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)*.