Chen, M.-L., Shu, C.-H. & Chang, Y.-S. (1993) Detection of Epstein-Barr virus and human papillomavirus in head and neck tumors. *J. clin. Microbiol.*, **31**, 53-56

Tynell, E., Aurelius, E., Brandell, A., Julander, I., Wood, M., Yao, Q.-Y., Rickinson, A., Åkerlund, B. & Andersson, J. (1996) Acyclovir and prednisolone treatment of acute infectious mononucleosis: A multicenter, double-blind, placebo-controlled study. *J. infect. Dis.*, **174**, 324-331

Uccini, S., Monardo, F., Ruco, L.P., Baroni, C.D., Faggioni, A., Agliano, A.M., Gradilone, A., Manzari, V., Vago, L., Costanzi, G., Carbone, A., Boiocchi, M. & De Re, V. (1989) High frequency of Epstein-Barr virus genome in HIV-positive patients with Hodgkin's disease (Letter to the Editor). *Lancet*, **i**, 1458

Uccini, S., Monardo, F., Stoppacciaro, A., Gradilone, A., Agliano, A.M., Faggioni, A., Manzari, V., Vago, L., Costanzi, G., Ruco, L.P. & Baroni, C.D. (1990) High frequency of Epstein-Barr virus genome detection in Hodgkin's disease of HIV-positive patients. *Int. J. Cancer*, **46**, 581-585

Uen, W.C., Luka, J. & Pearson, G.T. (1988) Development of an enzyme-linked immunosorbent assay (ELISA) for detecting IgA antibodies to the Epstein-Barr virus. *Int. J. Cancer*, **41**, 479-482

Uhara, H., Sato, Y., Mukai, K., Akao, I., Matsuno, Y., Furuya, S., Hoshikawa, T., Shimosato, Y. & Saida, T. (1990) Detection of Epstein-Barr virus DNA in Reed-Sternberg cells of Hodgkin's disease using the polymerase chain reaction and in situ hybridization. *Jpn. J. Cancer Res.*, **81**, 272-278

Ulaeto, D., Wallace, L., Morgan, A., Morein, B. & Rickinson, A.B. (1988) In vitro T cell responses to a candidate Epstein-Barr virus vaccine: Human CD4$^+$ T cell clones specific for the major envelope glycoprotein gp340. *Eur. J. Immunol.*, **18**, 1689-1697

University Health Physicians and PHLS Laboratories (1971) A joint investigation: Infectious mononucleosis and its relationship to EB virus antibody. *Br. med. J.*, **iv**, 643-646

Van der Weid, T. & Langhorne, J. (1993) The role of cytokines produced in the immune response to the erythrocytic stages of mouse malarias. *Immunobiology*, **189**, 397-418

Vasef, M.A., Kamel, O.W., Chen, Y.-Y., Medeiros, L.J. & Weiss, L.M. (1995) Detection of Epstein-Barr virus in multiple sites involved by Hodgkin's disease. *Am. J. Pathol.*, **147**, 1408-1415

Vasef, M.A., Weiss, L.M., Chen, Y.-Y. & Medeiros, L.J. (1996) Gastric lymphoepithelioma-like carcinoma and jejunal B-cell MALT lymphoma with large cell transformation. *Am. J. Clin. Pathol.*, **105**, 560-566

Vaughan, T.L., Strader, C., Davis, S. & Daling, J.R. (1986a) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: I. Occupational exposures. *Int. J. Cancer*, **38**, 677–683

Vaughan, T.L., Strader, C., Davis, S. & Daling, J.R. (1986b) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: II. Residential exposures. *Int. J. Cancer*, **38**, 685–688

Vaughan, T.L., Shapiro, J.A., Burt, R.D., Swanson, G.M., Bewick, M., Lynch, C.F. & Lyon, J.L. (1996) Nasopharyngeal cancer in a low-risk population: Defining risk factors by histological type. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 587–593

Veltri, R.W., Shah, S.H., McClung, J.E., Klingberg, W.G. & Sprinkle, P.M. (1983) Epstein-Barr virus, fatal infectious mononucleosis, and Hodgkin's disease in siblings. *Cancer*, **51**, 509–520

Venkitaraman, A.R., John, T.J., Rangad, F., Singh, A.D., Date, A. & Lenoir, G. (1983) Epstein-Barr virus-associated Burkitt's lymphoma in India. *Trop. geogr. Med.*, **35**, 273–277

Vestlev, P.M., Pallesen, G., Sandvej, K., Hamilton-Dutoit, S.J. & Bendtzen, S.M. (1992) Prognosis of Hodgkin's disease is not influenced by Epstein-Barr virus latent membrane protein (Letter to the Editor). *Int. J. Cancer*, **50**, 670–671

Vieira, P., de Waal-Malefyt, R., Dang, M.-N., Johnson, K.E., Kastelein, R., Fiorentino, D.F., deVries, J.E., Roncarolo, M.-G., Mosmann, T.R. & Moore, K.W. (1991) Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: Homology to Epstein-Barr virus open reading frame BCRFI. *Proc. natl Acad. Sci. USA*, **88**, 1172–1176

Voevodin, A.F., Lapin, B.A., Yakovleva, A., Ponomarjeva, T.I. & Agrba, Z. (1979) Prevalence of antibodies to soluble antigen of Epstein-Barr virus producing cells (P3HR-1) in the sera of hamadryas baboons of the high lymphoma incidence stock. *Exp. Pathol.*, **17**, 517–520

Voevodin, A.F., Yakovleva, L.A., Lapin, B.A. & Ponomarjeva, T.I. (1983) Increased antibody responses to Herpes virus papio (HVP) antigens in pre-lymphomatous baboons (*Papio hamadryas*) of the Sukhumi high lymphoma stock. *Int. J. Cancer*, **32**, 637–639

Voevodin, A.F., Ponomarjeva, T.I. & Lapin, B.A. (1985) Seroepizootiology of the Herpesvirus Papio (HVP) infection in healthy baboons (*Papio hamadryas*) of high and low lymphoma risk populations. *Exp. Pathol.*, **27**, 33–39

Vonka, V., Vlcková, I., Závadová, H., Kouba, K., Lazovská, J. & Duben, J. (1972) Antibodies to EB virus capsid antigen and to soluble antigen of lymphoblastoid cells in infectious mononucleosis patients. *Int. J. Cancer*, **9**, 529–535

Wakasugi, N., Tagaya, Y., Wakasugi, H., Mitsui, A., Maeda, M., Yodoi, J. & Tursz, T. (1990) Adult T-cell leukemia-derived factor/thioredoxin, produced by both human T-lymphotropic virus type I- and Epstein-Barr virus-transformed lymphocytes, acts as an autocrine growth factor and synergizes with interleukin 1 and interleukin 2. *Proc. natl Acad. Sci. USA*, **87**, 8282–8286

Walker, R.C., Marshall, W.F., Strickler, J.G., Wiesner, R.H., Velosa, J.A., Habermann, T.M., McGregor, C.G. & Paya, C.V. (1995) Pretransplantation assessment of the risk of lymphoproliferative disorder. *Clin. infect. Dis.*, **20**, 1346–1353

Wallace, L.E., Moss, D.J., Rickinson, A.B., McMichael, A.J. & Epstein, M.A. (1981) Cytotoxic T cell recognition of Epstein-Barr virus-infected B cells. II. Blocking studies with monoclonal antibodies to HLA determinants. *Eur. J. Immunol.*, **11**, 694–699

Wallace, L.E., Rickinson, A.B., Rowe, M. & Epstein, M.A. (1982a) Epstein-Barr virus-specific cytotoxic T-cell clones restricted through a single HLA antigen. *Nature*, **297**, 413–415

Wallace, L.E., Rickinson, A.B., Rowe, M., Moss, D.J., Allen, D.J. & Epstein, M.A. (1982b) Stimulation of human lymphocytes with irradiated cells of the autologous Epstein-Barr virus-transformed cell line. I. Virus-specific and nonspecific components of the cytotoxic response. *Cell. Immunol.*, **67**, 129–140

Wallace, L.E., Rowe, M., Hill Gaston, J.S., Rickinson, A.B. & Epstein, M.A. (1982c) Cytotoxic T cell recognition of Epstein-Barr virus-infected B cells. III. Establishment of HLA-restricted cytotoxic T cell lines using interleukin 2. *Eur. J. Immunol.*, **12**, 1012–1018

Wallace, L.E., Wright, J., Ulaeto, D.O., Morgan, A.J. & Rickinson, A.B. (1991) Identification of two T-cell epitopes on the candidate Epstein-Barr virus vaccine glycoprotein gp340 recognized by CD4+ T-cell clones. *J. Virol.*, **65**, 3821–3828

Waller, E.K., Ziemianska, M., Bangs, C.D., Cleary, M., Weissman, I. & Kamel, O.W. (1993) Characterization of posttransplant lymphomas that express T-cell-associated markers: Immunophenotypes, molecular genetics, cytogenetics, and heterotransplantation in severe combined immunodeficient mice. *Blood*, **82**, 247–261

Walling, D.M., Neese Edmiston, S.N., Sixbey, J.W., Abdel-Hamid, M., Resnick, L. & Raab-Traub, N. (1992) Coinfection with multiple strains of the Epstein-Barr virus in human immunodeficiency virus-associated hairy leukoplakia. *Proc. natl Acad. Sci. USA*, **89**, 6560–6564

Walls, D. & Perricaudet, M. (1991) Novel downstream elements upregulate transcription initiated from an Epstein-Barr virus latent promoter. *EMBO J.*, **10**, 143–151

Walsh, S.V., Egan, L.J., Connolly, C.E., Stevens, F.M., Egan, E.L. & McCarthy, C.F. (1995) Enteropathy-associated T-cell lymphoma in the west of Ireland: Low-frequency of Epstein-Barr virus in these tumors. *Mod. Pathol.*, **8**, 753-757

Walter, J., Möller, P., Moldenhauer, G., Schirrmacher, V., Pawlita, M. & Wolf, J. (1992) Local growth of a Burkitt's lymphoma versus disseminated invasive growth of the autologous EBV-immortalized lymphoblastoid cells and their somatic cell hybrids in SCID mice. *Int. J. Cancer*, **50**, 265–273

Waltzer, L., Logeat, F., Brou, C., Israel, A., Sergeant, A. & Manet, E. (1994) The human J kappa recombination signal sequence binding protein (RBP-J kappa) targets the Epstein-Barr virus EBNA2 protein to its DNA responsive elements. *EMBO J.*, **13**, 5633–5638

Waltzer, L., Bourillot, P.Y., Sergeant, A. & Manet, E. (1995) RBP-Jκ repression activity is mediated by a co-repressor and antagonized by the Epstein-Barr virus transcription factor EBNA2. *Nucleic Acids Res.*, **23**, 4939–4935

Waltzer, L., Perricaudet, M., Sergeant, A. & Manet, E. (1996) Epstein-Barr virus EBNA3A and EBNA3C proteins both repress RBP-Jκ-EBNA2-activated transcription by inhibiting the binding of RBP-Jκ to DNA. *J. Virol.*, **70**, 5909–5915

Wang, D., Liebowitz, D. & Kieff, E. (1985) An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell*, **43**, 831–840

Wang, F., Petti, L., Braun, D., Seung, S. & Kieff, E. (1987a) A bicistronic Epstein-Barr virus mRNA encodes two nuclear proteins in latently infected, growth-transformed lymphocytes. *J. Virol.*, **61**, 945–954

Wang, F., Gregory, C.D., Rowe, M., Rickinson, A.B., Wang, D., Birkenbach, M., Kikutani, H., Kishimoto, T. & Kieff, E. (1987b) Epstein-Barr virus nuclear antigen 2 specifically induces expression of the B-cell activation antigen CD23. *Proc. natl Acad. Sci. USA*, **84**, 3452–3456

Wang, D., Liebowitz, D. & Kieff, E. (1988a) The truncated form of the Epstein-Barr virus latent-infection membrane protein expressed in virus replication does not transform rodent fibroblasts. *J. Virol.*, **62**, 2337–2346

Wang, D., Liebowitz, D., Wang, F., Gregory, C., Rickinson, A., Larson, R., Springer, T. & Kieff, E. (1988b) Epstein-Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: Deletion of the amino terminus abolishes activity. *J. Virol.*, **62**, 4173–4184

Wang, F., Gregory, C., Sample, C., Rowe, M., Liebowitz, D., Murray, R., Rickinson, A. & Kieff, E. (1990a) Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. *J. Virol.*, **64**, 2309–2318

Wang, F., Tsang, S.-F., Kurilla, M.G., Cohen, J.I. & Kieff, E. (1990b) Epstein-Barr virus nuclear antigen 2 transactivates latent membrane protein LMP1. *J. Virol.*, **64**, 3407–3416

Wang, F., Kikutani, H., Tsang, S.-F., Kishimoto, T. & Kieff, E. (1991) Epstein-Barr virus nuclear protein 2 transactivates a *cis*-acting CD23 DNA element. *J. Virol.*, **65**, 4101–4106

Wara, W.M., Wara, D.W., Phillips, T.L. & Ammann, A.J. (1975) Elevated IgA in carcinoma of the nasopharynx. *Cancer*, **35**, 1313–1315

Waterhouse, J., Muir, C.S., Shanmugaratnam, K. & Powell, J., eds (1982) *Cancer Incidence in Five Continents, Vol. IV* (IARC Scientific Publications No. 42), Lyon, IARC

Watry, D., Hedrick, J.A., Siervo, S., Rhodes, G., Lamberti, J.J., Lambris, J.D. & Tsoukas, C.D. (1991) Infection of human thymocytes by Epstein-Barr virus. *J. exp. Med.*, **173**, 971–980

Weber, B., Brunner, M., Preiser, W. & Doerr, H.W. (1996) Evaluation of 11 enzyme immunoassays for the detection of immunoglobulin M antibodies to Epstein-Barr virus. *J. virol. Meth.*, **57**, 87–93

Wedderburn, N. (1970) Effect of concurrent malarial infection on development of virus-induced lymphoma in Balb/c mice. *Lancet*, **ii**, 1114–1116

Wedderburn, N., Edwards, J.M.B., Desgranges, C., Fontaine, C., Cohen, B. & de Thé, G. (1984) Infectious mononucleosis-like response in common marmosets infected with Epstein-Barr virus. *J. infect. Dis.*, **150**, 878–882

Wei, M.X., Moulin, J.C., Decaussin, G., Berger, F. & Ooka, T. (1994) Expression and tumorigenicity of the Epstein-Barr virus *BARF1* gene in human Louckes B-lymphocyte cell line. *Cancer Res.*, **54**, 1843–1848

Weinberg, E., Hoisington, S., Eastman, A.Y., Rice, D.K., Malfetano, J. & Ross, J.S. (1993) Uterine cervical lymphoepithelial-like carcinoma. Absence of Epstein-Barr virus genomes. *Am. J. clin. Pathol.*, **99**, 195–199

Weinreb, M., Day, P.J.R., Niggli, F., Green, E.K., Nyong'o, A.O., Othieno-Abinya, N.A., Riyat, M.S., Raafat, F. & Mann, J.R. (1996a) The consistent association between Epstein-Barr virus and Hodgkin's disease in children in Kenya. *Blood*, **87**, 3828–3836

Weinreb, M., Day, P.J., Niggli, F., Powell, J.E., Raafat, F., Hesseling, P.B., Schneider, J.W., Hartley, P.S., Tzortzatou-Stathopoulou, F., Khalek, E.R.A., Mangoud, A., El-Safy, U.R., Madanat, F., Al Sheyyab, M., Mpofu, C., Revesz, T., Rafii, R., Tiedemann, K., Waters, K.D., Barrantes, J.C., Nyongo, A., Riyat, M.S. & Mann, J.R. (1996b) The role of Epstein-Barr virus in Hodgkin's disease from different geographical areas. *Arch. Dis. Child.*, **74**, 27–31

Weis, K., Rambaud, S., Lavau, C., Jansen, J., Carvalho, T., Carmo-Fonseca, M., Lamond, A. & Dejean, A. (1994) Retinoic acid regulates aberrant nuclear localization of PML-RARα in acute promyelocytic leukemia cells. *Cell*, **76**, 345–356

Weiss, L.M. & Movahed, L.A. (1989) In situ demonstration of Epstein-Barr viral genomes in viral-associated B cell lymphoproliferations. *Am. J. Pathol.*, **134**, 651–659

Weiss, L.M., Strickler, J.G., Warnke, R.A., Purtilo, D.T. & Sklar, J. (1987) Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am. J. Pathol.*, **129**, 86–91

Weiss, L.M., Warnke, R.A. & Sklar, J. (1988) Clonal antigen receptor gene rearrangements and Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Hematol. Oncol.*, **6**, 233–238

Weiss, L.M., Movahed, L.A., Warnke, R.A. & Sklar, J. (1989a) Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *New Engl. J. Med.*, **320**, 502-506

Weiss, L.M., Movahed, L.A., Butler, A.E., Swanson, S.A., Frierson, H.F., Jr, Cooper, P.H., Colby, T.V. & Mills, S.E. (1989b) Analysis of lymphoepithelioma and lymphoepithelioma-like carcinomas for Epstein-Barr viral genomes by in situ hybridization. *Am. J. surg. Pathol.*, **13**, 625–631

Weiss, L.M., Chen, Y.-Y., Liu, X.-F. & Shibata, D. (1991) Epstein-Barr virus and Hodgkin's disease: A correlative in situ hybridization and polymerase chain reaction study. *Am. J. Pathol.*, **139**, 1259–1265

Weiss, L.M., Gaffey, M.J., Chen, Y.-Y. & Frierson, H.F., Jr (1992a) Frequency of Epstein-Barr viral DNA in 'western' sinonasal and Waldeyer's ring non-Hodgkin's lymphomas. *Am. J. surg. Pathol.*, **16**, 156-162

Weiss, L.M., Jaffe, E.S., Liu, X.F., Chen, Y.-Y., Shibata, D. & Medeiros, L.J. (1992b) Detection and localization of Epstein-Barr viral genomes in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. *Blood*, **79**, 1789-1795

Weissmann, D.J., Ferry, J.A., Harris, N.L., Louis, D.N., Delmonico, F. & Spiro, I. (1995) Post-transplantation lymphoproliferative disorders in solid organ recipients and predominantly aggressive tumors of host origin. *Am. J. Pathol.*, **103**, 748–755

Wen, S., Shimizu, N., Yoshiyama, H., Mizugaki, Y., Shinozaki, F. & Takada, K. (1996) Association of Epstein-Barr virus (EBV) with Sjögren's syndrome: Differential EBV expression between epithelial cells and lymphocytes in salivary glands. *Am. J. Pathol.*, **149**, 1511–1517

Wen, S., Mizugaki, Y., Shinozaki, F. & Takada, K. (1997) Epstein-Barr virus (EBV) infection in salivary gland tumors: Lytic EBV infection in nonmalignant epithelial cells surrounded by EBV-positive T-lymphoma cells. *Virology*, **227**, 484–487

Weng, Y.-M., Hotchkiss, J.H. & Babish, J.G. (1992) *N*-Nitrosamine and mutagenicity formation in Chinese salted fish after digestion. *Food Addit. Contam.*, **9**, 29–37

Werner, J., Wolfe, H., Apodaca, J. & zur Hausen, H. (1975) Lymphoproliferative disease in a cotton-top marmoset after inoculation with infectious mononucleosis derived Epstein-Barr virus. *Int. J. Cancer*, **15**, 1000–1008

West, S., Hildesheim, A. & Dosemeci, M. (1993) Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: Results from a case–control study. *Int. J. Cancer*, **55**, 722–727

Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W. & Kieff, E., (1987) Expression of the Epstein-Barr virus gp350/220 gene in rodent and primate cells. *J. Virol.*, **61**, 1796–1807

White, C.A., Cross, S.M., Kurilla, M.G., Kerr, B.M., Schmidt, C., Misko, I.S., Khanna, R. & Moss, D.J. (1996) Recruitment during infectious mononucleosis of CD3⁺CD4⁺CD8⁺ virus-specific cytotoxic T cells which recognise Epstein-Barr virus lytic antigen BHRF1. *Virology*, **219**, 489–492

Whittle, H.C., Brown, J., Marsh, K., Greenwood, B.M., Seidelin, P., Tighe, H. & Wedderburn, L. (1984) T-Cell control of Epstein-Barr virus infected B-cells is lost during *P. falciparum* malaria. *Nature*, **312**, 449–450

WHO (1969) Burkitt's lymphoma. *WHO Bull.*, **40**

Williams, A.O. (1966) Haemoglobin genotypes, ABO blood groups and Burkitt's tumour. *J. med. Genet.*, **3**, 177–179

Williams, M.C. (1967) Implications of the geographical distribution of Burkitt's lymphoma. In: Burchenal, J.H. & Burkitt, D.P., eds, *Treatment of Burkitt's Lymphoma* (UICC Monograph Series Vol. 8), Berlin, Springer-Verlag, pp. 42–51

Williams, A.O. (1975) Tumours of children in Ibadan, Nigeria. *Cancer*, **36**, 370–378

Williams, D.L. & Kowalski, D. (1993) Easily unwound DNA sequences and hairpin structures in the Epstein-Barr virus origin of plasmid replication. *J. Virol.*, **67**, 2707–2715

Williams, E.H. & de Thé, G. (1974) Familial aggregation in nasopharyngeal carcinoma (Letter to the Editor). *Lancet*, **ii**, 295

Williams, E.H., Spit, P. & Pike, M.C. (1969) Further evidence of space–time clustering of Burkitt's lymphoma patients in the West Nile District of Uganda. *Br. J. Cancer*, **23**, 235–246

Williams, L.L., Rooney, C.M., Conley, M.E., Brenner, M.K., Krance, R.A. & Heslop, H.E. (1993) Correction of Duncan's syndrome by allogeneic bone marrow transplantation. *Lancet*, **342**, 587–588

Wilson, J.B., Weinberg, W., Johnson, R., Yuspa, S. & Levine, A.J. (1990) Expression of the BNLF-1 oncogene of Epstein-Barr virus in the skin of transgenic mice induces hyperplasia and aberrant expression of keratin 6. *Cell*, **61**, 1315–1327

Wilson, A.D., Shooshstari, M., Finerty, S., Watkins, P. & Morgan, A.J. (1996a) Virus-specific cytotoxic T cell responses are associated with immunity of the cottontop tamarin to Epstein-Barr virus (EBV). *Clin. exp. Immunol.*, **103**, 199–205

Wilson, W.H., Kingma, D.W., Raffeld, M., Wittes, R.E. & Jaffe, E.S. (1996b) Association of lymphomatoid granulomatosis with Epstein-Barr viral infection of B lymphocytes and response to interferon-α2b. *Blood*, **87**, 4531–4537

Wilson, J.B., Bell, J.L. & Levine, A.J. (1996c) Expression of Epstein-Barr virus nuclear antigen-1 induces B cell neoplasia in transgenic mice. *EMBO J.*, **15**, 3117–3126

Winnett, A., Thomas, S.J., Brabin, B.J., Bain, C., Alpers, M.A. & Moss, D.J. (1997) Familial Burkitt's lymphoma in Papua New Guinea. *Br. J. Cancer*, **75**, 757–761

Witter, R.L., Nazerian, K., Purchase, H.G. & Burgoyne, G.H. (1970) Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. *Am. J. vet. Res.*, **31**, 525–538

Wöckel, W., Höfler, G., Popper, H.H. & Morresi, A. (1995) Lymphoepithelioma-like carcinoma of the lung. *Pathol. Res. Pract.*, **191**, 1170–1174

Woisetschlaeger, M., Yandava, C.N., Furmanski, L.A., Strominger, J.L. & Speck, S.H. (1990) Promoter switching in Epstein-Barr virus during the initial stages of infection of B lymphocytes. *Proc. natl Acad. Sci. USA*, **87**, 1725–1729

Wolf, H., zur Hausen, H. & Becker, V. (1973) EB viral genomes in epithelial nasopharyngeal carcinoma cells. *Nature new Biol.*, **244**, 245–257

Wolf, H., Haus, M. & Wilmes, E. (1984) Persistence of Epstein-Barr virus in the parotid gland. *J. Virol.*, **51**, 795–798

Wong, K.M. & Levine, A.J. (1986) Identification and mapping of Epstein-Barr virus early antigens and demonstration of a viral gene activator that functions in trans. *J. Virol.*, **60**, 149–156

Wong, M.P., Chung, L.P., Yuen, S.T., Leung, S.Y., Chan, S.Y., Wang, E. & Fu, K.H. (1995) In situ detection of Epstein-Barr virus in non-small cell lung carcinomas. *J. Pathol.*, **177**, 233–240

Worth, R.M. & Valentine, R. (1967) Nasopharyngeal carcinoma in New South Wales, Australia. In: Muir, C.S. & Shanmugaratnam, K., eds, *Cancer of the Nasopharynx* (UICC Monograph Series, Vol. 1), Geneva, UICC, pp. 73–76

Wright, D.H. (1964) Burkitt's tumor — A post mortem study of 50 cases. *Br. J. Surg.*, **51**, 245–251

Wright, D.H. (1966) Burkitt's tumour in England. A comparison with childhood lymphosarcoma. *Int. J. Cancer*, **1**, 503–514

Wright, D.H. (1970) Gross distribution and hematology. In: Burkitt, D.P. & Wright, D.H., eds, *Burkitt's Lymphoma*, Edinburgh, E. & S. Livingstone, pp. 64–81

Wright, D.H. & Roberts, M. (1966) The geographical distribution of Burkitt's tumour compared with the geographical distribution of other types of malignant lymphoma in Uganda. *Br. J. Cancer*, **20**, 469–474

Wu, S.-B., Hwang, S.-J., Chang, A.-S., Hsieh, T., Hsu, M.-M., Hsieh R.-P. & Chen, C.-J. (1989) Human leukocyte antigen (HLA) frequency among patients with nasopharyngeal carcinoma in Taiwan. *Anticancer Res.*, **9**, 1649–1654

Wu, T.-C., Mann, R.B., Charache, P., Hayward, S.D., Staal, S., Lambe, B.C. & Ambinder, R.F. (1990) Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin's disease. *Int. J. Cancer*, **46**, 801–804

Wu, T.-C., Mann, R.B., Epstein, J.I., MacMahon, E., Lee, W.A., Charache, P., Hayward, S.D., Kurman, R.J., Hayward, G.S. & Ambinder, R.F. (1991) Abundant expression of EBER-1 small nuclear RNA in nasopharyngeal carcinoma. A morphologically distinctive target for detection of Epstein-Barr virus in formalin-fixed paraffin-embedded carcinoma specimens. *Am. J. Pathol.*, **138**, 1461–1469

Wu, T.T., Swerdlow, S.H., Locker, J., Bahler, D., Randhawa, P., Yunis, E.J., Dickman, P.S. & Nalesnik, M.A. (1996) Recurrent Epstein-Barr virus-associated lesions in organ transplant recipients. *Hum. Pathol.*, **27**, 157–164

Wutzler, P., Farber, I., Sprossig, M., Sauerbrei, A., Wutke, K., Hoche, D., Rudiger, K.D., Wockel, W. & Scheibner, K. (1983) Antibodies against herpesviruses in patients with Hodgkin's disease. *Arch. Geschwulstforsch.*, **53**, 417–422

Wutzler, P., Meerbach, A., Färber, I., Wolf, H. & Scheibner, K. (1995) Malignant lymphomas induced by an Epstein-Barr virus-related herpesvirus from *Macaca arctoides* — A rabbit model. *Arch. Virol.*, **140**, 1979–1995

Yalamanchili, R., Tong, X., Grossman, S., Johannsen, E., Mosialos, G. & Kieff, E. (1994) Genetic and biochemical evidence that EBNA 2 interaction with a 63-kDa cellular GTG-binding protein is essential for B lymphocyte growth transformation by EBV. *Virology*, **204**, 634–641

Yamamoto, N., Mitsuma, T. & Vogt, A. (1982) Tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate: Effect on complement and Epstein-Barr virus receptors in human lymphoblastoid cell lines. *J. natl Cancer Inst.*, **68**, 385–390

Yang, C.-S., Hsu, Y.V., Chan, T.-K., Yen, Y.-S., Chu, C.-T. & Tu, S.-M. (1982) Expression of Epstein-Barr virus genome in nude mouse grown nasopharyngeal carcinoma cells. *J. Formosan med. Assoc.*, **81**, 882–891

Yao, Q.Y., Rickinson, A.B. & Epstein, M.A. (1985a) Oropharyngeal shedding of infectious Epstein-Barr virus in healthy virus-immune donors. A prospective study. *Chin. med. J.*, **98**, 191–196

Yao, Q.Y., Rickinson, A.B. & Epstein, M.A. (1985b) A re-examination of the Epstein-Barr virus carrier state in healthy seropositive individuals. *Int. J. Cancer*, **35**, 35-42

Yao, Q.Y., Rickinson, A.B., Gaston, J.S.H. & Epstein, M.A. (1985c) *In vitro* analysis of the Epstein-Barr virus: Host balance in long-term renal allograft recipients. *Int. J. Cancer*, **35**, 43–49

Yao, Q.Y., Ogan, P., Rowe, M., Wood, M. & Rickinson, A.B. (1989a) Epstein-Barr virus-infected B cells persist in the circulation of acyclovir-treated virus carriers. *Int. J. Cancer*, **43**, 67–71

Yao, Q.Y., Ogan, P., Rowe, M., Wood, M. & Rickinson, A.B. (1989b) The Epstein-Barr virus: Host balance in acute infectious mononucleosis patients receiving acyclovir anti-viral therapy. *Int. J. Cancer*, **43**, 61–66

Yao, G.Q., Grill, S., Egan, W. & Cheng, Y.C. (1993) Potent inhibition of Epstein-Barr virus by phosphorothioate oligodeoxynucleotides without sequence specification. *Antimicrob. Agents Chemother.*, **37**, 1420–1425

Yao, G.-Q., Liu, S.-H., Chou, E., Kukhanova, M., Chu, C.K. & Cheng, Y.-C. (1996) Inhibition of Epstein-Barr virus replication by a novel L-nucleoside, 2′-fluoro-5-methyl-beta-L-arabinofuranosyluracil. *Biochem. Pharmacol.*, **51**, 941–947

Yates, J.L. & Guan, N. (1991) Epstein-Barr virus-derived plasmids replicate only once per cell cycle and are not amplified after entry into cells. *J. Virol.*, **65**, 483–488

Yates, J., Warren, N., Reisman, D. & Sugden, B. (1984) A *cis*-acting element from the Epstein-Barr viral genome that permits stable replication of recombinant plasmids in latently infected cells. *Proc. natl Acad. Sci. USA*, **81**, 3806–3810

Yates, J.L., Warren, N. & Sugden, B. (1985) Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature*, **313**, 812–815

Ye, B.H., Rao, P.H., Chaganti, R.S. & Dalla-Favera, R. (1993) Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. *Cancer Res.*, **53**, 2732–2735

Yeung, W.M., Zong, Y.S., Chiu, C.T., Chan, K.H., Sham, J.S., Choy, D.T. & Ng, M.H. (1993) Epstein-Barr virus carriage by nasopharyngeal carcinoma *in situ*. *Int. J. Cancer*, **53**, 746–750

Yokoyama, S., Staunton, D., Fisher, R., Amiot, M., Fortin, J.J. & Thorley-Lawson, D.A. (1991) Expression of the blast-1 activation/adhesion molecule and its identification as CD48. *J. Immunol.*, **146**, 2192–2200

Yoneda, N. Tatsumi, E., Kawanishi, M., Teshigawara, K., Masuda, S., Yamamura, Y., Inui, A., Yoshino, G., Oimomi, M., Baba, S. & Yamaguchi, N. (1990) Detection of Epstein-Barr virus genome in benign polyclonal proliferative T cells of a young male patient. *Blood*, **76**, 172–177

Yoshiyama, H., Shimizu, N. & Takada, K. (1995) Persistent Epstein-Barr virus infection in a human T-cell line: Unique program of latent virus expression. *EMBO J.*, **14**, 3706–3711

Yoshizaki, T., Takimoto, T., Takeshita, H., Tanaka, S., Furukawa, M., Seiki, M. & Sato, H. (1994) Epstein-Barr virus lytic cycle spreads via cell fusion in a nasopharyngeal carcinoma hybrid cell line. *Laryngoscope*, **104**, 91–94

Yoshizawa, K., Kiyosawa, K., Yamada, S., Furuta, K., Yabu, K., Kitano, K., Akamatsu, T., Nakayama, J., Katsuyama, T., Matsunami, H., Kawasaki, S., Makuuchi, M., Nanba, K. & Furuta, S. (1993) Establishment of Epstein-Barr virus-associated lymphoma cell line SUBL with t(2;3)(p11;q27) from a liver transplant patient. *Cancer Genet. Cytogenet.*, **71**, 155–163

Young, L.S., Clark, D., Sixbey, J.W. & Rickinson, A.B. (1986) Epstein-Barr virus receptors on human pharyngeal epithelia. *Lancet*, **i**, 240–242

Young, L.S., Yao, Q.Y., Rooney, C.M., Sculley, T.B., Moss, D.J., Rupani, H., Laux, G., Bornkamm, G.W. & Rickinson, A.B. (1987) New type B isolates of Epstein-Barr virus from Burkitt's lymphoma and from normal individuals in endemic areas. *J. gen. Virol.*, **68**, 2853–2862

Young, L.S., Dawson, C.W., Clar, D., Rupani, H., Busson, P., Tursz, T., Johnson, A. & Rickinson, A.B. (1988) Epstein-Barr virus gene expression in nasopharyngeal carcinoma. *J. gen. Virol.*, **69**, 1051–1065

Young, L.S., Alfieri, C., Hennessy, K., Evans, H., O'Hara, C., Anderson, K.C., Ritz, J., Shapiro, R.S., Rickinson, A., Kieff, E. & Cohen, J.I. (1989a) Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. *New Engl. J. Med.*, **321**, 1080–1085

Young, L.S., Finerty, S., Brooks, L., Scullion, F., Rickinson, A.B. & Morgan, A.J. (1989b) Epstein-Barr virus gene expression in malignant lymphomas induced by experimental virus infection of cottontop tamarins. *J. Virol.*, **63**, 1967–1974

Young, L.S., Dawson, C.W., Brown K.W. & Rickinson, A.B. (1989c) Identification of a human epithelial cell surface protein sharing an epitope with the C3d/Epstein-Barr virus receptor molecule of B lymphocytes. *Int. J. Cancer*, **43**, 786–794

Young, L.S., Lau, R., Rowe, M., Niedobitek, G., Packham, G., Shanahan, F., Rowe, D.T., Greenspan, D., Greenspan, J.S., Rickinson, A.B. & Farrell, P.J. (1991) Differentiation-associated expression of the Epstein-Barr virus BZLF1 transactivator protein in oral hairy leukoplakia. *J. Virol.*, **65**, 2868–2874

Yu, M.C., Ho, J.H.C., Ross, R.K. & Henderson, B.E. (1981) Nasopharyngeal carcinoma in Chinese — Salted fish or inhaled smoke? *Prev. Med.*, **10**, 15–24

Yu, M.C., Ho, J.H.C., Lai, S.-H. & Henderson, B.E. (1986) Cantonese-style salted fish as a cause of nasopharyngeal carcinoma: Report of a case–control study in Hong Kong. *Cancer Res.*, **46**, 956–961

Yu, M.C., Mo, C.-C., Chong, W.-X., Yeh, F.-S. & Henderson, B.E. (1988) Preserved foods and nasopharyngeal carcinoma: A case–control study in Guangxi, China. *Cancer Res.*, **48**, 1954–1959

Yu, M.C., Huang, T.-B. & Henderson, B.E. (1989a) Diet and nasopharyngeal carcinoma: A case–control study in Guangzhou, China. *Int. J. Cancer*, **43**, 1077–1082

Yu, M.C., Nichols, P.W., Zou, X.-N., Estes, J. & Henderson, B.E. (1989b) Induction of malignant nasal cavity tumours in Wistar rats fed Chinese salted fish. *Br. J. Cancer*, **60**, 198–201

Yu, M.C., Garabrant, D.H., Huang, T.-B. & Henderson, B.E. (1990) Occupational and other non-dietary risk factors for nasopharyngeal carcinoma in Guangzhou, China. *Int. J. Cancer*, **45**, 1033–1039

Yuen, S.T., Chung, L.P., Leung, S.Y., Luk, I.S.C., Chan, S.Y. & Ho, J. (1994) In situ detection of Epstein-Barr virus in gastric and colorectal adenocarcinomas. *Am. J. surg. Pathol.*, **18**, 1158–1163

Zajac-Kaye, M., Yu, B. & Ben-Baruch, N. (1990) Downstream regulatory elements in the c-myc gene. *Curr. Top. Microbiol. Immunol.*, **166**, 279–284

Zalani, S., Holley-Guthrie, E. & Kenney, S. (1996) Epstein-Barr viral latency is disrupted by the immediate-early BRLF1 protein through a cell-specific mechanism. *Proc. natl Acad. Sci. USA*, **93**, 9194–9199

Zarate-Osorno, A., Roman, L.N., Kingma, D.W., Meneses-Garcia, A. & Jaffe, E.S. (1994) Hodgkin's disease in Mexico. Prevalence of Epstein-Barr virus sequences and correlations with histologic subtype. *Cancer*, **75**, 1360–1366

Zech, L., Haglund, U., Nilsson, K. & Klein, G. (1976) Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int. J. Cancer*, **17**, 47–56

Zeng, Y. (1985) Seroepidemiological studies on nasopharyngeal carcinoma in China. *Adv. Cancer Res.*, **44**, 121–138

Zeng, Y. (1987) Prospective studies on nasopharyngeal carcinoma and Epstein-Barr virus inducers. In: Wagner, G. & Zhang, Y.-H., eds, *Cancer of the Liver, Esophagus, and Nasopharynx*, New York, Springer-Verlag, pp. 164–169

Zeng, Y., Shang, M., Liu, C.R., Chen, S.W., Wei, J.N., Zhu, J.S. & Zai, H.G. (1979) Detection of anti Epstein-Barr virus IgA in NPC patients in 8 provinces and cities in China. *Chin. Oncol.*, **1**, 2–7

Zeng, Y., Liu, Y., Liu, C., Chen, S., Wei, J., Zhu, J. & Zai, H. (1980) Application of an immunoenzymatic method and an immunoautoradiographic method for a mass survey of nasopharyngeal carcinoma. *Intervirology*, **13**, 162–168

Zeng, Y., Shen, S., Pi, G., Ma, J.L., Zhang, Q., Zhao, M.G. & Dong, H.J. (1981) Application of anticomplement immunoenzymatic method for the detection of EBNA in carcinoma cells and normal epithelial cells from the nasopharynx. In: Grundmann, E., Krueger, G.R.F. & Ablashi, D.V., eds, *Cancer Campaign*, Vol. 5, *Nasopharyngeal Carcinoma*, Stuttgart, Gustav Fischer Verlag, pp. 2–7

Zeng, Y., Zhang, L.G., Li, H.Y., Jan, M.G., Zhang, Q., Wu, Y.C., Wang, Y.S. & Su, G.R. (1982) Serological mass survey for early detection of nasopharyngeal carcinoma in Wuzhou City, China. *Int. J. Cancer*, **29**, 139–141

Zeng, Y., Zhong, J.M. & Li, L.Y. (1983a) Antibody-positive persons in Zangwu County, China. *Intervirology*, **20**, 190–194

Zeng, Y., Zhong, J.M., Mo, Y.K. & Miao, X.C. (1983b) Epstein-Barr virus early antigen induction in Raji cells by Chinese medicinal herbs. *Intervirology*, **19**, 201–204

Zeng, Y., Zhang, L.G. & Wu, Y.C. (1985) Prospective studies on nasopharyngeal carcinoma in Epstein-Barr virus IgA/VCA antibodies-positive persons in Wuzhon City. *China Int. J. Cancer*, **36**, 545–547

Zeng, Y., Hong, D., Jianming, J., Naiqin, H., Pingjun, L., Wenjun, P., Yuying, H., Yue, L., Peizhong, W. & de Thé, G. (1993) A 10-year prospective study on nasopharygneal carcinoma in Wuzhou City and Zangwu County, Guangxi, China. In: Tursz, T., Pagano, J., Ablashi, D.V., de Thé, G., Lenoir, G. & Pearson, G.R., eds, *The Epstein-Barr Virus and Associated Diseases* (Colloque INSERM No. 225), Paris, INSERM/John Libbey Eurotext Ltd, pp. 735–741

Zhang, P.F., Klutch, M., Armstrong, G., Qualtiere, L., Pearson, G. & Marcus-Sekura, C.J. (1991) Mapping of the epitopes of Epstein-Barr virus gp350 using monoclonal antibodies and recombinant proteins expressed in *Escherichia coli* defines three antigenic determinants. *J. gen. Virol.*, **72**, 2747–2755

Zhang, Q., Brooks, L., Busson, P., Wang, F., Charron, D., Kieff, E., Rickinson, A.B. & Tursz, T. (1994a) Epstein-Barr virus (EBV) latent membrane protein 1 increases HLA class II expression in an EBV-negative B cell line. *Eur. J. Immunol.*, **24**, 1467–1470

Zhang, Q., Gutsch, D. & Kenney, S. (1994b) Functional and physical interaction between p53 and BZLF1: Implications for Epstein-Barr virus latency. *Mol. cell. Biol.*, **14**, 1929–1938

Zhao, B., Marshall, D.R. & Sample, C.E. (1996) A conserved domain of the Epstein-Barr virus nuclear antigens 3A and 3C binds to a discrete domain of Jκ. *J. Virol.*, **70**, 4228–4236

Zheng, Y., Ohshima, H., Bouvier, G., Roy, P., Zhong, J., Li, B., Brouet, I., de Thé, G. & Bartsch, H. (1993) Urinary excretion of nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for nasopharyngeal carcinoma in southern China. *Cancer Epidemiol. Biomarkers Prev.*, **2**, 195–200

Zheng, X., Yan, L., Nilsson, B., Eklund, G. & Drettner, B. (1994a) Epstein-Barr virus infection, salted fish and nasopharyngeal carcinoma. A case–control study in southern China. *Acta oncol.*, **33**, 867–872

Zheng, Y.M., Tuppin, P., Hubert, A., Jeannel, D., Pan, Y.J., Zeng, Y. & de Thé, G. (1994b) Environmental and dietary risk factors for nasopharyngeal carcinoma: A case–control study in Zangwu County, Guangxi, China. *Br. J. Cancer*, **69**, 508–514

Zheng, X., Hu, L., Chen, F. & Christensson, B. (1994c) Expression of Ki67 antigen, epidermal growth factor receptor and Epstein-Barr virus-encoded latent membrane protein (LMP1) in nasopharyngeal carcinoma. *Eur. J. Cancer B Oral Oncol.*, **30B**, 290–295

Zheng, X., Luo, Y., Christensson, B. & Drettner, B. (1994d) Induction of nasal and nasopharyngeal tumours in Sprague-Dawley rats fed with Chinese salted fish. *Acta otolaryngol.*, **114**, 98–104

Zhou, X.-G., Hamilton-Dutoit, S.J., Yan, Q.-H. & Pallesen, G. (1993) The association between Epstein-Barr virus and Chinese Hodgkin's disease. *Int. J. Cancer*, **55**, 359–363

Zhou, X.-G., Hamilton-Dutoit, S.J., Yan, Q.-H. & Pallesen, G. (1994) High frequency of Epstein-Barr virus in Chinese peripheral T-cell lymphoma. *Histopathology*, **24**, 115–122

Zhu, K., Levine, R.S., Brann, E.A., Gnepp, D.R. & Baum, M.K. (1995) A population-based case–control study of the relationship between cigarette smoking and nasopharyngeal cancer (United States). *Cancer Causes Control*, **6**, 507–512

Ziegler, J.L., Bluming, A.Z., Morrow, R.H., Fass, L. & Carbone, P.P. (1970) Central nervous system involvement in Burkitt's lymphoma. *Blood*, **36**, 718–728

Ziegler, J.L., Andersson, M., Klein, G. & Henle, W. (1976) Detection of Epstein-Barr virus DNA in American Burkitt's lymphoma. *Int. J. Cancer*, **17**, 701–706

Ziegler, J.L., Newton, R., Katongole-Mbidde, E., Mbulataiye, S., DeCock, K., Wobinga, H., Mugerwa, J., Katabira, E., Jaffe, H., Parkin, D.M., Reeves, G., Weiss, R. & Beral, V. (1997) Risk factors for Kaposi's sarcoma in HIV positive subjects in Uganda. *AIDS*, **11**, 1619–1626

Zimber, U., Adldinger, H.K., Lenoir, G.M., Vuillaume, M., Knebel-Doeberitz, M.V., Laux, G., Desgranges, C., Wittmann, P., Freese, U.-K., Schneider, U. & Bornkamm, G.W. (1986) Geographical prevalence of two types of Epstein-Barr virus. *Virology*, **154**, 56–66

Zimber-Strobl, U., Suentzenich, K.O., Laux, G., Eick, D., Cordier, M., Calender, A., Billaud, M., Lenoir, G.M. & Bornkamm, G.W. (1991) Epstein-Barr virus nuclear antigen 2 activates transcription of the terminal protein gene. *J. Virol.*, **65**, 415–423

Zimber-Strobl, U., Kremmer, E., Grässer, F., Marschall, G., Laux, G. & Bornkamm, G.W. (1993) The Epstein-Barr virus nuclear antigen 2 interacts with an EBNA2 responsive *cis*-element of the terminal protein 1 gene promoter. *EMBO J.*, **12**, 167–175

Zimber-Strobl, U., Strobl, L.J., Meitinger, C., Hinrichs, R., Sakai, T., Furukawa, T., Honjo, T. & Bornkamm, G.W. (1994) Epstein-Barr virus nuclear antigen 2 exerts its transactivating function through interaction with recombination signal binding protein RBP-Jκ, the homologue of Drosophila suppressor of hairless. *EMBO J.*, **13**, 4973–4982

Zimber-Strobl, U., Kempkes, B., Marschall, G., Zeidler, R., van Kooten, C., Bancherau, J., Bornkamm, G.W. & Hammerschmidt, W. (1996) Epstein-Barr virus latent membrane protein (LMP1) is not sufficient to maintain proliferation of B cells but both it and activated CD40 can prolong their survival. *EMBO J.*, **15**, 7070–7078

Zutter, M.M., Martin, P.J., Sale, G.E., Shulman, H.M., Fisher, L., Thomas, E.D. & Durnam, D.M. (1988) Epstein-Barr virus lymphoproliferation after bone marrow transplantation. *Blood*, **72**, 520–529