KAPOSI'S SARCOMA HERPESVIRUS/HUMAN HERPESVIRUS 8

1. Virus–Host Interactions

1.1 Taxonomy, structure and biology

1.1.1 *Taxonomy*

A new human herpesvirus was detected by Chang *et al.* (1994) in Kaposi's sarcomas associated with the acquired immune deficiency syndrome (AIDS; see IARC, 1996) by representational difference analysis. As it is detectable in virtually all cases of all the epidemiological forms of Kaposi's sarcoma, it is known as Kaposi's sarcoma-associated herpesvirus (KSHV). Recent seroepidemiological findings (see below) support a strong association between Kaposi's sarcoma and infection with this virus. In keeping with the nomenclature adopted for the two other recently discovered human herpesviruses (HHV), HHV6 and 7, however, and to allow for the fact that this virus is also associated with primary effusion lymphoma (body cavity-based lymphoma) and some cases of multicentric Castleman's disease, the formal designation human herpesvirus 8 (HHV8) was proposed by the herpesvirus subcommittee of the International Committee on the Taxonomy of Viruses. In this monograph, the term KSHV/HHV8 is used throughout to accommodate the two nomenclatures.

On the basis of phylogenetic analyses (Moore *et al.*, 1996a; Russo *et al.*, 1996), KSHV/HHV8 is a gamma-2 herpesvirus (rhadinovirus; see Table 2 in the Introduction) and represents the first 'human' member of this group. Of the rhadinoviruses of other species, KSHV/HHV8 appears so far to be most closely related to *Herpesvirus saimiri* and *Herpesvirus ateles*, two rhadinoviruses of New World monkeys, two herpesviruses of two macaque species, *Macaca nemestrina* and *Macaca mulatta*, murid herpesvirus 4 and bovine herpesvirus 4 (see Section 3).

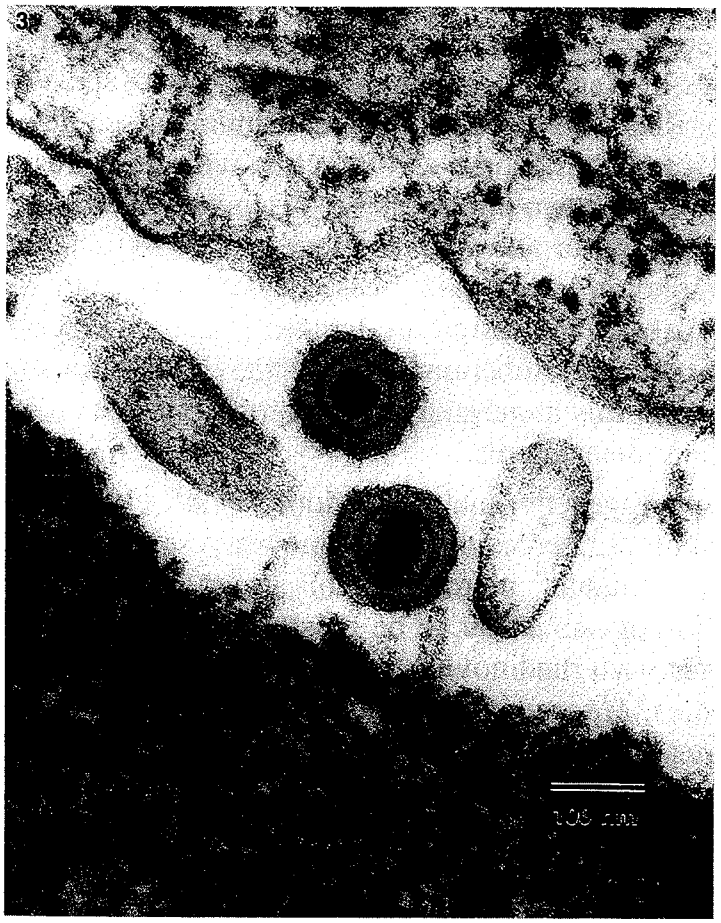
1.1.2 *Structure*

1.1.2.1 *Morphology*

KSHV/HHV8 has been shown to have some of the typical morphological characteristics of a herpesvirus in electron micrographs of KSHV/HHV8-infected primary effusion lymphoma cell lines (Arvanitakis *et al.*, 1996; Renne *et al.*, 1996a; Said *et al.*, 1996a), a transiently infected kidney-cell line (Foreman *et al.*, 1997) and biopsy samples of Kaposi's sarcomas (Walter *et al.*, 1984; Ioachim, 1995; Orenstein *et al.*, 1997) (Figure 1), with 100–150-nm particles surrounded by a lipid envelope and an electron-dense central core (Renne *et al.*, 1996a). Capsid substructures, such as ring-shaped capsomers of approximately 9 nm in diameter arranged in linear arrays, have also been

observed in viral preparations (Arvanitakis *et al.*, 1996; Said *et al.*, 1996a). Herpesviral particles have been observed in two Kaposi's sarcoma biopsy specimens (Walter *et al.*, 1984; Orenstein *et al.*, 1997). Hexagonal intranuclear capsids of 110 nm in diameter, with or without an internal core, and mature envelope virions of 140 nm in diameter located mainly within cytoplasmic cisternae and vacuoles were seen in these samples (Orenstein *et al.*, 1997) and are likely to represent KSHV/HHV8 particles. Before the discovery of KSHV/HHV8 (Chang *et al.*, 1994), herpesvirus particles were described in short-term cultures from Kaposi's sarcoma lesions (Giraldo *et al.*, 1972); however, these were later identified as cytomegalovirus (CMV) (Giraldo *et al.*, 1980).

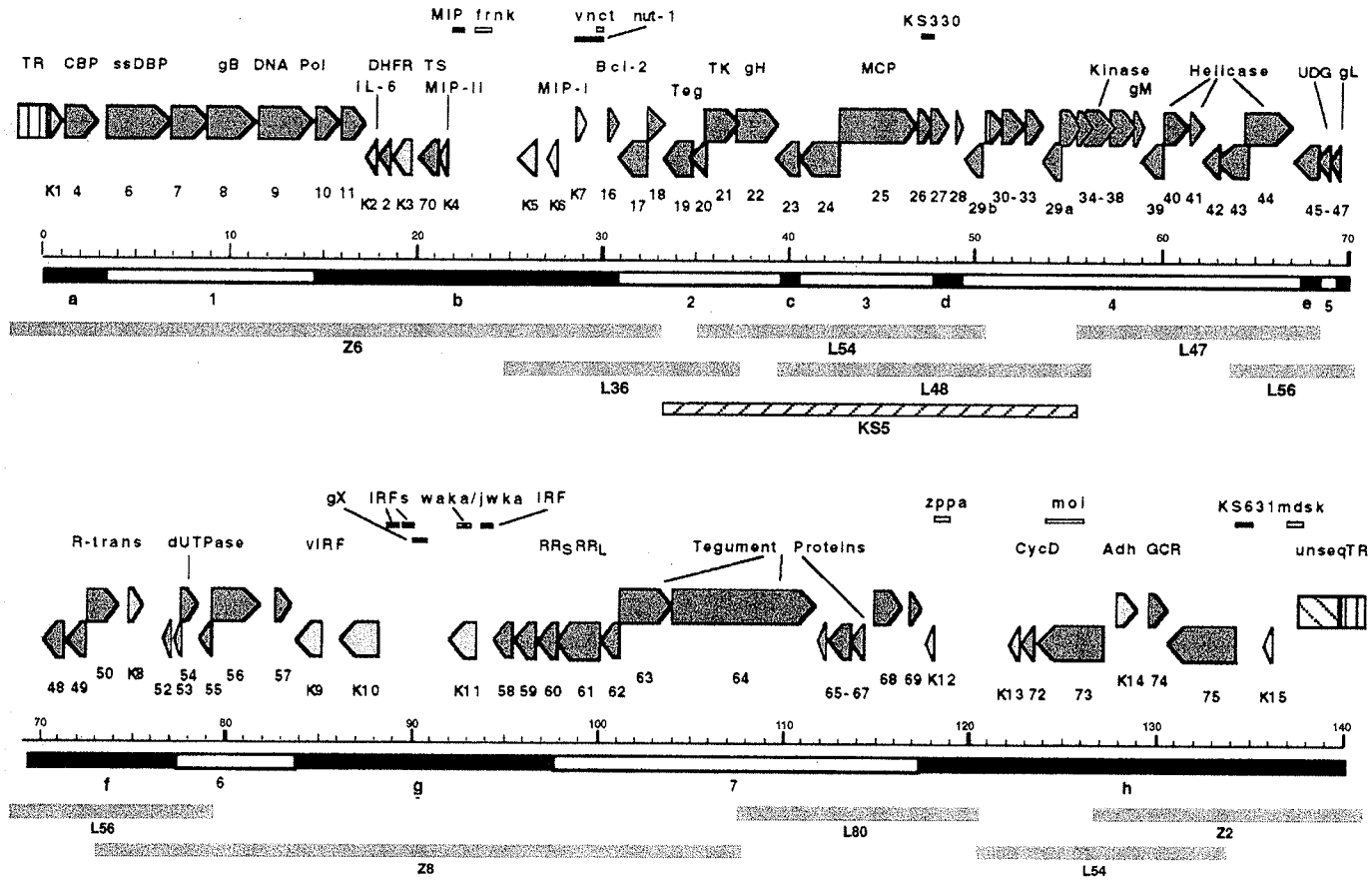
Figure 1. Electron microscopic view of KSHV/HHV8 capsid structure surrounded by a lipid envelope with an electron-dense central core representing viral DNA



1.1.2.2 *Genomic structure and properties of gene products*

The genomic structure of the virus (Russo *et al.*, 1996; Neipel *et al.*, 1997a) is similar to that of *Herpesvirus saimiri* (HVS) (Albrecht *et al.*, 1992), with a single, contiguous 140.5-kb long unique region containing all identified coding regions (Russo *et al.*, 1996; Neipel *et al.*, 1997a; Figure 2). This region is flanked on either side by a variable length terminal-repeat region composed of repeat units with a high G:C (84.5%) content, of approximately 800 base pairs.

Figure 2. Annotated long unique region and terminal repeats of the KSHV genome



From Russo *et al.* (1996)

The orientation of identified open reading frames in the long unique region is denoted by the direction of arrows, with *Herpesvirus saimiri* homologous with open reading frames as shaded areas and those not homologous as lighter areas. Seven blocks (numbered) of conserved herpesviral genes with nonconserved interblock regions (lettered) are shown under the kilobase marker. Features and putative coding regions not specifically designated are shown above the open reading frame map. Repeat regions (*frnk*, *vnct*, *waka/jwka*, *zppa*, *moi*, *mdsk*) are shown as light lines, and putative coding regions and other features not designated as open reading frames are shown as solid lines.

The size of the KSHV/HHV8 genome is calculated to be approximately 165 kb on the basis of studies of the genome banded from productive primary effusion lymphoma cells (Arvanitakis *et al.*, 1996; Renne *et al.*, 1996b) and confirmed by Gardella gel electrophoresis (Decker *et al.*, 1996) and mapping of the whole genome (Russo *et al.*, 1996). Larger estimates made earlier (~ 270 kb) (Mesri *et al.*, 1996; Moore *et al.*, 1996a) were based on analyses of the primary effusion lymphoma-derived BC-1 cell line, which contains a large (> 30 kb) genomic duplication. This genomic duplication was also found in another cell line, independently derived from the same tumour, and may therefore have been present in the parental lymphoma (Russo *et al.*, 1996).

(*a*) *Terminal-repeat region*

The terminal-repeat region is a conserved feature of herpesviruses and is involved in packaging of the viral DNA into new virions during the lytic cycle of replication. KSHV/HHV8 has approximately 30 terminal-repeat units. In the BC-1 strain, insertions of long unique region fragments have been observed in the terminal repeats. None of the open reading frames (ORFs) present in the Epstein-Barr virus (EBV; see monograph on Epstein-Barr virus) have so far been identified within the terminal-repeat region of KSHV/HHV8. Hybridization with the terminal-repeat region used as a probe is a sensitive method for detecting viral DNA (Russo *et al.*, 1996). In EBV, circularized viral genomes in latently infected cells maintain the length of their terminal repeats, and the presence of a clonal cell population arising from a single latently infected cell can therefore be demonstrated by Southern blot analysis with restriction enzymes that do not cut into the terminal repeat (Raab-Traub & Flynn, 1986). The restriction enzyme Taq I frequently cuts within the long unique region of the KSHV/HHV8 genome but not within the terminal repeat (Russo *et al.*, 1996). The potential application of this approach to determine the clonality of Kaposi's sarcoma lesions is discussed in section 4.1.1.

The mechanism of replication of KSHV/HHV8 is probably similar to that of other herpesviruses (Roizman, 1993), in which the genome is replicated as a rolling circle, monomeric genomes being cleaved within the terminal-repeat region to form linear genomes. Sequences homologous (similar) to HVS packaging and cleaving sites are present in the terminal-repeat unit sequence (Russo *et al.*, 1996). Linear genomes are packaged into virions, as demonstrated by pulse-field and Gardella gel electrophoresis studies of viral particles (Renne *et al.*, 1996b), and most likely recircularize at the terminal repeat after entry into the recipient cell.

(*b*) *Long unique region*

The 140.5-kb KSHV/HHV8 long unique region is larger than the corresponding regions of HVS and EBV, encoding at least 81 predicted ORFs (Russo *et al.*, 1996; Neipel *et al.*, 1997a). The ORFs were named according to the corresponding HVS genes with which they share a significant level of homology. Unique genes that are not homologous with HVS have a K prefix. It is likely that additional genes and alternatively transcribed ORFs will be identified experimentally. The long unique region has blocks of genes conserved among all subfamilies of herpesviruses (Chee *et al.*, 1990), which include genes that encode herpesvirus structural proteins and replication enzymes.

Homologues to several major herpesvirus glycoproteins (gB [encoded by *ORF 8*], gH [*ORF 22*], gM [*ORF 39*] and gL [*ORF 47*]) are encoded by KSHV/HHV8; and conserved capsid proteins (major capsid protein [*ORF 25*], VP23 [*ORF 26*]), other capsid proteins encoded by *ORFs 17, 43* and *65*, tegument proteins encoded by *ORFs 19, 63, 64, 67* and *75*, replication enzymes (DNA polymerase [*ORF 9*], helicases [*ORFs 40, 41* and *44*], DNA replication proteins [*ORFs 56* and *59*]) and enzymes involved in nucleic acid metabolism (thymidylate synthetase [*ORF 70*], thymidine kinase [*ORF 21*], uracil glucosidase [*ORF 46*], dUTPase [*ORF 54*], ribonucleotide reductases [*ORFs 60* and *61*]) are found in the long unique region (Russo *et al.*, 1996; see Table 1). These conserved proteins are likely to play an important role during the lytic replication cycle of KSHV/HHV8.

Between the conserved herpesvirus gene blocks lie blocks of genes that are either found only in rhadinoviruses or are unique to KSHV/HHV8 (Russo *et al.*, 1996). The majority of genes in this category share significant sequence similarity with cellular genes and were presumably pirated at some point during the evolution of these viruses. Thus, KSHV/HHV8 encodes a complement binding protein (encoded by *ORF 4*) that is related to a family of mammalian complement regulatory proteins and a similar protein in HVS, an interleukin (IL)-6 homologue (*ORF K2*) which is unique to KSHV/HHV8, two chemokine homologues related to macrophage inflammatory protein (MIP)-1α (viral [v]-MIP-I and v-MIP-II; encoded by *ORFs K6* and *K4*), a possible third chemokine homologue (encoded by a putative *ORF K4.1*), a bcl-2 homologue (*ORF 16*), a homologue of interferon regulatory factor (v-IRF; *ORF K9*), a homologue of a D-type cyclin (v-cyclin; *ORF 72*), an adhesion molecule homologue (OX-2; *ORF K14*) and a chemokine receptor homologue (*ORF 74*) (Russo *et al.*, 1996; Cesarman *et al.*, 1996a; Neipel *et al.*, 1997a,b; Nicholas *et al.*, 1997a,b). Also in this category are several proteins of unknown function: Unique to KSHV/HHV8 are the putative type-I transmembrane protein encoded by *ORF K1* and a putative small hydrophobic protein ('kaposin') encoded by *ORF K12*. Also found in some (e.g. HVS, bovine herpesvirus 4 [BHV-4], murine herpesvirus 68 [MHV-68]), but not other (e.g. equine herpesvirus 2) rhadinoviruses, is the latency-associated nuclear antigen (LANA) protein encoded by *ORF 73* (Russo *et al.*, 1996; Neipel *et al.*, 1997a; Rainbow *et al.*, 1997). The probable function of these genes in the virus life cycle and tumour formation is discussed in section 4.1.

1.1.3 *Strain variation*

As expected for a DNA virus, different KSHV/HHV8 isolates have highly conserved genomes. Several partial and complete genomic KSHV/HHV8 sequences have recently been reported for viruses found in both Kaposi's sarcoma lesions and primary effusion lymphoma cell lines, which are highly conserved (Russo *et al.*, 1996; Moore *et al.*, 1996a; Nicholas *et al.*, 1997a; Neipel *et al.*, 1997a). A comparison of a 20-kb region sequenced from both a Kaposi's sarcoma lesion and a primary effusion lymphoma cell line showed less than 0.1% nucleotide variation (Russo *et al.*, 1996). Several groups have reported a limited degree of sequence variation in *ORF 26* (Boshoff *et al.*, 1995a; Collandre *et al.*, 1995; Huang *et al.*, 1995; Moore & Chang, 1995; Marchioli *et al.*, 1996;

Table 1. KSHV/HHV8 open reading frames (ORFs) with homology to genes in other herpesviruses

Name	Pol.	Start	Stop	Size (aa)	KSHV/HHV8 vs. HVS		KSHV/HHV8 vs. EBV			Putative function
					% Sim.	% Id.	EBV name	% Sim.	% Id.	
K1	+	105	974	289						
ORF 4	+	1 142	2 794	550						Complement binding protein
(to 4a)					45.3	31.2				
(to 4b)					46.4	34.0				
ORF 6	+	3 210	6 611	1 133	74.1	55.2	*BALF2*	65.6	42.1	Single-strand DNA binding protein
ORF 7	+	6 628	8 715	695	65.0	44.7	*BALF3*	59.9	41.3	Transport protein
ORF 8	+	8 699	11 236	845	72.5	54.9	*BALF4*	62.1	42.6	Glycoprotein B
ORF 9	+	11 363	14 401	1 012	77.6	62.1	*BALF5*	70.9	55.6	DNA polymerase
ORF 10	+	14 519	15 775	418	50.4	26.2				
ORF 11	+	15 790	17 013	407	49.4	28.9	*Raji LF2*	44.4	27.9	
K2	–	17 875	17 261	204						IL-6 homologue
ORF 02	–	18 553	17 921	210	65.8	48.4				Dihydrofolate reductase
K3	–	19 609	18 608	333						BHV-4-IE1 homologue
ORF 70	–	21 104	20 091	337	79.5	66.4				Thymidylate synthase
K4	–	21 832	21 548	94						v-MIP-II
K5	–	26 483	25 713	257						BHV-4-IE1 homologue
K6	–	27 424	27 137	95						v-MIP-I
K7	+	28 622	29 002	126						
ORF 16	+	30 145	30 672	175	50.0	26.7	*BHRF1*	46.3	22.8	Bcl-2 homologue
ORF 17	–	32 482	30 821	553	60.3	42.9	*BVRF2*	58.8	34.3	Capsid protein
ORF 18	+	32 424	33 197	257	70.6	48.4				
ORF 19	–	34 843	33 194	549	62.8	43.8	*BVRF1*	62.5	42.0	Tegument protein
ORF 20	–	35 573	34 611	320	59.6	42.7	*BXRF1*	54.7	34.6	
ORF 21	+	35 383	37 125	580	50.9	32.5	*BXLF1*	50.7	28.2	Thymidine kinase
ORF 22	+	37 113	39 305	730	53.9	35.1	*BXLF2*	48.3	26.5	Glycoprotein H
ORF 23	–	40 516	39 302	404	57.4	33.7	*BTRF1*	51.0	31.0	

Table 1 (contd)

Name	Pol.	Start	Stop	Size (aa)	KSHV/HHV8 vs. HVS		KSHV/HHV8 vs. EBV			Putative function
					% Sim.	% Id.	EBV name	% Sim.	% Id.	
ORF 24	–	42 778	40 520	752	65.8	45.6	*BcRF1*	56.4	37.7	
ORF 25	+	42 777	46 907	1 376	80.9	65.8	*BcLF1*	74.8	56.8	Major capsid protein
ORF 26	+	46 933	47 850	305	76.8	58.3	*BDLF1*	73.4	48.8	Capsid protein
ORF 27	+	47 873	48 745	290	49.6	29.6	*BDLF2*	43.3	19.6	
ORF 28	+	48 991	49 299	102	42.2	21.7	*BDLF3*			
ORF 29b	–	50 417	49 362	351	41.8	17.0	*BDRF1*	43.3	16.3	Packaging protein
ORF 30	+	50 623	50 856	77	52.1	31.0	*BDLF3.5*			
ORF 31	+	50 763	51 437	224	63.0	43.5	*BDLF4*	58.9	36.4	
ORF 32	+	51 404	52 768	454	51.7	30.1	*BGLF1*	47.0	26.6	
ORF 33	+	52 761	53 699	312	58.6	36.4	*BGLF2*	52.8	32.2	
ORF 29a	–	54 676	53 738	312	41.9	15.8	*BGRF1*	57.1	40.6	Packaging protein
ORF 34	+	54 675	55 658	327	58.9	42.7	*BGLF3*	54.8	33.0	
ORF 35	+	55 639	56 091	151	60.0	31.7	*BGLF3.5*			
ORF 36	+	55 976	57 310	444	49.4	31.1	*BGLF4*	50.0	30.2	Kinase
ORF 37	+	57 273	58 733	486	65.9	50.4	*BGLF5*	60.1	42.7	Alkaline exonuclease
ORF 38	+	58 688	58 873	61	58.6	39.7	*BBLF1*	52.5	23.0	
ORF 39	–	60 175	58 976	399	73.2	52.1	*BBRF3*	65.2	43.6	Glycoprotein M
ORF 40	+	60 308	61 681	457	51.9	28.1	*BBLF2*	47.1	23.3	Helicase-primase
ORF 41	+	61 827	62 444	205	53.4	29.2	*BBLF3*			Helicase-primase
ORF 42	–	63 272	62 436	278	55.8	38.9	*BBRF2*	52.9	33.0	
ORF 43	–	64 953	63 136	605	74.9	60.5	*BBRF1*	67.6	50.1	Capsid protein
ORF 44	+	64 892	67 258	788	75.5	61.4	*BBLF4*	67.8	51.1	Helicase-primase
ORF 45	–	68 576	67 353	407	50.2	30.7	*BKRF4*	48.9	26.2	
ORF 46	–	69 404	68 637	255	73.0	59.5	*BKRF3*	69.2	54.8	Uracil DNA glucosidase
ORF 47	–	69 915	69 412	167	53.0	29.9	*BKRF4*	53.8	24.2	Glycoprotein L
ORF 48	–	71 381	70 173	402	47.3	24.4	*BRRF2*	46.1	18.8	
ORF 49	–	72 538	71 630	302	45.4	21.2	*BRRF1*	49.8	28.0	
ORF 50	+	72 734	74 629	631	46.5	24.9	*BRLF1*	41.4	19.0	Transactivator

Table 1 (contd)

Name	Pol.	Start	Stop	Size (aa)	KSHV/HHV8 vs. HVS		KSHV/HHV8 vs. EBV			Putative function
					% Sim.	% Id.	EBV name	% Sim.	% Id.	
K8	+	74 850	75 569	239						
ORF 52	–	77 197	76 802	131	50.0	33.3	*BLRF2*	54.6	36.9	
ORF 53	–	77 665	77 333	110	59.6	36.0	*BLRF1*	58.1	40.9	
ORF 54	+	77 667	78 623	318	55.0	35.5	*BLLF3*	53.7	32.4	dUTPase
ORF 55	–	79 448	78 765	227	64.4	46.4	*BSRF1*	61.6	44.0	
ORF 56	+	79 436	81 967	843	62.5	44.3	*BSLF1*	56.6	35.4	DNA replication protein
ORF 57	+	82 717	83 544	275	56.9	31.5	*BMLF1*	45.1	22.0	Immediate-early protein
K9	–	85 209	83 860	449						v-IRF1
K10	–	88 164	86 074	696						
K11	–	93 367	91 964	467						
ORF 58	–	95 544	94 471	357	55.9	28.7	*BMRF2*	50.6	25.3	
ORF 59	–	96 739	95 549	396	54.1	32.3	*BMRF1*	50.7	28.3	DNA replication protein
ORF 60	–	97 787	96 870	305	79.3	64.6	*BaRF1*	74.8	57.3	Ribonucleotide reductase, small
ORF 61	–	100 194	97 816	792	69.4	52.4	*BORF2*	64.1	43.6	Ribonucleotide reductase, large
ORF 62	–	101 194	100 199	331	64.6	40.2	*BORF1*	57.7	34.7	Assembly/DNA maturation
ORF 63	+	101 208	103 994	927	53.1	32.1	*BOLF1*	47.0	24.5	Tegument protein
ORF 64	+	104 000	111 907	2 635	50.1	29.7	*BPLF1*	46.6	26.1	Tegument protein
ORF 65	–	112 443	111 931	170	60.4	40.3	*BFRF3*	49.4	27.8	Capsid protein
ORF 66	–	113 759	112 470	429	58.7	34.7	*BFRF2*	50.0	28.0	
ORF 67	–	114 508	113 693	271	71.8	53.0	*BFRF1*	62.8	39.5	Tegument protein
ORF 68	+	114 768	116 405	545	64.7	45.4	*BFLF1*	58.3	36.2	Glycoprotein
ORF 69	+	116 669	117 346	225	71.1	53.6	*BFLF2*	60.7	41.7	
K12	–	118 101	117 919	60						Kaposin
K13	–	122 710	122 291	139						
ORF 72	–	123 566	122 793	257	53.0	32.5				Cyclin D homologue
ORF 73	–	127 296	123 808	1 162	51.2	31.8				Immediate-early protein

Table 1 (contd)

Name	Pol.	Start	Stop	Size (aa)	KSHV/HHV8 vs. HVS		KSHV/HHV8 vs. EBV			Putative function
					% Sim.	% Id.	EBV name	% Sim.	% Id.	
K14	+	127 883	128 929	348						OX-2 membrane glycoprotein homologue
ORF 74	+	129 371	130 399	342	57.8	34.1				G-Protein coupled receptor
ORF 75	–	134 440	130 550	1 296	54.8	36.3	*BNRF1*			Tegument protein/FGARAT
K15	–	136 279	135 977	100						

From Russo *et al.* (1996)

Pol., polarity; aa, amino acid; % Sim., percentage similar; % Id., percentage identical; ss, single-stranded; IL, interleukin; v, viral; MIP, macrophage inflammatory protein; IE, immediate-early; IRF, interferon regulatory factor; FGARAT, *N*-formylglycinamide ribotide amidotransferase

Zong *et al.*, 1997). In comparison with the corresponding genes in EBV and HVS, *ORF 26* is among the more highly conserved genes and is therefore probably not a very informative locus (Moore *et al.*, 1996a). Limited sequence variation has also been found within two regions of *ORF 75*. In a combined analysis of several genomic regions, Zong *et al.* (1997) found up to 1.5% overall nucleotide variation between isolates, which can be grouped into three different main variants, provisionally termed A, B and C. The high concentration of sequences in the genome in homosexual men in the United States suggests the recent introduction of predominant strains of KSHV/HHV8; however, further sequence and phylogenetic analyses are required to confirm this conclusion.

In HVS, the greatest degree of sequence variation between different isolates is found towards the left end of the genome, in a region that encodes the *STP* and *Tip* genes, which are essential for the transformation of T cells by this virus (Albrecht *et al.*, 1992). A comparative analysis of the KSHV/HHV8 sequence shows that *ORF K1* of KSHV/-HHV8, located at the left end of the genome, may vary more than the structural genes studied so far (Russo *et al.*, 1996; Lagunoff & Ganem, 1997; Neipel *et al.*, 1997a).

1.1.4 *Host range*

Humans appear to be the natural host for KSHV/HHV8. Recent seroepidemiological data (see below) indicate that KSHV/HHV8 is more prevalent in some regions (e.g. Africa, southern Europe) than in northern Europe and the United States (Gao *et al.*, 1996a; Kedes *et al.*, 1996; Lennette *et al.*, 1996; Simpson *et al.*, 1996). Whether KSHV/-HHV8 was recently introduced into the human population or is an ancient human herpesvirus remains to be determined.

No published evidence for natural infection of animal species by KSHV/HHV8 is currently available. The tropism of KSHV/HHV8 for individual cell lineages is discussed in section 1.1.6.

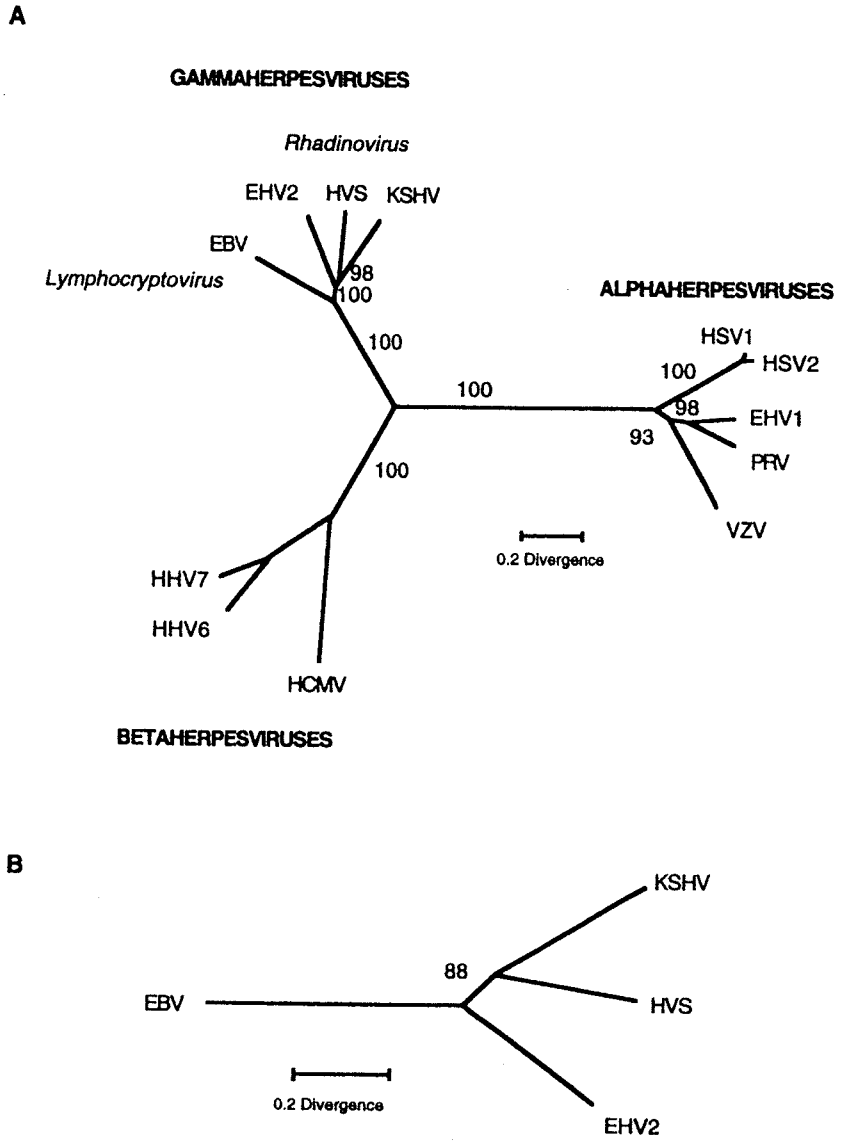
1.1.5 *Related non-human viruses*

The phylogenetic relationship of KSHV/HHV 8 to other rhadinoviruses is shown in Figure 3. As discussed in Section 3, KSHV/HHV8 is closely related to *Herpesvirus saimiri* of squirrel monkeys and to other rhadinoviruses of cattle and mice. Captive macaques belonging to two species, *M. nemestrina* and *M. mulatta*, have been shown to harbour two distinct viruses which are closely related to KSHV/HHV8 (Rose *et al.*, 1997) (see Section 3).

1.1.6 *Tropism and persistence in infected cells* in vivo

KSHV/HHV8 has been detected by the polymerase chain reaction (PCR), PCR–in-situ hybridization or conventional in-situ hybridization and immunohistochemistry, in endothelial and spindle cells of Kaposi's sarcoma lesions, in circulating endothelial cells, primary effusion lymphoma cells, B cells, macrophages, dendritic cells and prostatic glandular epithelium (Ambroziak *et al.*, 1995; Boshoff *et al.*, 1995b; Cesarman *et al.*, 1995a; Moore & Chang, 1995; Corbellino, 1996a; Li *et al.*, 1996; Moore *et al.*, 1996b; Rainbow *et al.*, 1997; Sirianni *et al.*, 1997; Staskus *et al.*, 1997; Stürzl *et al.*, 1997).

Figure 3. Phylogenetic trees of KSHV based on comparison of aligned amino-acid sequences in herpesviruses for the *MCP* gene and for a concatenated nine-gene set



From Moore *et al.* (1996a)

PRV, pseudorabies virus; VZV, varicella-zoster virus; HCMV, human cytomegalovirus; HHV6 and HHV7, human herpesviruses 6 and 7.

(A) *MCP* sequences were compared by the neighbour-joining method. The sequence is shown in unrooted form, with branch lengths proportional to divergence (mean number of substitution events per site) between the nodes bounding each branch. Comparable results (not shown) were obtained by maximum-parsimony analysis. The number of times (of 100 bootstrap samplings) that the division indicated by each internal branch was obtained is shown next to each branch; bootstrap values below 75 are not shown.

(B) Phylogenetic tree of gammaherpesvirus sequences based on a nine-gene set demonstrates that KSHV is most closely related to the gamma-2 herpesvirus sublineage, genus *Rhadinovirus*. The amino-acid sequence was used to infer a tree by the Protml maximum-likelihood method; comparable results (not shown) were obtained with the neighbour-joining and maximum-parsimony methods. The bootstrap value for the central branch is marked. On the basis of the *MCP* analysis, the root must lie between EBV and the other three species.

1.1.6.1 *Persistence and gene expression in infected endothelial cells*

Experiments with PCR–in-situ hybridization indicate that KSHV/HHV8 can infect the atypical endothelial cells lining the ectatic vascular spaces in Kaposi's sarcoma lesions and endothelial tumour (spindle) cells of fully developed, nodular Kaposi's sarcoma lesions, but is not generally present in normal endothelial cells (Boshoff *et al.*, 1995b; Li *et al.*, 1996). This has been confirmed by microdissection (Boshoff *et al.*, 1995b). More recently, in-situ hybridization (Staskus *et al.*, 1997) and immunohistochemistry with LANA (Rainbow *et al.*, 1997) have been used to confirm KSHV/HHV8 gene expression in Kaposi's sarcoma spindle cells within tumours. KSHV/HHV8 establishes a persistent infection in most of these spindle cells, as demonstrated by the expression of genes *ORF K12*, *ORF 72* and *ORF 73* (Rainbow *et al.*, 1997; Staskus *et al.*, 1997; Stürzl *et al.*, 1997). These genes are expressed in latently infected primary effusion lymphoma cell lines, and their expression is not increased by agents known to induce lytic viral replication (Cesarman *et al.*, 1996a; Renne *et al.*, 1996a). *ORF 72* encodes a homologue of mammalian D-type cyclins shown to be functionally active in phosphorylating the retinoblastoma tumour suppressor protein (Rb) in association with cyclin-dependent kinases (Chang *et al.*, 1996a; Li *et al.*, 1997). *ORF 73* encodes LANA, the function of which is still unknown (Rainbow *et al.*, 1997), and *ORF K12* may encode a small 60-amino acid, hydrophobic protein (Zhong *et al.*, 1996), also of unknown function. The KSHV/HHV8 homologue to IL-6 (encoded by *ORF K2*) is expressed during latency in primary effusion lymphoma, but is not generally expressed in Kaposi's sarcoma lesions (Moore *et al.*, 1996b).

The atypical flat endothelial cells that are found in nodular Kaposi's sarcoma and in the early stages (patch) of the disease have so far been found to express only the latent *ORF K12/T0.7* transcript (Rainbow *et al.*, 1997; Staskus *et al.*, 1997; Stürzl *et al.*, 1997); however, as *ORF K12/T0.7* transcripts are particularly abundant in both primary effusion lymphoma cell lines and Kaposi's sarcoma lesions (Renne *et al.*, 1996a; Zhong *et al.*, 1996), it is at present unclear whether *ORFs 72* and *73* are not expressed in endothelial cells or only more weakly expressed. A subpopulation (approximately 10%) of Kaposi's sarcoma spindle cells also expresses a polyadenylated nuclear RNA, *T1.1*, which may not encode a protein and is abundantly expressed in primary effusion lymphoma cell lines induced into lytic replication (Renne *et al.*, 1996a; Sun *et al.*, 1996; Zhong *et al.*, 1996; Staskus *et al.*, 1997). The distribution pattern of *T1.1*-expressing cells in Kaposi's sarcoma tissue is similar to that of a few lytically infected spindle cells, which can be defined by their expression of mRNA for the major capsid protein (encoded by *ORF 25*) (Staskus *et al.*, 1997). Expression of *T1.1* may therefore be indicative of lytic replication within Kaposi's sarcoma lesions. This suggests that a subpopulation of the spindle cells can produce KSHV/HHV8 virions, as suggested by three reports describing the presence of intranuclear herpesvirus-like particles of 120 nm in diameter or intranuclear inclusions characteristic of herpesviruses in Kaposi's sarcoma tissues (Walter *et al.*, 1984; Ioachim, 1995; Orenstein *et al.*, 1997). In addition to these viral genes, *ORF 74*, which encodes a homologue of a mammalian chemokine receptor, has been found to be expressed in

Kaposi's sarcoma lesions by reverse transcriptase (RT)-PCR (Cesarman *et al.*, 1996a), but the cell type that expresses this gene has not yet been identified.

Several independent lines of evidence therefore suggest that KSHV/HHV8 infects and persists in spindle cells *in vivo*. In contrast, all primary cell cultures established from Kaposi's sarcoma lesions lose detectable KSHV/HHV8 (Ambroziak *et al.*, 1995; Lebbé *et al.*, 1995; Aluigi *et al.*, 1996; Flamand *et al.*, 1996), and few cultures have been reported to maintain detectable KSHV/HHV8 for several passages (Lebbé *et al.*, 1995; Aluigi *et al.*, 1996). Two permanent Kaposi's sarcoma cell lines that are tumorigenic in severe combined immunodeficiency (SCID) and nude mice and contain chromosomal abnormalities (Siegal *et al.*, 1990; Lunardi-Iskandar *et al.*, 1995) also lack detectable KSHV/HHV8 (Flamand *et al.*, 1996).

1.1.6.2 *Persistence in haematopoietic cells*

The most highly expressed KSHV/HHV8 transcripts in primary effusion lymphoma cell lines are polyadenylated transcripts that encode *ORF K12* (*T 0.7*) and a nuclear untranslated RNA (*T1.1*) (Renne *et al.*, 1996a; Sun *et al.*, 1996). The *ORFs 16* (*bcl-2*), *72* (*CV-cyc*), *73* (*LANA*), *74* (*v-GCR*), *K2* (*v-IL-6*), *K4* (*v-MIP-II*), *K6* (*v-MIP-I*) and *K9* (*v-IRF*) are also expressed in primary effusion lymphoma cell lines but at lower levels (Cesarman *et al.*, 1996a; Moore *et al.*, 1996b; Rainbow *et al.*, 1997; Sarid *et al.*, 1997). A polyclonal antibody mono-specific for v-IL-6 has been used to demonstrate expression of this viral cytokine in KSHV/HHV8-infected haematopoetic cells in lymph nodes and in uninduced primary effusion lymphoma cell lines. Expression of *v-IL-6*, *v-MIP-I*, *v-MIP-II* and *v-IRF* can be induced by treatment with phorbol esters (Moore *et al.*, 1996b). Limited data are available on the persistence of KSHV/HHV8 in peripheral blood mononuclear cells (PBMC); B cells and macrophages may harbour KSHV/HHV8 genomes (Ambroziak *et al.*, 1995; Sirianni *et al.*, 1997). The presence of circular and linear KSHV/HHV8 genomes in PBMC was reported in one study, reflecting the presence of both latently and productively infected cells (Decker *et al.*, 1996).

1.1.6.3 *Presence in other tissues*

Several studies suggest the presence of KSHV/HHV8 in prostatic tissues of some infected men (Monini *et al.*, 1996a; Corbellino *et al.*, 1996b; Staskus *et al.*, 1997; see section 2.1.3), and KSHV/HHV8 is preferentially detected in semen rather than spermatocytes, suggesting secretion into seminal fluids (Monini *et al.*, 1996a; Howard *et al.*, 1997), although it cannot be excluded that KSHV/HHV8-infected mononuclear cells occasionally represent the source of KSHV/HHV8 in semen. In a survey of tissues from AIDS patients with Kaposi's sarcoma, Corbellino *et al.* (1996b) found that prostate tissues harboured the viral genome, suggesting that the prostate is a major site of infection in these patients. In-situ hybridization of prostatic glandular epithelium for a latent KSHV/HHV8 gene showed that viral gene expression is common in prostate biopsy samples from men without Kaposi's sarcoma (Staskus *et al.*, 1997), lending support to the supposition that the virus is widely disseminated in the healthy male population. Studies by PCR have not shown that prostatic tissue from men without Kaposi's sarcoma

is infected with KSHV/HHV8 (Corbellino *et al.*, 1996c; Tasaka *et al.*, 1996; Blackbourn & Levy, 1997; Rubin *et al.*, 1997).

It was suggested in one report that dorsal root ganglia in patients with AIDS and Kaposi's sarcoma harbour viral DNA (Corbellino *et al.*, 1996a).

KSHV/HHV8 has been detected in sputum, saliva, throat washing and bronchoalveolar lavage fluid, predominantly in patients with Kaposi's sarcoma (Howard *et al.*, 1995; Boldogh *et al.*, 1996; Koelle *et al.*, 1997).

1.2 Methods of detection

1.2.1 *Nucleic acids*

PCR is widely used to detect KSHV/HHV8 in clinical samples. The virus is detected consistently by PCR in biopsy samples from all epidemiological forms of Kaposi's sarcoma, including that in AIDS patients (Chang *et al.*, 1994), in persons who are not infected with human immunodeficiency virus (HIV) ('classic' Kaposi's sarcoma), in both Mediterranean countries and other geographical regions (Boshoff *et al.*, 1995a; Dupin *et al.*, 1995a; Moore & Chang, 1995; Schalling *et al.*, 1995; Chang *et al.*, 1996b; Buonaguro *et al.*, 1996; Luppi *et al.*, 1996a; Noel *et al.*, 1996), in iatrogenically immunosuppressed transplant recipients (Boshoff *et al.*, 1995a; Buonaguro *et al.*, 1996; Noel *et al.*, 1996) and in HIV-negative homosexual men (Boshoff *et al.*, 1995a; Moore & Chang, 1995). Unaffected tissues proximal to Kaposi's sarcoma lesions are more likely to have detectable viral genome than more distant tissues (Chang *et al.*, 1994; Boshoff *et al.*, 1995a; Dupin *et al.*, 1995a; Moore & Chang, 1995), suggesting that the virus is localized primarily to Kaposi's sarcoma lesions; however, it is also found in undiseased tissues, as discussed above.

The detection rate of KSHV/HHV8 DNA in all forms of Kaposi's sarcoma was about 95% in over 500 cases tested by PCR by numerous groups (for review, see Olsen & Moore, 1997; see Table 3 in section 2.1.3). A specific signal is almost always detected by PCR in DNA extracted from fresh or frozen Kaposi's sarcoma tissue samples after 30–35 amplification cycles. Detection can be enhanced by Southern blotting for the PCR product, but this does not reduce the likelihood of a false-positive result due to contamination. Formaldehyde-fixed, paraffin-embedded Kaposi's sarcoma tissue must often be tested by nested PCR in order to obtain a positive signal; this also dramatically increases the likelihood for intraexperimental contamination. It has been suggested that the PCR detection rate also depends on the histological stage of a Kaposi's sarcoma lesion and is higher in plaque and fully developed nodular lesions than in early patch lesions (Noel *et al.*, 1996). It has also been suggested that a decrease in viral DNA may precede the regression of Kaposi's sarcoma lesions in iatrogenically immunosuppressed patients, but these findings must be confirmed in larger case series (Aluigi *et al.*, 1996). As discussed in more detail in section 1.3, KSHV/HHV8 is also detected consistently in primary effusion lymphoma and some cases of multicentric Castleman's disease, as well as in lymphatic tissue, peripheral blood and semen from a proportion of KSHV/HHV8-infected individuals. The detection rates, even by nested PCR, of KSHV/HHV8 in

PBMC from patients with Kaposi's sarcoma are 50–60% (Whitby *et al.*, 1995; Moore *et al.*, 1996c; Blauvelt *et al.*, 1997; Lebbé *et al.*, 1997a; see section 2.1.3).

1.2.2 *Serology*

Immunofluorescence, western blot and enzyme-linked immunosorbent assays to detect antibodies against latent and lytic antigens of KSHV/HHV8 have been described. Most of the serological assays for KSHV/HHV8 used currently are based on B-cell lines derived from primary effusion lymphomas (Cesarman *et al.*, 1995b; Arvanitakis *et al.*, 1996; Gaidano *et al.*, 1996a; Gao *et al.*, 1996a; Renne *et al.*, 1996a; Said *et al.*, 1996a). These cell lines are latently infected with KSHV/HHV8. In the first report on immunofluorescence-based assays, the cell lines used (HBL-6, BC-1) were dually infected with KSHV/HHV8 and EBV (Moore *et al.*, 1996a), requiring absorption of EBV-specific antibodies to avoid cross-reactivity; however, lytic replication of KSHV/HHV8, but not EBV, could be induced in these cell lines with sodium butyrate, allowing the detection of antibodies to a prominent, 40-kDa, lytic (structural) antigen (Miller *et al.*, 1996). Although of limited use for determining the seroprevalence of KSHV/HHV8 in the general population, the results obtained with these early assays indicated that most individuals with AIDS-associated Kaposi's sarcoma and a much smaller proportion of HIV-infected individuals without Kaposi's sarcoma had antibodies to KSHV/HHV8 (Miller *et al.*, 1996; Moore *et al.*, 1996c).

When the nuclei of one of these dually infected cell lines (BC-1) were examined by western blot for the presence of KSHV/HHV8-specific nuclear antigens, a nuclear protein of high molecular mass (226/234 kDa) was found to react specifically with sera from Kaposi's sarcoma patients or those at increased risk for Kaposi's sarcoma (Gao *et al.*, 1996b): 80% of AIDS-associated Kaposi's sarcoma patients had antibodies to this 'latent nuclear antigen', whereas no sera from United States blood donors or HIV-infected patients with haemophilia were reactive. This antigen is not cross-reactive with EBV-specific antibodies. Thus, dually infected cell lines can be used that are readily amenable to large-scale culture.

Widespread screening of groups at risk for Kaposi's sarcoma and of the general population became possible when the first primary effusion lymphoma cell lines infected with KSHV/HHV8 alone were established. These cell lines express a latency-associated nuclear antigen (LANA), which is characterized by a typical speckled nuclear pattern (Gao *et al.*, 1996a; Kedes *et al.*, 1996). Detection of LANA by immunofluorescence assay correlates closely with reactivity to the 226/234-kDa nuclear antigen on western blots (Gao *et al.*, 1996b). The 226/234-kDa nuclear protein is encoded by KSHV/HHV8 *ORF 73*. Studies by immunoadsorption and recombinant antigens indicate that LANA is in part, and perhaps entirely, composed of the ORF 73 protein (Rainbow *et al.*, 1997). There is no homologue to the *ORF 73*-encoded nuclear protein in EBV, the most closely related known human gammaherpesvirus (Russo *et al.*, 1996).

Examination of panels of sera from populations at high and low risk for Kaposi's sarcoma suggests that antibodies to LANA predict the likelihood of Kaposi's sarcoma developing in AIDS patients. Only 0–3% of blood donors in the United States and United

Kingdom have antibodies to this latent nuclear protein (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Lennette *et al.*, 1996; Simpson *et al.*, 1996), but 80–90% of sera from AIDS patients with Kaposi's sarcoma and about 95% of sera from (non-immunosuppressed) 'classic' cases of Kaposi's sarcoma react with it under optimal circumstances (Gao *et al.*, 1996a; Kedes *et al.*, 1996; Simpson *et al.*, 1996). The assay is less sensitive when serum samples that have been repeatedly frozen and thawed are examined (Gao *et al.*, 1996b). Non-specific cross-reactive antibodies to cytoplasmic antigens interfere with the immunofluorescence at low serum dilutions, requiring either the isolation of whole nuclei (Kedes *et al.*, 1996) or use of diluted sera, usually 1/100–1/160 (Gao *et al.*, 1996b; Simpson *et al.*, 1996).

Lytic cycle (structural) KSHV/HHV8 antigens have also been found to react with sera from Kaposi's sarcoma patients. In addition to the 40-kDa structural protein discussed above, recognized by 67% of the sera from patients with Kaposi's sarcoma, some patients have antibodies to other lytic (structural) KSHV/HHV8 proteins of approximately 27 and 60 kDa (Miller *et al.*, 1996, 1997). While there is so far no indication that these proteins cross-react with the corresponding EBV proteins, their specificity requires further investigation. A 19-kDa capsid-related protein encoded by *ORF 65* has been used as a recombinant protein in enzyme-linked immunosorbent and western blot assays and is recognized by about 80% of sera from AIDS patients with Kaposi's sarcoma and 85–90% of those from patients with 'classic' Kaposi's sarcoma (Simpson *et al.*, 1996; Lin *et al.*, 1997). About 3–5% of blood donors in the United Kingdom and United States show reactivity to this protein. Its immunogenic determinants are located within the 80 amino acids in the carboxy terminal, and this region is 21% identical to the corresponding region in the EBV homologue *BFRF3*. Although vp19/ORF 65 is thus not recognized by most sera from EBV-positive individuals and did not react with a set of high-titre EBV-positive sera, the question of whether there may be occasional cross-reactivity with EBV is not completely resolved. Concordance between the recombinant vp19/ORF 65 antigen and latent immunofluorescence antigen is high (around 80–85%) in sera from patients with, or at risk for, Kaposi's sarcoma but lower in sera from blood donors in non-endemic regions (see below) and Africa. A combination of two or more antigen assays is likely to be required for optimal sensitivity (Simpson *et al.*, 1996).

Other recombinant lytic-phase proteins are being investigated as potential serological antigens. The minor capsid protein vp23, encoded by *ORF 26*, which was part of one of the original representational difference analysis fragments that characterize the virus, has been expressed as a recombinant protein and used as a serological antigen (André *et al.*, 1997; Davis *et al.*, 1997). Significantly more sera from AIDS patients with Kaposi's sarcoma than from HIV-negative controls react with this antigen, and no clear-cut evidence for EBV cross-reactivity was seen, despite the relatively high homology (49% amino acid identity) with its EBV homologue BDLF1. As this protein is recombinant, the antigen reacts with only about one-third of sera from AIDS patients with Kaposi's sarcoma (André *et al.*, 1997); however, a synthetic peptide from this region was reported to be recognized by 60% of sera from this group of patients (Davis *et al.*, 1997). The recombinant ORF 26 antigen was reported to react with the sera of only a few German blood donors (André *et al.*, 1997), whereas the ORF 26-derived synthetic peptide was

reactive with 20% of sera from United States blood donors (Davis *et al.*, 1997). A recombinant carboxy-terminal fragment of the major capsid protein encoded by *ORF 25* has also been investigated and shown to cross-react with high-titre EBV antibodies (André *et al.*, 1997). This observation is in line with the high homology (56% amino acid identity) of the ORF 25 protein with its EBV homologue, BclF1 (Moore *et al.*, 1996a).

Other assays involve the detection of antibodies to unknown lytic antigens. These include an immunofluorescence assay on a primary effusion lymphoma cell line treated with phorbol esters to induce the lytic replication cycle (Lennette *et al.*, 1996; Smith *et al.*, 1997; Ablashi *et al.*, 1997). With these assays, antibodies can be detected in nearly 100% of Kaposi's sarcoma patients and in 0–20% of United States blood donors. The specificity of this lytic antigen assay should be clarified. While Lennette *et al.* (1996), using a serum dilution of 1:10, found antibodies to lytic KSHV/HHV8 antigens in 20% of United States blood donors, Smith *et al.* (1997), using a 1:40 serum dilution and Evan's blue to reduce nonspecific background staining, found no antibodies in 52 blood donors. Smith *et al.* (1997) noted that sera that are reactive by immunofluorescence at a dilution of < 1:40 cannot be confirmed as reactive by radioimmunoprecipitation. The demonstration that cytoplasmic seroreactivity to primary effusion lymphoma cells infected with KSHV/HHV8 can be cross-adsorbed by EBV-containing cell lines not infected with KSHV/HHV8 (Gao *et al.*, 1996a) suggests that nonspecific reactivity in lytic antigen assays could be a major problem. Whole virion antigens may be of use in the design of competitive assays that are less sensitive to cross-reactive antibodies.

Experience with these assays suggests that LANA is a very specific antigen and can be used to detect most but not all cases of KSHV/HHV8 infection. Of the lytic KSHV/-HHV8 antigens, the recombinant vp19/ORF 65 protein gives comparable detection rates. The specificity of immunofluorescence in induced primary effusion lymphoma cells varies with the assay protocol used, and the optimal conditions for these assays should be established. While the correlation among these different assays is good for sera from Kaposi's sarcoma patients, there is considerable variation with regard to sera from blood donors in non-endemic areas.

1.2.3 *Culture* in vitro

No efficient culture system that results in a high titre of KSHV/HHV8 transmission is presently available. Some latently infected primary effusion lymphoma cell lines can be induced into lytic replication of KSHV/HHV8 by treatment with phorbol esters or sodium butyrate (Renne *et al.*, 1996a; Miller *et al.*, 1997). Some transmission of viral DNA from the BC-1 cell line to Raji, BJAB, Molt-3 and OMK cell lines and to cord blood lymphocytes (Mesri *et al.*, 1996; Moore *et al.*, 1996a) has been described; however, the replication competence of KSHV/HHV8 from BC-1 is unclear, given its large genomic duplication. KSHV/HHV8 has been directly cultured from Kaposi's sarcoma lesions in embryonal kidney 293 recipient cells (Foreman *et al.*, 1997). While 293 cells allow serial propagation of KSHV/HHV8, viral amplification is limited, requiring PCR amplification for virus detection.

1.3 Epidemiology of infection

Limited prevalence estimates in various risk and geographic groups are available from case–control studies and surveys. PCR-based estimates of prevalence in PBMC or semen are of limited value because of the low copy number of KSHV/HHV8 in these samples, which results in underestimates of the true prevalence. Measurement of KSHV/HHV8 infection in various tissues by PCR is discussed more fully in the context of case–control studies (section 2.1.3). The advantage of examining PBMC by PCR is that samples are readily acquired from various populations; serological studies to examine prevalence have the advantage that any antibodies detected may reflect both past and current viral infection. As indicated in section 1.2.2, test methods may differ in sensitivity and specificity, resulting in widely different estimates.

1.3.1 *Prevalence in peripheral blood mononuclear cells*

Several groups have attempted to detect KSHV/HHV8 in PBMC from healthy individuals, usually studied as control groups for patients with conditions linked to infection with KSHV/HHV8. When PBMC from healthy individuals in countries with a low prevalence of Kaposi's sarcoma (e.g. France, the United Kingdom, the United States; see below) were studied, no KSHV/HHV8 genomes were detected by nested PCR (Ambroziak *et al.*, 1995; Whitby *et al.*, 1995; Lefrère *et al.*, 1996; Marchioli *et al.*, 1996). In Italy, a country where 'classic' Kaposi's sarcoma is present, KSHV/HHV8 was detected in 9% of PBMC and lymphoid tissues from HIV-uninfected individuals (Bigoni *et al.*, 1996). In Uganda, where endemic Kaposi's sarcoma is common, KSHV/HHV8 was detected in 14% of patients with tumours other than Kaposi's sarcoma (Chang *et al.*, 1996b). These studies were consistent in finding a higher prevalence of KSHV/HHV8 in PBMC in populations at higher risk of developing Kaposi's sarcoma. The correlation between the detection of KSHV/HHV8 in peripheral blood of individuals and the presence of or risk for Kaposi's sarcoma is discussed in Section 2.

1.3.2 *Prevalence in semen*

On the assumption that, if KSHV/HHV8 can be sexually transmitted, the virus may be present at high copy number in semen, prevalence studies based on semen samples might result in higher rates of detection than those based on PBMC.

The presence of KSHV/HHV8 in the semen of healthy men is controversial. It is detected in some semen samples from HIV-infected patients with or without Kaposi's sarcoma, but the reported detection rates in the United Kingdom and the United States vary from 0 (only four samples tested) to 33% (Ambroziak *et al.*, 1995; Gupta *et al.*, 1996; Marchioli *et al.*, 1996; Monini *et al.*, 1996b; Howard *et al.*, 1997). The results obtained in semen samples from healthy, HIV-seronegative donors are even more controversial in view of the implications of the prevalence of KSHV/HHV8 in the general population. Samples from Italian semen donors were initially reported to be 91% positive (Monini *et al.*, 1996a) but later to be 23% positive (Monini *et al.*, 1996b), whereas not a single positive sample was found among 115 semen donors in the United Kingdom (Howard *et al.*, 1997) or in 20 in Milan, Italy (Corbellino *et al.*, 1996c). Some of these

discrepant results probably reflect regional differences in KSHV/HHV8 prevalence (as shown by serological studies) and/or selection of semen donors. A high detection rate was reported in HIV-negative semen donors in the United States (Lin *et al.*, 1995). [The Working Group noted that this rate may be flawed and requires confirmation.]

1.3.3 *Seroprevalence and geographical distribution*

The use of different serological assays for KSHV/HHV8-specific antibodies has resulted in uncertainty about the exact seroprevalence of this virus in different populations and geographical areas. Most groups agree, however, that antibodies to KSHV/-HHV8 are found in most, if not all, patients with Kaposi's sarcoma and are more common in individuals at risk for this disease than in the general population; it is also generally agreed that the virus is not as widespread in the west as, for example, EBV. Antibodies to the latent nuclear antigen are found in about 85% of AIDS patients with Kaposi's sarcoma and in more than 90% of individuals with 'classic' Kaposi's sarcoma (Gao *et al.*, 1996b; Kedes *et al.*, 1996; Simpson *et al.*, 1996). Among individuals studied in Denmark, the United Kingdom and the United States, about one-third of HIV-infected homosexual men without Kaposi's sarcoma (Simpson *et al.*, 1996; Melbye *et al.*, 1998), 8% of HIV-uninfected persons attending sexually transmitted disease clinics (10 out of 130), 0–3% of HIV-uninfected blood donors, 0–3 % of patients with haemophilia and no intravenous drug users had antibodies to this antigen (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996). Women in the United States, who are at low risk for AIDS-associated Kaposi's sarcoma, have correspondingly low LANA antibody titres, regardless of HIV status (Kedes *et al.*, 1997). Antibody positivity to vp19/ORF 65 shows a very similar distribution: 81% of patients with AIDS-associated Kaposi's sarcoma, 94% of those with 'classic' Kaposi's sarcoma, 31% of HIV-infected homosexual men without Kaposi's sarcoma, 2–5% of HIV-negative blood donors, 1% of patients with haemophilia and 3% of intravenous drug users (Simpson *et al.*, 1996). Therefore, the distribution of antibodies to both these antigens would suggest that KSHV/HHV8 is an uncommon infection in the general populations of those countries where it is likely to be sexually transmitted.

The phorbol ester-induced lytic immunofluorescence antigen assay may be even more sensitive (> 95%) than either vp19/ORF 65 or LANA assays. Lennette *et al.* (1996) found that the prevalence of antibodies to this antigen in the adult North American population ranged from 16 to 28%; that for patients with AIDS-associated Kaposi's sarcoma was 96–100%, that for HIV-infected homosexual men, 90%, and that for intravenous drug users, 23%. It is unclear whether the higher values represent increased sensitivity, cross-reactivity with EBV or other human herpesviruses or both. Nonetheless, results from all of these assays broadly concur in suggesting that in Europe and North America KSHV/HHV8 is markedly more common among homosexual men than in other risk groups for HIV transmission. Thus, the distribution of KSHV/HHV8 mirrors that of Kaposi's sarcoma, which has long been known to occur more frequently among HIV-infected homosexual men than among patients with haemophilia, transfusion recipients or intravenous drug users (Beral *et al.*, 1990).

Although lower seroprevalences for antibodies to vp19/ORF 65 and LANA/ORF 73 are found in the general populations of northern Europe and North America than for lytic immunofluorescence antibodies, much higher rates are found in several Mediterranean countries. In Milan, Italy, a region with a relatively low incidence of Kaposi's sarcoma, the seroprevalence among blood donors was found to be 4% by latent immunofluorescence antigen and western blot assays (Gao *et al.*, 1996a). In Greece, 12% of HIV-negative surgical patients without Kaposi's sarcoma were seropositive for ORF 65/vp19 and/or ORF 73/LANA (Simpson *et al.*, 1996), suggesting that the seroprevalence in endemic countries may be much higher than in northern Europe or the United States.

The KSHV/HHV8 seroprevalence to these two antigens is much higher (> 50%) in countries of East, Central and West Africa (Gao *et al.*, 1996a; Lennette *et al.*, 1996; Simpson *et al.*, 1996). This suggests that infection with KSHV/HHV8 may approach near universal levels in some African populations (50–70% seroprevalence). Within Europe, KSHV/HHV8 appears to be more common in regions known for their higher incidence of endemic Kaposi's sarcoma, although a careful comparison of the incidence of Kaposi's sarcoma with KSHV/HHV8 seroprevalence in southern Europe is required before definitive conclusions can be reached. These marked differences in seroprevalence within Europe may also help to explain some of the discrepant reports on KSHV/HHV8 genome prevalence detected by PCR in various populations.

Reports from other parts of the world are limited. The prevalence rates in the Caribbean and Central America have been found to be between 0% as determined by latent antigen assays and up to 29% by lytic antigen assays (Lennette *et al.*, 1996).

1.3.4 *Routes of transmission*

Serological testing is currently being used to evaluate risk factors for KSHV/HHV8 transmission. Several serological studies have indicated that, irrespective of the type of antigen used, KSHV/HHV8 infection may be more common among people attending sexually transmitted disease clinics than among blood donors (Kedes *et al.*, 1996; Lennette *et al.*, 1996; Simpson *et al.*, 1996).

A recent detailed analysis of the behavioural risk factors among Danish homosexual men revealed that variables such as promiscuity and receptive anal intercourse, but not oral–anal contact, increased the risk for KSHV/HHV8 infection (Melbye *et al.*, 1998). Furthermore, in the United States in the early 1980s, contact with homosexual men markedly enhanced the likelihood of having or acquiring antibodies to KSHV/HHV8, suggesting that KSHV/HHV8 was introduced into that community in the late 1970s or early 1980s (Melbye *et al.*, 1998). The rate of new infections decreased during the 1980s. Detailed studies will be needed to delineate the precise mechanisms by which KSHV/-HHV8 is transmitted among homosexual men and other populations at increased risk for sexually transmitted diseases. These findings suggest that KSHV/HHV8 is sexually transmitted in countries of low prevalence, consistent with the finding by PCR that infectious virus is secreted into the semen of infected men. These studies demonstrate that the behavioural risk factors that were previously shown to increase the risk for Kaposi's sarcoma (Beral *et al.*, 1990) also increase the likelihood of being infected with

KSHV/HHV8, providing further evidence that KSHV/HHV8 is indeed the postulated 'Kaposi's sarcoma agent'. To what extent sexual transmission is an important route of infection with KSHV/HHV8 in countries of high prevalence is not yet resolved. Early indications that a significant proportion of pre-adolescent children in Central Africa may have antibodies to ORF 65/vp19 and/or ORF 73/LANA suggest that the occurrence of Kaposi's sarcoma among young children in highly endemic African countries (a condition which is exceedingly rare in developed countries) occurs by vertical viral transmission in childhood in some settings (Ziegler & Katongole-Mbidde, 1996).

The route of KSHV/HHV8 transmission in Italian organ transplant recipients was investigated in one study. Parravicini *et al.* (1997) found that 10 of 11 patients who developed Kaposi's sarcoma were seropositive before receiving the allograft, in comparison with two of 17 transplant recipients who did not develop the disease. This suggests that, in KSHV/HHV8 endemic areas, most cases of transplant-associated Kaposi's sarcoma are due to reactivation of a pre-existing KSHV/HHV8 infection; however, the authors also documented one case in which transmission of KSHV/HHV8 occurred from the allograft.

In the 1960s and 1970s, a new epidemiological type of Kaposi's sarcoma associated with immunosuppression was identified among organ transplant patients, constituting up to 5% of tumours (Penn, 1979, 1983, 1988a,b; Qunibi *et al.*, 1988). It has been reported that patients who develop Kaposi's sarcoma after a renal transplant in North America are often of Jewish or Mediterranean ancestry (Harwood *et al.*, 1979). A recent study from Scandinavia found only two cases among 5000 transplant patients (Birkeland *et al.*, 1995).

1.4 Control and prevention

The role of antiviral agents in the prevention of Kaposi's sarcoma is discussed in section 4.6.

2. Studies of Cancer in Humans

Because KSHV/HHV8 was discovered only recently, few analytical data are available on its possible association with cancer in humans. Most of the available information derives from case series and case–control and cohort studies. This field of research is rapidly evolving, and the information reported below will be up-to-date for only a limited time.

2.1 Kaposi's sarcoma

A recent monograph on the evaluation of carcinogenic risks to humans dealt with Kaposi's sarcoma and human immunodeficiency viruses in detail (IARC, 1996). For completeness, the sections on the descriptive epidemiology on Kaposi's sarcoma from

that monograph have been incorporated in modified and shortened versions in the present monograph.

2.1.1 *Pathology and clinical disease*

2.1.1.1 *Epidemiological and clinical presentation*

Epidemiologically, Kaposi's sarcoma has been classified into sporadic (classic), endemic (African), epidemic (AIDS-related) and immunosuppression-associated (usually in transplant recipients) types; however, the histopathology of all of these types of Kaposi's sarcoma is identical (Templeton, 1981; Cockerell, 1991). In 1872, Dr Moriz Kaposi, a Hungarian dermatologist, first described an idiopathic, multiple pigmented sarcoma, now called 'classic' or sporadic Kaposi's sarcoma (Kaposi, 1872; Breimer, 1994). For many years, Kaposi's sarcoma was thought to be a lesion that affected predominantly elderly men of Mediterranean and eastern European origin (Dörffel, 1932; Landman *et al.*, 1984; Franceschi & Geddes, 1995). The presence of Kaposi's sarcoma was first noted in Africa in the 1920s (Williams, 1992). In the 1960s, it was reported to comprise up to 8% of malignancies, with endemic foci in parts of Africa (Oettlé, 1962; MacLean, 1963; Hutt & Burkitt, 1965; Williams, 1975). 'Endemic' Kaposi's sarcoma, like the 'classic' type, predominates in men but also occasionally affects children (Hutt & Birkitt, 1965; Williams, 1975; Ziegler & Katongole-Mbidde, 1996). The geographic distribution of endemic Kaposi's sarcoma in Africa prior to the AIDS epidemic was reported to be similar but not identical to that of Burkitt's lymphoma. In the early 1980s, a fourth variant of Kaposi's sarcoma, the 'epidemic' type, heralded the onset of the AIDS epidemic (Hymes *et al.*, 1981). Today, Kaposi's sarcoma is an AIDS-defining condition in HIV-infected individuals.

'Classic' or endemic Kaposi's sarcoma affects predominantly the skin of the lower limbs; internal organs are rarely involved. The disease typically follows an indolent course, patients surviving for an average of 10–15 years (Tappero *et al.*, 1993). Young children tend to have more severe disease than adults, the lesions often affecting the lymphatic system and internal organs rather than the skin, and shorter survival (Oettlé, 1962; Ziegler & Katongole-Mbidde, 1996). Kaposi's sarcoma in immunocompromised individuals — mainly transplant recipients and long-term users of steroids and cytotoxic drugs — often involves internal organs, lymph nodes and the face, mimicking the 'epidemic' type (Tappero *et al.*, 1993). In transplant recipients, Kaposi's sarcoma appears before most other tumours and may regress completely when immunosuppressive therapy is terminated (Penn, 1988a,b). In the epidemic form, the lesions are usually multiple, progress rapidly and may affect any area of the skin as well as internal organs. The tumours frequently begin as dusky-red or violet macules, progressing over weeks or months to plaques and raised, usually painless, firm nodules and plaques. Although the tumour may affect the legs, as seen with 'classic' Kaposi's sarcoma, lesions of the trunk, arms, genitalia and face are also common (Smith & Spittle, 1987). Lymph nodes and the oral cavity, most notably the palate, may be extensively involved. Oral Kaposi's sarcoma is often associated with involvement elsewhere in the gastrointestinal tract (Levine, 1993; Regezi *et al.*, 1993). Pulmonary Kaposi's sarcoma generally presents with short-

ness of breath and cough and is clinically difficult to distinguish from other pulmonary complications of AIDS (Levine, 1993). Median survival following a diagnosis of epidemic Kaposi's sarcoma is 14–18 months (Jacobson *et al.*, 1993; Lundgren *et al.*, 1994, 1995; Luo *et al.*, 1995).

2.1.1.2 *Histology*

The early patch-stage macular lesions contain abnormally shaped, dilated vessels surrounded by a mononuclear-cell infiltrate containing plasma cells; nuclear atypia and mitoses are rarely seen. In the plaque-stage lesions, there is proliferation of spindle-shaped cells in the superficial-to-deep dermis, with rare proliferation of spindle-shaped cells, nuclear atypia and mitoses. Spindle cells, which often surround slit-like vascular spaces, are characteristic of more advanced nodular lesions. The presence of KSHV/-HHV8 in spindle and endothelial cells and the expression of individual viral genes is discussed in section 1.1.6.1.

2.1.2 *Epidemiology*

2.1.2.1 *Incidence and geographical distribution*

The epidemiology of Kaposi's sarcoma was drastically influenced by the onset of the AIDS epidemic in the late 1970s and early 1980s. From being an exceedingly rare condition outside sub-Saharan Africa, its incidence suddenly increased dramatically among certain populations, such as homo- and bisexual men. Throughout the world, the incidence of Kaposi's sarcoma today reflects the burden of the AIDS epidemic, and as such varies considerably. Whereas the incidence appears to have reached a plateau or even a decline in parts of Europe and the United States (Dal Maso *et al.*, 1995), it is apparently rising in some African countries, such as Uganda (Wabinga *et al.*, 1993; Basset *et al.*, 1995; see Table 2).

As mentioned above, Kaposi's sarcoma represented up to 8% of all tumours in some parts of sub-Saharan Africa before the appearance of HIV infection in the 1980s. Relatively high incidence rates were reported from Israel (1970–79, 1.5/100 000 in people of each sex combined; Landman *et al.*, 1984), from Italy (1976–84, 1.05/100 000 in men, 0.27/100 000 in women; Geddes *et al.*, 1994), particularly in the south, and from Sardinia (1977–82, 1.6/100 000 in people of each sex combined; Cottoni *et al.*, 1996). Recently, high rates have also been described in two other island societies, those of Iceland and the Faeroe Islands in the North Atlantic (Hjalgrim *et al.*, 1998). Much lower age-adjusted rates are reported in Australia (1972–82, 0.065/100 000 in men, 0.029/100 000 in women; Kaldor *et al.*, 1994), England and Wales (0.014/100 000 in both men and women) and the United States (1973–79, 0.297/100 000 in men, 0.07/100 000 in women; Biggar *et al.*, 1984). On the basis of data in the Nordic cancer registries, the incidence rose among men from 0.05/100 000 in 1953–57 to 0.18/100 000 in 1978–79; in Nordic women, the corresponding rates were 0.02/100 000 and 0.08/100 000, respectively (Hjalgrim *et al.*, 1996a). Thus, in some countries, modest increases in the incidence of Kaposi's sarcoma were already occurring before the onset of the AIDS epidemic (Dictor & Attewell, 1988; Hjalgrim *et al.*, 1996a).

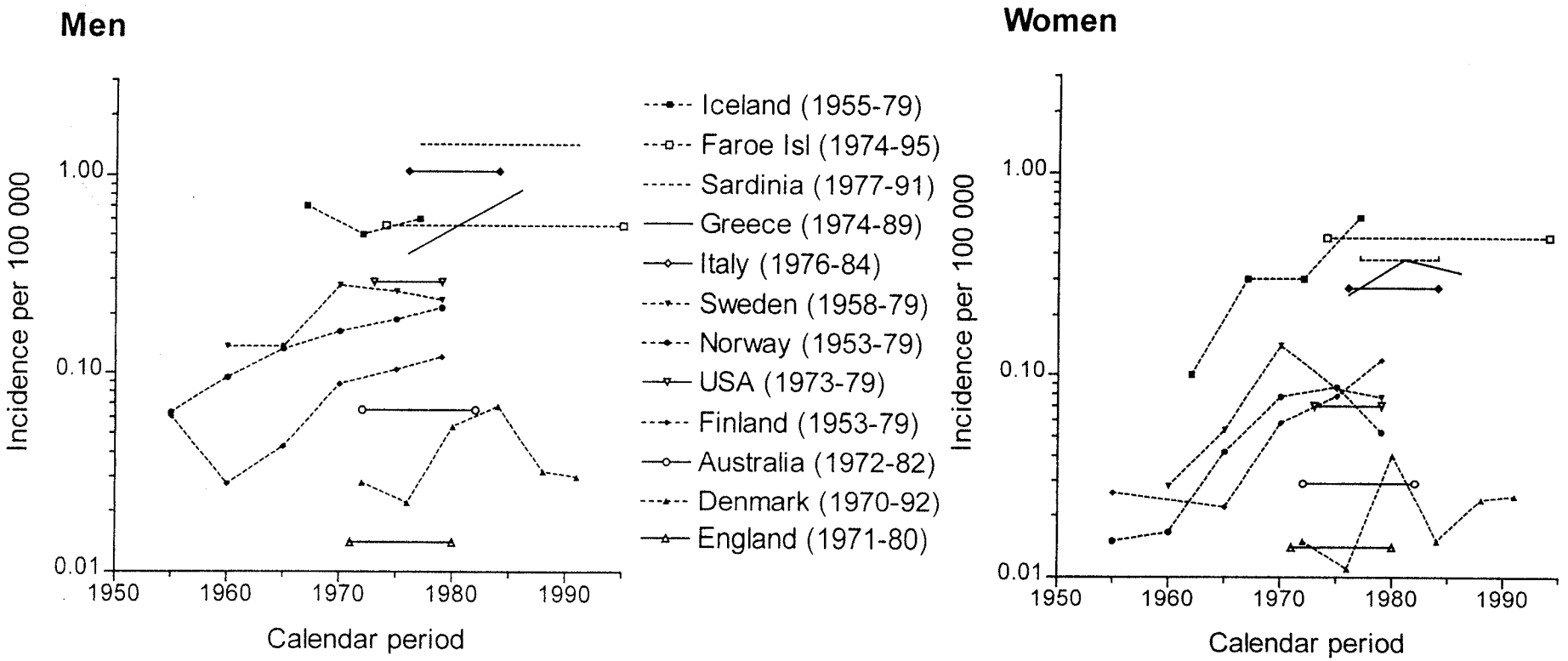
Table 2. Frequency of Kaposi's sarcoma in relation to all cancers in various areas of Africa

Reference	Location	Year(s) of study or report	Percentage of all cancers		
			Men	Women	Both
Oettlé (1962)	Former French Equatorial Africa	1953	–	–	5
	Former French West Africa	1954	–	–	1
	Ghana	1956	–	–	1
	Kenya	1948–61	–	–	2–4
	Mozambique	1958	–	–	2
	Nigeria	1934–44	–	–	2
	South Africa	1951 and 1960	–	–	1–3
	South Africa (Natal)	1957	–	–	1
	United Republic of Tanzania	1960	–	–	3
	Tunisia	1960	–	–	< 1
	Zaire	1956–57	–	–	9–13
	Zambia and Zimbabwe	1949	–	–	1
Hutt & Burkitt (1965)	Uganda	1964	–	–	4
Bayley (1984)	Zaire	1983	–	–	9
Otu (1986)	Nigeria	1986	–	–	15–20
Melbye *et al.* (1987)	Zaire	1984	16	–	–
Ngendahayo *et al.* (1989)	Rwanda	1979–86	–	–	6
Wabinga *et al.* (1993)	Uganda (registry)	1989–91	49	18	–
	Zambia				
Patil *et al.* (1992)	Children	1980–89	–	–	8.8
Patil *et al.* (1995)	Adults	1980–89	–	–	7.0
Bassett *et al.* (1995)	Zimbabwe (registry)	1990–92	23	10	–
Newton *et al.* (1996)	Rwanda (registry)	1991–93	10	3	–
Sitas *et al.* (1996)	South Africa (registry)				
	Black	1990–91	0.54	0.14	0.3
	White	1990–91	0.12	0.03	0.1

–, not reported

Studies in Australia, Denmark, the United Kingdom and the United States have shown an increased risk for Kaposi's sarcoma among persons of certain ethnicities from Central and East Africa, eastern Europe and Mediterranean countries and people of Jewish descent (Laor & Schwartz, 1979; DiGiovanna & Safai, 1981; Friedman-Birnbaum *et al.*, 1990; Grulich *et al.*, 1992; Kaldor *et al.*, 1994; Hjalgrim *et al.*, 1996b; Figure 4).

Figure 4. Reported incidence rates of 'classic' Kaposi's sarcoma



From Hjalgrim *et al.* (1998)

Dotted lines indicate world-standardized rates and solid lines the rates standardized to local populations; calendar period reflects period of observations.

2.1.2.2 *Demographic variations*

Formerly a tumour affecting predominantly the elderly (Oettlé, 1962; Templeton, 1981; Hutt, 1984, Geddes *et al.*, 1994; Hjalgrim *et al.*, 1996a), Kaposi's sarcoma has shown a substantial alteration in age distribution in recent years, in both developed and developing countries. Whereas the median age in developed countries before the AIDS epidemic was over 70 years, it is now in the late thirties.

In Europe and the United States, childhood Kaposi's sarcoma is very rare, even since the advent of the AIDS epidemic. In the early 1990s, the age-specific incidence rates in African countries such as Uganda and Zimbabwe showed a modest peak for children aged zero to four years, a decline until age 15 years and then the main peak at age 35–39 years in men and 25–29 years in women (Wabinga *et al.*, 1993; Basset *et al.*, 1995).

Studies based on registry data have found a male:female ratio of 'classic' Kaposi's sarcoma of 2–3:1 (Biggar *et al.*, 1984; Franceschi & Geddes, 1995; Hjalgrim *et al.*, 1996a). In a study based on data from the Nordic cancer registries, the male excess was primarily restricted to men over 60 years of age (Hjalgrim *et al.*, 1996a). In Africa, male:female ratios greater than 10 reported in early studies (Wahman *et al.*, 1991) have since declined to about 3:1 (Wabinga *et al.*, 1993; Basset *et al.*, 1995; Newton *et al.*, 1996).

2.1.2.3 *Behavioural factors*

Case reports suggest that Kaposi's sarcoma may occur more frequently than expected in HIV-uninfected homo- and bisexual men (Friedman-Kien *et al.*, 1990; Peterman *et al.*, 1991) and at a rate equivalent to the total number of cases diagnosed among all men under 50 years of age per year before the AIDS epidemic (Biggar *et al.*, 1984). [The Working Group noted that surveillance bias could entirely explain this observation.] Furthermore, in an analysis of 'classic' Kaposi's sarcoma in Denmark, men who had never married (used as a rough surrogate for homosexuality) were 19 times more at risk for the disease than men who had married (Hjalgrim *et al.*, 1996b); a similar analysis of data in the United States, however, showed no such difference (Biggar & Melbye, 1996).

The risk for Kaposi's sarcoma varies greatly among the different groups at risk for HIV transmission, being particularly high in homo- and bisexual men (IARC, 1996). This elevated risk is seen even among men aged 13–24 and suggests a rapid increase in risk after homosexual contact. Beral *et al.* (1990) found that 13 616 of 88 739 (15%) AIDS patients in the United States developed Kaposi's sarcoma, the proportion varying from 21% of homo- or bisexual men to 3% of heterosexuals, 2% of intravenous drug users, 3% of transfusion recipients, 1% of haemophiliacs and 1% of children infected by perinatal transmission. Furthermore, women with AIDS who were sexual partners of bisexual men were more likely to have Kaposi's sarcoma than women who were partners of intravenous drug users (Peterman *et al.*, 1993; Serraino *et al.*, 1995). Even among homo- and bisexual men, the risk for Kaposi's sarcoma is not uniform: Schechter *et al.* (1991) conducted an analysis of all AIDS-associated cases of Kaposi's sarcoma among homo- and bisexual men in Canada between 1980 and 1989 and found that the pro-

portion of cases among AIDS patients had a strong geographical association with the original centres of the AIDS epidemic in Canada. Furthermore, homosexual men born between 1945 and 1954 were more likely to present with Kaposi's sarcoma, consistent with the hypothesis of an environmental cofactor with higher levels of exposure. In another study from the same group, Archibald *et al.* (1990) found that 56% of Canadian homosexual men with AIDS who developed Kaposi's sarcoma and only 21% of those who developed AIDS but not Kaposi's sarcoma reported that they had had more than 20 sexual partners from large cities in the United States (odds ratio, 4.6; 95% confidence interval [CI], 1.6–13). Similarly, homosexual men with AIDS in the United Kingdom were more likely to have Kaposi's sarcoma if they had had sexual contact with an American (31%) or African (26%) man than if they had not (19%) ($p < 0.05$) (Beral *et al.*, 1991). Furthermore, Peterman et *al.* (1993) found that Kaposi's sarcoma was more frequently part of the AIDS definition in homosexual men from California and New York than in homosexual men from the rest of the United States.

Most analyses of the number of sexual partners of homo- and bisexual men with Kaposi's sarcoma and of those with other manifestations of AIDS (Haverkos *et al.*, 1985; Goedert *et al.*, 1987; Archibald *et al.*, 1990; Armenian *et al.*, 1993), but not all (Lifson *et al.*, 1990a,b), found that patients with Kaposi's sarcoma had had a larger number of sexual partners. Patients with this cancer have also been reported to be more likely to have a history of sexually transmitted disease (Goedert *et al.*, 1987; Armenian *et al.*, 1993). In a case–control study in New York City, United States, Kaposi's sarcoma was found to be significantly associated with receptive anal intercourse (Marmor *et al.*, 1982; Jaffe *et al.*, 1983). Several authors have subsequently reported an increased risk for Kaposi's sarcoma among HIV-positive men whose sexual practices involve faecal contact (Beral *et al.*, 1992; Darrow *et al.*, 1992). The possible association between insertive oral–anal contact and the risk for Kaposi's sarcoma remains controversial, some studies showing a possible association (Archibald *et al.*, 1990; Beral *et al.*, 1992; Darrow *et al.*, 1992) and others not (Lifson *et al.*, 1990b; Elford *et al.*, 1992; Page-Bodkin *et al.*, 1992; Armenian *et al.*, 1993; Kaldor et al., 1993). Casabona *et al.* (1991) noted that the fraction of AIDS cases with Kaposi's sarcoma was similar in southern Europe, with a relatively high incidence of 'classic' Kaposi's sarcoma, and in northern Europe, with a relatively low incidence. In Uganda, increased risk for Kaposi's sarcoma was seen in HIV-seropositive adults of each sex who had one rather than several spouses or a history of sexually transmitted diseases, and especially those who were relatively affluent, well-educated, had travelled and had spent increasing time in contact with water (Ziegler *et al.*, 1997).

In conclusion, men who develop Kaposi's sarcoma tend to be more sexually active and to have more sexual partners from epicentres of the AIDS epidemic. In conjunction with the much higher risk for Kaposi's sarcoma among homosexual men than among other HIV transmission groups, these data indicate that an infectious sexually transmitted agent (independent of HIV) is associated with Kaposi's sarcoma. Transmission of such an agent via the blood is apparently less common, since Kaposi's sarcoma occurs in only 3% of people who acquire HIV through a blood transfusion.

2.1.2.4 *Second primary malignancies after Kaposi's sarcoma*

An association between Kaposi's sarcoma and lymphomas has been suspected for many years. Both tumours occur in association with immunosuppression and can occur in the same individual. In a hospital-based cohort of 72 patients with 'classic' Kaposi's sarcoma in New York, United States, Safai *et al.* (1980) counted a total of nine lymphoid malignancies, including four non-Hodgkin's lymphomas, during 581 person–years of follow-up. This corresponds to a significantly (20-fold) increased risk for these malignancies over that of the background population. Few studies have addressed 'classic' Kaposi's sarcoma because of its infrequency. Three deaths from non-Hodgkin's lymphoma were reported among 68 patients with Kaposi's sarcoma in the United Kingdom (Grulich *et al.*, 1992). In contrast, two larger population-based studies of 492 American and 204 Italian subjects with 'classic' Kaposi's sarcoma did not confirm the suspected association (Biggar *et al.*, 1994; Franceschi *et al.*, 1996).

2.1.3 *Case series and case–control studies*

2.1.3.1 *Detection of KSHV/HHV8 DNA in tumour tissue*

Published case series and case–control studies on the detection of KSHV/HHV8 DNA in Kaposi's sarcoma tissue are summarized in Table 3. Many of these studies are small and/or included heterogeneous controls.

KSHV/HHV8 DNA is found in nearly all Kaposi's sarcoma tissues, despite differences in detection methods and in the quality or preservation of tumour material. In 28 studies in which the detection of KHSV/HHV8 DNA was described in Kaposi's sarcoma tissues (Table 3), KSHV/HHV8 was identified in 735 of 794 (91%) Kaposi's sarcoma analysed. The rates reported in one of the studies (Noel *et al.*, 1996) were very different from those in the other studies; when these results were excluded, the percentage positivity rose to 96% (686/716). The detection rate was similar whether the patients were HIV-infected (391/417; 94%) or uninfected (335/368; 91%). Furthermore, KSHV/HHV8 was found in all four epidemiological forms of Kaposi's sarcoma, with no indication of significant differences in the detection rate in the four types. With the exception of a few studies (Rady *et al.*, 1995; Gyulai *et al.*, 1996a,b), little or no evidence of KSHV/HHV8 DNA has been found in tumours other than Kaposi's sarcoma. The exceptions include primary effusion lymphomas and Castleman's disease. The recent identification of KSHV/HHV8 in bone-marrow dendritic cells of myeloma patients awaits confirmation (Rettig *et al.*, 1997; see section 2.2.3). These and other conditions potentially associated with KSHV/HHV8 are discussed in subsequent sections (see also Table 3).

A major difficulty in assessing associations with disease on the basis of detection of DNA is in selecting appropriate control tissues in order to identify differences in infection rate. In the initial description of the virus, Chang *et al.* (1994) found that 25 of 27 AIDS-associated Kaposi's sarcomas contained KSHV/HHV8 DNA in comparison with three of 27 lymphomas from AIDS patients [odds ratio, 100, $p < 10^{-7}$] (some of whom may have had Kaposi's sarcoma as a secondary malignancy), none of 29 lymphomas from non-AIDS patients and none of 49 consecutive surgical biopsy samples ($p < 10^{-5}$).

Table 3. Presence of KSHV/HHV8 DNA in Kaposi's sarcoma (KS) tissue, other tissues from Kaposi's sarcoma patients and tissues from subjects without Kaposi's sarcoma, detected by polymerase chain reaction (PCR)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Chang *et al.* (1994)	AIDS-KS	25/27			AIDS		Fresh frozen
					Lymphoma	3/27	
					Lymph node	3/12	
					Total	6/39	
					Non-AIDS		
					Lymphoma	0/29	
					Lymph node	0/7	
					Vascular tumour	0/5	
					Opportunistic infections	0/13	
					Surgical biopsy	0/49	
					Total	0/103	
Su *et al.* (1995)	AIDS-KS	4/4	None		AIDS lymph node	0/5	
	Non-AIDS KS	2/3			Benign hyperplasia	0/10	
	Total	6/7			B-Cell lymphoma	0/12	
					T-Cell lymphoma	0/10	
					Total	0/37	
Dupin *et al.* (1995a)	Classic KS	5/5	Skin, classic	3/3	Various tissues, HIV-negative	0/6	Snap-frozen
	AIDS-KS	4/4	Skin, AIDS	2/3			
	Total	9/9	Total	5/6			
Boshoff *et al.* (1995a)	Classic KS	16/17	None		Angioma/angiosarcoma	0/4	Fresh-frozen or
	Transplant KS	8/8			Skin naevi	0/3	paraffin-embedded
	AIDS-KS	14/14			Granulomatous tissue	0/4	(nested PCR)
	HIV-negative homosexual man	1/1			Total	0/11	
	Total	39/40					

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Ambroziak *et al.* (1995)	AIDS-KS, homosexual men	12/12	None		None		
	HIV-negative homosexual man	1/1					
	Total	13/13					
Moore & Chang (1995)	AIDS-KS	10/11	Skin, AIDS	1/7	Skin from healthy subjects	1/11	Fresh-frozen
	Classic KS	6/6	Skin, classic	1/5	PBMC from healthy subjects	0/10	
	HIV-negative homosexual men	4/4	Skin, HIV-negative	1/2	Total	1/21	
	Total	20/21					
Lebbé *et al.* (1995)	Immunosuppressed KS	1/1	Skin, HIV-negative	3/9	None		
	Classic KS	10/10					
	African KS	3/3					
	AIDS-KS	2/2					
	Total	16/16					
Schalling *et al.* (1995)	AIDS-KS	25/25	Pyothorax-related B-cell lymphoma-KS, HIV-positive	0/3	HIV-positive		
	African KS	18/18			Pyothorax-related B-cell lymphoma (PBMC)	0/13	
	Classic KS	3/3			Lymph node	0/8	
	Total	46/46			Total	0/21	
			Skin, HIV-negative	0/2	HIV-negative		
					Pyothorax-related B-cell lymphoma (PBMC)	0/12	
					Skin, non-KS patient	0/1	
					Haemangioma	0/1	
					Pyogenic granuloma	0/1	
					Total	0/15	

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Chang *et al.* (1996b)	AIDS-KS African KS Total	22/24 17/20 39/44	None		HIV-positive HIV-negative Total	1/7 2/15 3/22	Paraffin-embedded Negatives retested by nested PCR
Chuck *et al.* (1996)	African KS HIV-negative homosexual men Total	4/4 1/2 5/6	None		None		Fresh-frozen (endemic) or paraffin-embedded (HIV-negative homosexual men)
O'Neill *et al.* (1996)	AIDS-KS	7/7			HIV-negative	0/1	Nested PCR Fresh-frozen or paraffin-embedded
Buonaguro *et al.* (1996)	African KS Classic KS Immunosuppressed KS AIDS-KS Total	12/12 28/28 2/2 19/19 61/61	Skin, HIV-negative	9/13	Reduction mammoplasty Penile carcinoma biopsies Xeroderma pigmentosum skin cancer Xeroderma pigmentosum autologous normal skin PBMC of HIV-positive patients Total	0/3 0/4 0/5 0/5 0/15 0/32	Snap-frozen
Cathomas *et al.* (1996)	AIDS-KS Classic KS Transplant KS Total	9/9 12/12 1/1 22/22	None		Other skin lesions, HIV-positive Other skin lesions, HIV-negative Total	0/4 0/10 0/14	Paraffin-embedded Nested PCR

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Gaidano *et al.* (1996b)	AIDS-KS	35/35	AIDS, skin (PCR; only 3/6 by Southern blot)	6/6	Hodgkin's disease Primary effusion lymphoma Other non-Hodgkin's lymphoma Persistent generalized lymph-adenopathy Anogenital neoplasia Total	0/3 3/3 0/28 0/15 0/14 3/63	Fresh-frozen (a few paraffin-embedded)
Jin *et al.* (1996a)	AIDS-KS Classic KS Total	5/5 12/12 17/17	None		Haemangiosarcoma Haemangioma Lymphangioma Lymphangiomatosis Pyogenic granuloma Haemangiopericytoma Kimura's disease Lymphangiomyomatosis Total	0/15 0/75 0/15 0/2 0/25 0/3 0/2 0/1 0/138	Paraffin-embedded
Dictor *et al.* (1996)	Classic KS AIDS-KS Total	35/40 14/14 49/54	None		Endothelial lesions	0/86	Paraffin-embedded
Marchioli *et al.* (1996)	AIDS-KS Classic KS African KS HIV-negative homosexual men Total	28/28 7/8 7/10 2/2 44/48	HIV-positive Serum Plasma	 3/28 0/13	Normal PBMC Normal skin Paediatric lymphomas Adult lymphomas Carcinomas Total	0/163 0/10 0/8 0/37 0/12 0/230	Fresh-frozen and paraffin embedded

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Luppi *et al.* (1996a)	Classic KS AIDS-KS Total	15/22 3/4 18/26	None		Normal PBMC Normal salivary glands Normal saliva samples Hyperplastic tonsils Total	0/13 0/9 0/6 2/11 2/39	Paraffin-embedded
McDonagh *et al.* (1996)	KS	9/9	None		Angiosarcoma Haemangioma Haemangiopericytoma Total	7/24 1/20 0/6 8/50	Fresh-frozen and paraffin-embedded
Corbellino *et al.* (1996a,b)	AIDS-KS	7/7	HIV-positive Lymphoid tissue Prostate glands Uninvolved skin Bone marrow Paravertebral sensory lumbar ganglion	 7/7 5/5 3/5 2/3 7/7	AIDS patients HIV-positive Paravertebral sensory lumbar ganglion HIV-negative Paravertebral sensory lumbar ganglion	0/6 0/4 0/3	Snap-frozen
Lebbé *et al.* (1997a)	Classic KS African KS Castleman's disease HIV-negative homosexual men Immunosuppressed/ transplant KS Total	16/16 3/3 1/1 3/3 2/2 25/25	Skin, classic Skin, African Skin, HIV-negative homosexual men Skin, induced Total	10/13 2/3 1/3 1/1 14/20	HIV-negative Dermatology biopsies Reduction mammaplasties Total	 0/10 0/5 0/15	Fresh-frozen[a] Nested PCR
Huang *et al.* (1997)	AIDS-KS HIV-negative	12/12 2/2	HIV-positive Normal skin HIV-negative Normal skin	 5/12 1/2	HIV-positive Intravenous drug users (PBMC) HIV-negative Healthy (PBMC)	 0/5 0/5	Fresh frozen

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Albini *et al.* (1996a)	AIDS-KS African KS Classic KS Transplant KS	59/59 21/21 32/33 6/7	Skin, HIV-positive Skin, HIV-negative	0/3 3/9	HIV-positive Skin Other tissue Lymphoma HIV-negative Lymphoma	 0/4 0/2 2/10 1/34	Fresh or paraffin embedded
Uthman *et al.* (1996)	AIDS-KS Classic KS	23/23 5/5	None		HIV-positive Skin lesions HIV-negative Leiomyoma Melanoma Basal-cell carcinoma Pityriasis rosea Molluscum contagiosum Psoriasis vulgaris Viral warts Pseudolymphoma Total	 0/28 0/3 0/8 0/11 0/4 0/6 0/6 0/8 0/7 0/53	Nested PCR
Decker *et al.* (1996)	AIDS-KS	5/5	None		HIV-negative Allograft (PBMC)[b] Healthy (PBMC)[b]	 4/5 3/5	Fresh tissue

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Li *et al.* (1996)	AIDS-KS Classic KS	6/6 3/3	None		HIV-negative Skin Verrucea vulgaris Total	 0/3 0/2 0/5	Fresh tissue
Noel *et al.* (1996)	AIDS-KS HIV-negative	41/61 8/17	Cutaneous and others HIV-negative	1/19	HIV-negative Cutaneous and others	 1/26	Paraffin-embedded

Modified from Olsen and Moore (1997)
PBMC, peripheral blood mononuclear cells; HIV, human immunodeficiency virus; PCR, polymerase chain reaction
[a] Positivity rate dependent on amount of added template DNA
[b] Testing of multiple samples from same individual

Similarly, Boshoff *et al.* (1995a) found KSHV/HHV8 in 39 of 40 Kaposi's sarcoma lesions of all types but in none of 11 pathologically similar tissues (angioma/angiosarcoma, skin naevi and granulomatous tissues). In a case–control analysis of Kaposi's sarcoma tissues from HIV-positive and HIV-negative persons and skin and PBMC from HIV-negative persons, Moore and Chang (1995), who were unaware of the case or control status of the subjects, found viral DNA in 20 of 21 Kaposi's sarcoma lesions and in only one of 21 control tissues (odds ratio, 400; 95% CI, 19–17 000). Jin *et al.* (1996a) and Dictor *et al.* (1996) compared Kaposi's sarcomas from HIV-positive and HIV-negative persons with a wide variety of tissues resembling Kaposi's sarcoma, including those of endothelial origin and angiogenic and skin tumours. They found viral DNA in 88–100% of 71 Kaposi's sarcoma lesions and none of 224 control tissues. Table 3 also gives a partial list of studies in which control tissues from nearly all organ systems were examined by PCR. Overall, 34 of 1128 (3%) tissues not from Kaposi's sarcomas contained KSHV/HHV8 DNA. Several of these samples were primary effusion lymphomas (described in section 2.2.1), which are also associated with KSHV/HHV8 (Chang *et al.*, 1994; Gaidano *et al.*, 1996b), and the results in two studies (Decker *et al.*, 1996; McDonagh *et al.*, 1996) accounted for nearly half of all the positive findings.

The amount of viral DNA detected by Southern blot in Kaposi's sarcoma lesions averages from undetectable to an estimated 10–20 viral genome copies per cell equivalent. Similarly, KSHV/HHV8 DNA is readily detectable by PCR in DNA extracted from fresh tissue, whereas nested PCR is often required to obtain positive results from fixed, paraffin-embedded tissue. These conditions probably play a role with respect to the differences in positivity rate observed by different investigators.

Several groups reported a higher detection rate of KSHV/HHV8 by PCR in late plaque or nodular stages than in early or patch-stage Kaposi's sarcoma (Luppi *et al.*, 1996a; Noel *et al.*, 1996).

2.1.3.2 *Detection of KSHV/HHV8 DNA in peripheral blood mononuclear cells*

The rate of detection of HHV8 in PBMC from Kaposi's sarcoma patients varies widely (Table 4); however, most of the larger studies suggest that about 50% of PBMC samples from Kaposi's sarcoma patients give positive results when tested by nested PCR under standard conditions, e.g. using 100–500 ng of PBMC DNA (Whitby *et al.*, 1995; Bigoni *et al.*, 1996; Lefrère *et al.*, 1996; Moore *et al.*, 1996c; Lebbé *et al.*, 1997a). When assaying for the presence of KSHV/HHV8 DNA in PBMC, it is important to use sufficient DNA to detect a low copy number of viral DNA (Decker *et al.*, 1996; Blackbourn *et al.*, 1997).

As shown in Table 4, 42% (161/386) of HIV-positive patients and 53% (47/89) of HIV-negative patients with Kaposi's sarcoma had detectable KSHV/HHV8 DNA in their PBMC. Albini *et al.* (1996a) reported an exceptionally low positivity rate (3/54) for KSHV/HHV8 in PBMC from HIV-infected Kaposi's sarcoma patients. If these exceptional results are excluded, the positivity rate among HIV-infected patients in the remaining studies was 48% (158/332). In a study of Mediterranean Kaposi's sarcoma patients, Brambilla *et al.* (1996) reported a particularly high concordance for patients with

Table 4. Detection of KSHV/HHV8 DNA in peripheral blood mononuclear cells (PBMC)

Reference	KS type	KSHV/HHV8 (positive/ total)	Control PBMCs	KSHV/HHV8 (positive/ total)	Odds ratio	95% CI	Comments
Collandre *et al.* (1995)	AIDS-KS	2/10	HIV-positive, no KS	0/9			PCR Southern blot
Ambroziak *et al.* (1995)	AIDS-KS	7/7	HIV-positive, no KS	0/6			
	HIV-negative KS	3/3	HIV-negative, no KS	0/14			
	Total	10/10	Total	0/20			
Whitby *et al.* (1995)	AIDS-KS	24/46	HIV-positive, no KS	11/143			Nested PCR
			Oncology patients	0/26			
			Blood donors	0/134			
			Total	11/303			
Moore *et al.* (1996c) and correction by Parry & Moore (1997)	AIDS-KS	11/21	Homo- or bisexual AIDS patients, no KS	3/23	7.3	1.4–47.9	Nested PCR
			Haemophiliac AIDS patients, no KS	0/19	21.8	2.4–978	
			Total	3/42			
Marchioli *et al.* (1996)	AIDS-KS	46/99	HIV-positive	0/64			PCR Southern blot
	HIV-negative homosexual men	0/2	HIV-negative	0/163			
			Total	0/227			
	Total	46/101					
Humphrey *et al.* (1996)	AIDS-KS	34/98	HIV-positive, no KS	12/64	2.3	1.0–5.3	PCR Southern blot
			HIV-negative, no KS	0/11			
			Total	12/75			
Decker *et al.* (1996)	AIDS-KS	8/9	Allograft patients	4/5			Multiple samples tested to obtain positive results in controls
			Healthy donors	3/5			
			Total	7/10			

Table 4 (contd)

Reference	KS type	KSHV/HHV8 (positive/ total)	Control PBMCs	KSHV/HHV8 (positive/ total)	Odds ratio	95% CI	Comments
Lebbé *et al.* (1997a)	Classic KS	9/18	Blood donors	0/20			PCR Southern blot Nested PCR (32% of KS subjects positive with unnested PCR)
	African KS	2/3					
	Castleman's disease	0/1					
	HIV-negative homosexual men	1/4					
	Immunosuppressed or transplant KS	0/2					
	Total	12/28					
Albini *et al.* (1996a)	AIDS-KS	3/54	Healthy donors	0/4			
	Classic KS	1/6					
	Transplant	1/2					
	Total	5/62					
Brambilla *et al.* (1996)	Classic KS		None				
	Stage I	9/16					
	Stage II	1/5					
	Stage III	6/11					
	Stage IV	8/8					
	Total	24/40					
Heredia *et al.* (1996)	AIDS-KS	2/2	HIV-positive	0/2			
	HIV-negative KS	6/8	HIV-negative	0/8			
	Total	8/10	Total	0/10			
Howard *et al.* (1995)	AIDS-KS	11/17	AIDS, no KS	0/6			Nested PCR
Huang *et al.* (1997)	AIDS-KS	3/12	None				
Lefrère *et al.* (1996)	AIDS-KS	10/11	AIDS, no KS	1/14			
			HIV-positive (asymptomatic)	1/45			
			HIV-negative	0/20			

HIV, human immunodeficiency virus; PCR, polymerase chain reaction

disseminated Kaposi's sarcoma, 100% of whom had detectable KSHV/HHV8 DNA in their PBMC.

Under the same conditions, KSHV/HHV8 is only rarely detected in PBMC from blood donors or other subjects without Kaposi's sarcoma (Table 4; Ambroziak *et al.*, 1995; Whitby *et al.*, 1995; Bigoni *et al.*, 1996; Lefrère *et al.*, 1996; Moore *et al.*, 1996c; Blackbourn *et al.*, 1997).

2.1.3.3 *Detection of KSHV/HHV8 DNA in other tissues*

Similarly high rates of KSHV/HHV8 positivity have been observed in uninvolved skin of Kaposi's sarcoma patients (Moore & Chang, 1995; Albini *et al.*, 1996a; Buonaguro *et al.*, 1996; Corbellino *et al.*, 1996b; Huang *et al.*, 1997), with no significant difference between those who are HIV-infected and uninfected (Table 3). In contrast, skin biopsy samples from non-Kaposi's sarcoma patients very rarely have detectable KSHV/HHV8.

KSHV/HHV8 is also detected to varying degrees in other tissues (e.g. semen, serum, prostate glands, bone marrow) from Kaposi's sarcoma patients (Table 5; see also section 1.1.6). Corbellino *et al.* (1996a) detected KSHV/HHV8 DNA by nested PCR in all of seven paravertebral sensory lumbar ganglia from Kaposi's sarcoma patients and in none of similar materials from patients without this tumour. Howard *et al.* (1995) detected KSHV/HHV8 by nested PCR in bronchoalveolar lavage fluid from 11 of 14 HIV-positive men with both cutaneous and pulmonary Kaposi's sarcoma, but in none of six men with only cutaneous manifestations and in one of 19 HIV-positive men with no evidence of either cutaneous or pulmonary Kaposi's sarcoma; the last case presented three months later with pulmonary manifestations of Kaposi's sarcoma.

2.1.3.4 *Serology*

Serological assays have been developed to detect antibodies to either a LANA (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996) and/or a defined (Miller *et al.*, 1996; Simpson *et al.*, 1996) or undefined (Lennette *et al.*, 1996; Ablashi *et al.*, 1997) structural ('lytic') antigen of KSHV/HHV8 (Rickinson, 1996). The exact prevalence of KSHV/HHV8 infection in northern Europe and the United States, as measured by these assays, is still controversial and ranges from 0–20%. The available evidence indicates considerable geographical variation. Infection with KSHV/HHV8 seems to be widespread in several African countries (50–70%) and more common in some Mediterranean countries than in northern Europe or the United States (for a more detailed discussion of currently available serological assays and KSHV/HHV8 prevalence, see section 1.2.2).

Studies in which the association between Kaposi's sarcoma and KSHV/HHV8 antibodies was analysed are summarized in Table 6. Irrespective of differences in the assays used, most of the rates reported were more than 80% seropositivity in all epidemiological types of Kaposi's sarcoma.

Most studies in which antibodies to LANA were measured by immunofluorescence or western blotting (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996) are consistent in detecting antibodies in 80–90% of Kaposi's sarcoma patients but in only

Table 5. Detection of KSHV/HHV8 in semen and prostate tissue

Reference (country or region)	KS type	Positive/ total	Control/other populations	Positive/ total	Method
Semen					
Ambroziak *et al.* (1995) (USA)	AIDS-KS	0/4	–	–	PCR hybridization detection of amplicons
Monini *et al.* (1996a) (Italy)	–	–	Semen donors	5/10[a] 30/33[b]	Nested PCR
Corbellino *et al.* (1996c) (Italy)	–	–	Semen donors	0/20	Nested PCR
Monini *et al.* (1996b) (Italy)	AIDS-KS	1/5	AIDS without KS Semen donors	0/10 3/13	Nested PCR PCR
Marchioli *et al.* (1996) (USA)	AIDS-KS Classic KS	4/31 0/2	–	–	PCR[a]
Gupta *et al.* (1996) (USA)	AIDS-KS	2/14	AIDS without KS	0/10	Nested PCR; sampling at two times
Howard *et al.* (1997) (United Kingdom)	AIDS-KS	3/15	AIDS without KS Semen donors	3/9 0/115	Nested PCR
Huang *et al.* (1997) (USA)	AIDS-KS Classic KS	3/12 0/2	AIDS without KS HIV-positive intravenous drug users HIV-negative	0/4 0/5 0/7	PCR hybridization and in-situ PCR detection of amplicons
Viviano *et al.* (1997) (Sicily)	AIDS-KS	1/1	AIDS without KS HIV-negative	1/10 6/45	Nested PCR

Table 5 (contd)

Reference (country or region)	KS type	Positive/ total	Control/other populations	Positive/ total	Method
Prostate tissue					
Monini *et al.* (1996a) (Italy)	–	–	Benign hyperplasia and carcinoma	7/16	Nested PCR
Corbellino *et al.* (1996c) (Italy)	AIDS-KS	5/5	–	–	
Corbellino *et al.* (1996c) (Italy)	–	–	AIDS without KS HIV-negative	0/20 0/8	Nested PCR
Tasaka *et al.* (1996, 1997) (Italy and USA)	–	–	Prostate biopsy	0/52	Nested PCR
Monini *et al.* (1996b) (Italy)	–	–	Hyperplastic prostate biopsy	2/7	Nested PCR
Lebbé *et al.* (1997b) (France)	–	–	Benign hyperplasia and carcinoma	0/19	Nested PCR
Staskus *et al.* (1997) (USA)	–	–	Prostate biopsy	12/16	In-situ hybridization
Rubin *et al.* (1997) (USA)	–	–	Prostate biopsy	0/45	PCR[a]

Modified from Blackbourn & Levy (1997)
[a]Blinded analysis
[b]Unblinded analysis

Table 6. Serological studies of KSHV/HHV8 infection

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Gao *et al.* (1996a,b)	BC-1 LANA Western blot	USA		AIDS without KS, US homosexual men	7/40	ND
		AIDS (homosexuals)	32/40	HIV-positive US haemophiliacs	0/20	ND
		Italy		Ugandan cancer patients		
		AIDS	11/14	HIV-positive	25/35	ND
		Classic	11/11	HIV-negative	29/47	ND
		Uganda		Blood donors		
		AIDS	16/18	USA	0/122	ND
		Endemic	1/1	Italy	4/107	ND
	BCP-1 LANA IFA	USA		US EBV-positive	0/69	ND
		AIDS	35/40	AIDS without KS, US homosexual men	12/40	ND
		Italy		HIV-positive US haemophiliacs	0/20	ND
		AIDS	10/14	Ugandan cancer patients		
		Classic	11/11	HIV-positive	18/35	ND
		Uganda		HIV-negative	24/47	ND
		AIDS	14/18	Blood donors		
		Endemic	1/1	USA	0/122	ND
				Italy	4/107	ND
				US EBV-positive	0/69	ND
Kedes *et al.* (1996)	BCBL-1 LANA IFA	AIDS	37/45	HIV-positive US haemophiliacs	9/300	ND
		Classic	1/1	HIV-positive US transfusion recipients	2/44	ND
				Sexually transmitted disease clinic attendees		
				HIV-positive (bi/homosexual)	13/37	ND
				HIV-positive (heterosexual)	0/9	ND
				HIV-negative	10/130	ND
				US blood donors		
				HIV-positive	41/138	ND
				HIV-negative	2/141	ND
Kedes *et al.* (1997)	BCBL-1 LANA IFA	AIDS (women)	2/2	HIV-positive women	12/302	ND
				HIV-negative women	1/84	ND

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Lennette *et al.* (1996)	BCBL-1 LANA IFA	US (AIDS and a few classic)	47/91	HIV-positive, US homosexual men	19/94	87/94
				HIV-positive male intravenous drug users	0/13	3/13
		African endemic	28/28	HIV-positive women	0/33	7/33
	BCBL-1 TPA induced lytic IFA	US (AIDS and a few classic)	87/91	Children < 16 years	0/263	10/263
				Adults > 16 years	0/174	33/174
		African endemic	28/28	US blood donors	0/44	9/44
				US women	0/54	15/54
				Haemophiliacs	0/83	10/83
				Various tumours (Dominican Republic, Sweden, Malaysia and Netherlands)	0/147	19/147
				EBV-positive patients	0/40	8/40
				Rheumatoid arthritis	0/20	5/20
				Zimbabwe	4/37	12/37
				Nigeria	3/52	29/52
				Zaire	4/16	13/16
				Uganda	9/82	63/82
				The Gambia	11/45	38/45
				Ivory Coast	4/7	7/7
				Haiti	0/52	15/52
				Dominican Republic	0/40	5/40
				Guatemala	0/20	2/20

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Simpson *et al.* (1996)	ORF 65 ELISA	AIDS (USA, UK)	46/57	HIV-positive homosexual men	10/33	
		AIDS (Uganda)	14/17	HIV-positive women with sexually transmitted diseases	3/15	
		Classic (Greece)	17/18	Haemophiliacs		
				HIV-positive	0/26	
				HIV-negative	ND	
	BCP-1 LANA IFA	AIDS (US, UK)	84/103	Intravenous drug users		
		Classic (Greece)	17/18	HIV-positive	0/38	2/38
				HIV-negative	0/25	0/25
				HIV-negative homosexual men with sexually transmitted diseases	8/65	ND
				Heterosexual men	4/75	ND
				Heterosexual women	2/26	ND
				Children with rash or fever	0/24	ND
				Blood donors		
				UK	4/150	3/174
				USA	0/117	6 /117
				Greek age-/sex-matched controls	3/26	3/26
				Ugandan controls		
				HIV-positive	18/34	16/34
				HIV-negative	9/17	7/54
Miller *et al.* (1996)	Sodium butyrate-induced BC-1 western blot	AIDS	32/48	HIV-positive US homosexual men	ND	7/54
	Sodium butyrate-induced BC-1 IFA	AIDS	31/48	HIV-positive US homosexual men	ND	7/54
Davis *et al.* (1997)	ORF 26 peptide ELISA	AIDS	21/35	HIV-negative blood donors	ND	6/30
				HIV-positive homosexual men	ND	2/8
				HIV-positive haemophiliacs	ND	6/24

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Lin *et al.* (1997)	Recombinant whole ORF 65 western blot	AIDS	42/47	HIV-positive US adults	ND	11/54
				HIV-positive children	ND	0/12
				HIV-negative children	ND	0/10
				Haemophiliacs	ND	0/25
				Autoimmune patients	ND	0/25
				Children with acute illness	ND	0/25
				Healthy adults	ND	3/28
				Nasopharyngeal cancer patients (China)	ND	0/25
Smith *et al.* (1997)	BCBL-1 TPA-induced lytic IFA with Evans blue counterstain	AIDS	7/7	HIV-positive	ND	6/18
				US blood donors	ND	0/52

Modified from Olsen & Moore (1997)
ND, not determined; LANA, latency-associated nuclear antigen; IFA, immunofluorescent assay; ELISA, enzyme-linked immunosorbent assay; TPA, 12-*O*-tetradecanoylphorbol 13-acetate

30% of HIV-positive homosexual or bisexual men and less than 2% of HIV-positive patients with haemophilia or blood donors in the United Kingdom or the United States. Simpson *et al.* (1996) also found no LANA-reactive sera among 38 HIV-positive intravenous drug users. Gao *et al.* (1996a,b), in a nested case–control study within the cohort of a multicentre study on AIDS, compared 40 AIDS patients with Kaposi's sarcoma and 40 randomly selected AIDS patients without Kaposi's sarcoma, matched for $CD4^+$ count. The odds ratio for an association between LANA positivity and Kaposi's sarcoma was 16. A similar comparison of 45 AIDS patients with Kaposi's sarcoma and 37 HIV-positive homosexual and bisexual men showed an odds ratio for LANA positivity of 8.5 (Kedes *et al.*, 1996). Lennette *et al.* (1996), who found a lower LANA antibody detection rate among Kaposi's sarcoma patients (52%) than in other studies, obtained an odds ratio of 4.2 for a similar comparison.

In view of the rarity of Kaposi's sarcoma among HIV-infected haemophilia patients, the virtual absence of LANA antibodies in this group (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996) is an important finding. In these studies, a total of 346 HIV-positive persons with haemophilia from three defined cohorts were investigated.

The association between antibodies to KSHV/HHV8 structural proteins and Kaposi's sarcoma has also been examined. Miller *et al.* (1996) compared antibodies to a 40-kDa structural antigen by western blot in AIDS patients with Kaposi's sarcoma (67%) and in HIV-positive homosexual men (13%), giving an odds ratio of 13. For a recombinant capsid-related protein, vp19/ORF 65, Simpson *et al.* (1996) found 81% antibody reactivity among AIDS patients with Kaposi's sarcoma and 31% in HIV-positive homosexual men (odds ratio, 9.2). In contrast, Lennette *et al.* (1996), using an immunofluorescence assay for low titres of antibodies to undefined structural proteins, found positive results in 96% of AIDS patients with Kaposi's sarcoma and in 93% of HIV-positive homosexual men in the United States (odds ratio, 1.8). A comparison of antibody positivity for both LANA and structural antigens in cases of AIDS plus Kaposi's sarcoma and in blood donors and other groups at risk for HIV infection (haemophilia patients and intravenous drug users) yielded much higher odds ratios.

2.1.4 *Temporal associations*

The long-term consequences of infection with KSHV/HHV8 have been examined in a limited number of studies of few exposed individuals. These studies have addressed only the association between Kaposi's sarcoma and prior exposure to KSHV/HHV8, determined by either PCR or serology.

In a cohort study, Whitby *et al.* (1995) followed (for a median of 30 months) 143 HIV-positive patients who did not have Kaposi's sarcoma at the time their first or only blood sample was taken. Of the 11 men who initially had detectable KSHV/HHV8 in their PBMC by nested PCR, six (54%) developed Kaposi's sarcoma, whereas only 12 of 132 (9%) men who were KSHV/HHV8-negative developed the disease (odds ratio, 7.0; 95% CI, 2.8–13).

In a nested case–control study, Moore *et al.* (1996c) and Parry and Moore (1997) compared the detection of KSHV/HHV8 by PCR in paired samples of PBMC drawn

from 21 HIV-infected patients before and after a diagnosis of Kaposi's sarcoma with that in paired samples from 23 high-risk, HIV-infected homosexual men who later developed AIDS. Nine of the 21 Kaposi's sarcoma patients and one of the 23 homosexual controls were KSHV/HHV8-positive at the time the initial sample was taken (odds ratio, 17; 95% CI, 1.8–755). Overall, 11/21 patients and 3/23 controls (at any sample) had evidence of KSHV/HHV8 before the onset of Kaposi's sarcoma (odds ratio, 7.3; 95% CI, 1.4–48).

Lenette *et al.* (1996) analysed 13 pairs of sera collected before and after diagnosis of Kaposi's sarcoma. No clear association between seroconversion to KSHV/HHV8 (latent or lytic antibodies) and the development of Kaposi's sarcoma was found after a median interval of about 12 months.

In a longitudinal study of 40 patients who developed AIDS-associated Kaposi's sarcoma over a period of 13–103 months, 11 patients (28%) showed positive results at all visits, and 21 seroconverted to KSHV/HHV8 6–75 months before a diagnosis of Kaposi's sarcoma (Gao *et al.*, 1996b). The median duration of positivity for antibodies to LANA before the diagnosis was 33 months (Figure 5). The LANA antibody titres remained constant between seroconversion and Kaposi's sarcoma development, which the authors suggested was inconsistent with seroconversion to LANA reflecting reactivation of a pre-existing KSHV/HHV8 infection (Gao *et al.*, 1996a; Figure 6). In a subsequent study based on an indirect immunofluorescence assay for LANA on the EBV-negative KSHV/HHV8-infected cell line BCP-1, similar results were obtained (Gao *et al.*, 1996a).

2.2 Lymphoproliferative disorders

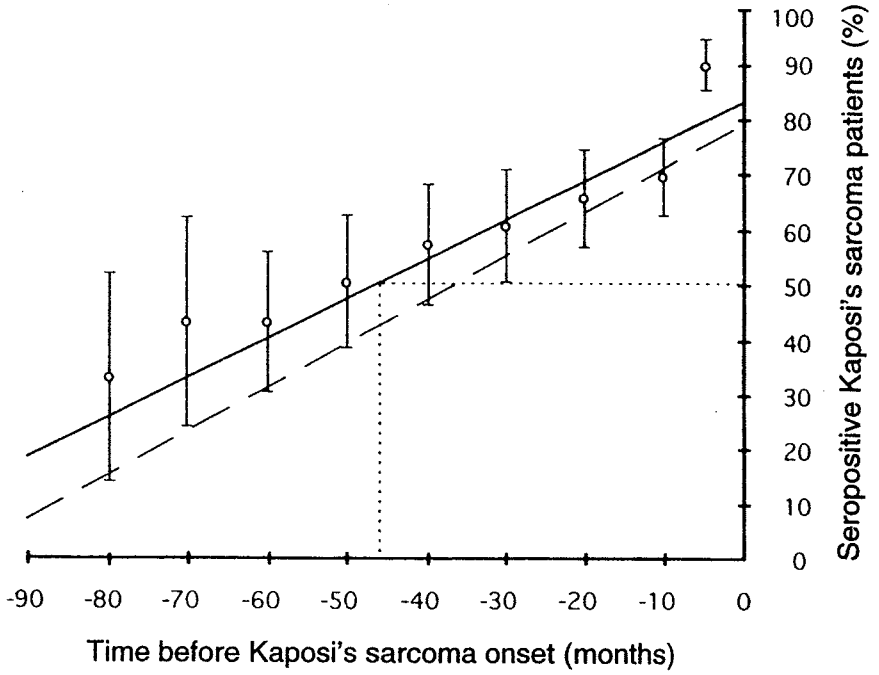
2.2.1 *Primary effusion lymphomas*

2.2.1.1 *Pathology and clinical presentation*

Another neoplastic condition associated with KSHV/HHV8 is primary effusion lymphoma. AIDS-related lymphomas presenting as primary malignant lymphomatous effusions in body cavities were first recognized in the late 1980s (Knowles *et al.*, 1989; Walts *et al.*, 1990; Karcher *et al.*, 1992). This lymphoma is a rare, distinct subtype of non-Hodgkin's lymphoma that has morphological features shared by large-cell immunoblastic lymphomas and anaplastic large-cell lymphoma (Ansari *et al.*, 1996; Carbone *et al.*, 1996a; Cesarman *et al.*, 1996b). Primary effusion lymphoma is defined by distinctive clinical, immunophenotypic and molecular genetic features (Cesarman *et al.*, 1995a). It presents predominantly as malignant effusions in the pleural, pericardial or peritoneal cavities, usually without significant tumour mass or lymphadenopathy; however, lymphomatous infiltration of serosal surfaces adjacent to the site of the primary malignant effusion is sometimes seen (Komanduri *et al.*, 1996).

Morphologically, the cells bridge the features of large-cell immunoblastic and anaplastic large-cell lymphomas. They are usually large and irregularly shaped, with abundant cytoplasm and variably chromatic and pleomorphic nuclei. One or more prominent nucleoli are usually present, and mitotic features are abundant (Ansari *et al.*, 1996).

Figure 5. Prevalence of seropositivity for BCP-1 immunofluorescence and for latent nuclear antigen in 39 homosexual AIDS patients before onset of Kaposi's sarcoma



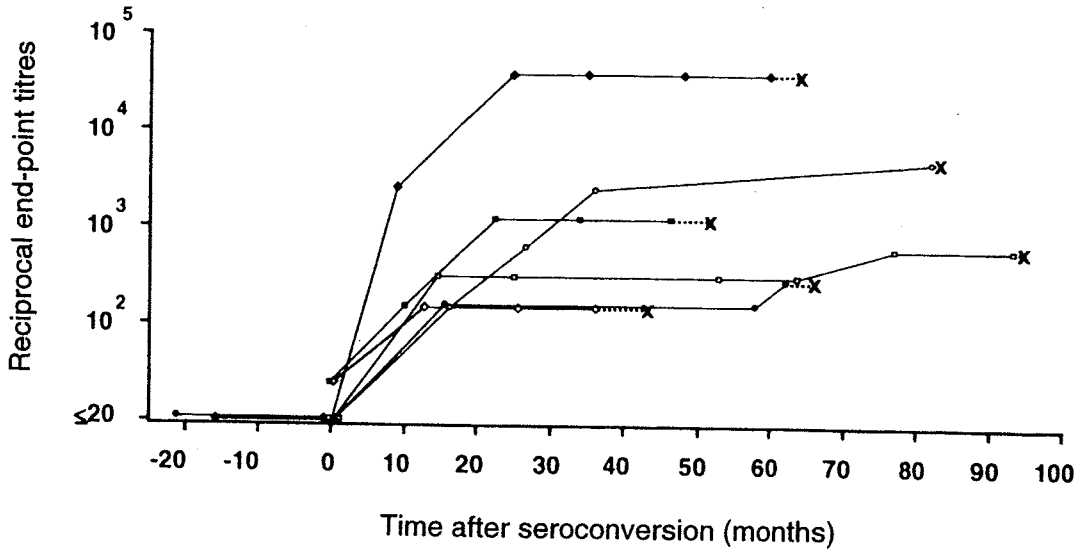
From Gao *et al.* (1996a,b)

Date of seroconversion was estimated to be the mid-point between last negative and first positive serological test. For comparison, seropositivity for KSHV/HHV8 by immunoassay for latent nuclear antigen (dashed line) is plotted against seropositivity by BCP-1 immunofluorescence (solid line). Fifty percent of the Kaposi's sarcoma patients were seropositive 46 months before onset of the disease by BCP-1 immunofluorescence assay. Error bars are standard errors of the mean calculated from a binomial distribution.

Under the electron microscope, the cells are large, with lobulated nuclei containing marginated heterochromatin and prominent rope-like nucleolonemas. The cytoplasm is moderate in amount and exhibits short, blunt, surface projections. KSHV/HHV8 particles are not identified in the cytoplasm, but nuclear particles measuring 110 nm have been observed (Renne *et al.*, 1996a; Said *et al.*, 1996a,b).

Primary effusion lymphoma cells have indeterminate (null) immunophenotypes, lacking expression of any lineage-associated B- or T-lymphocyte antigens (Table 7), but usually express the common leukocyte antigen CD45. A B-cell lineage is indicated by the presence of clonal immunoglobulin gene rearrangement (Knowles *et al.*, 1989; Cesarman *et al.*, 1995b; Komanduri *et al.*, 1996). The B-cell derivation is also supported by the monoclonal nature of primary effusion lymphoma, as demonstrated by a consistent rearrangement of the immunoglobulin genes and by expression of monotypic κ or λ mRNA in the cell cytoplasm (Nador *et al.*, 1996). Primary effusion lymphoma cells usually express activation markers such as CD30, CD38, CD71 and epithelial membrane antigen.

Figure 6. Immunoglobulin-γ end-point titres for six AIDS patients with Kaposi's sarcoma from whom three or more samples were drawn after seroconversion (immunofluorescence titre > 1:160 and a fourfold or greater rise in end-point titre)



From Gao *et al.* (1996a)

Titres remained elevated for 36–93 months after seroconversion, until onset of Kaposi's sarcoma (X), consistent with a prolonged antibody response after primary infection

These cells consistently lack the molecular defects commonly associated with neoplasia of mature B cells, including activation of the proto-oncogenes *c-myc*, *bcl-2*, *bcl-6*, *N-ras* and *K-ras* or mutations of *p53* (Cesarman *et al.*, 1995a; Carbone *et al.*, 1996a; Nador *et al.*, 1996). Cytogenetic studies have shown complex, hyperdiploid karyotypes. Alterations of the chromosomal region 1q21–q23 have been reported, which are also present in other EBV-positive AIDS-related lymphomas (Ansari *et al.*, 1996).

The levels of IL-6 and IL-10, which are involved in B-cell proliferation and differentiation, are both markedly elevated (340–16 000-fold higher than in normal human plasma) in primary effusion lymphoma. Expression of both IL-6 and IL-6 receptor transcripts in some cells suggests a paracrine mechanism for continued B-cell proliferation (Komanduri *et al.*, 1996).

In patients with AIDS, primary effusion lymphoma is a fulminant lymphoproliferation, and the median survival time is less than six months (Komanduri *et al.*, 1996; Nador *et al.*, 1996); however, a more indolent course has been documented in immunocompetent patients (Strauchen *et al.*, 1996).

Table 7. Immunophenotypic types of primary effusion lymphoma cells

CD45 (leukocyte common antigen)	+
TdT	–
Activation markers	
CD30	+
CD38	+
CD71	+
HLA-DR	+
Epithelial membrane antigen	+
T-Cell markers	
CD2	–
CD3	–
CD4	–
CD5	–
CD7	–
CD8	–
B-Cell markers	
CD19	–
CD20	–
CD22	–
CD23	–
Other markers	
CD10	–
CD14	–
CD15 (Reed-Sternberg antigen)	–

From Cesarman *et al.* (1995a,b), Ansari *et al.* (1996) and Nador *et al.* (1996)

2.2.1.2 *Descriptive epidemiology*

Very little is known about the distribution and epidemiological characteristics of primary effusion lymphoma. Because it is rare, its incidence remains to be established. Although primary effusion lymphoma was first described among AIDS patients (Knowles *et al.*, 1988), in whom it occurs mainly at an advanced stage of the disease (Komanduri *et al.*, 1996), it has also been reported in HIV-negative individuals (Nador *et al.*, 1995). In addition, like Kaposi's sarcoma, with which it is closely linked, it is seen primarily in homosexual men and seldom in other groups at risk for HIV infection (Jaffe, 1996; Nador *et al.*, 1996).

Primary effusion lymphoma is distinct from another body cavity-based lymphoma, pyothorax-related B-cell lymphoma. These large B-cell lymphomas occur in patients with long-standing pyothorax resulting from artificial pneumothorax for the treatment of pulmonary tuberculosis or tuberculous pleuritis (Iuchi *et al.*, 1987, 1989). This tumour has been identified most often in Japan, with more than 50 cases in the literature, in comparison with a single series of three cases reported from a western country, France

(Martin *et al.*, 1994). The geographical distribution of pyothorax-related B-cell lymphoma may be due to the fact that artificial pneumothorax is used more frequently as a treatment modality in Japan. In common with primary effusion lymphoma, the tumour cells in pyothorax-related B-cell lymphoma nearly always contain EBV (14 of 14 in a study by Cesarman *et al.*, 1996b); however, in pyothorax-related B-cell lymphoma, pleural mass lesions are seen, *c-myc* rearrangements are present and KSHV/HHV8 is absent (Cesarman *et al.*, 1996b).

2.2.1.3 *Case reports and case series*

Table 8 summarizes 30 case reports of primary effusion lymphoma reported in the literature. Four other cases were associated with *c-myc* gene rearrangements and thus molecularly resembled Burkitt-type lymphomas (Nador *et al.*, 1996). Since these cases also had cytomorphological features similar to those of Burkitt's or Burkitt-like lymphomas, and two of the four also involved systemic lymphoma, Nador *et al.* (1996), who originally reported these cases as primary effusion lymphomas, argued that they should be classified as Burkitt-type lymphomas, despite their body cavity involvement. [The Working Group concluded that these cases could not be considered primary effusion lymphomas.] Effusions from the 30 patients all contained KSHV/HHV8 and in 26 of these the PCR product was confirmed by Southern blot hybridization. Twenty-five of the described cases occurred in HIV-infected homosexual men and three in uninfected elderly men who did not belong to any established HIV risk group. Two cases of primary effusion lymphomas have been described in HIV-negative women (Said *et al.*, 1996b).

The median $CD4^+$ count in the HIV-infected persons was 65, indicating that they were severely immunosuppressed at the time of diagnosis of primary effusion lymphoma (Table 8). Of the patients reported to be infected with HIV, 10/25 had previously or at the same time received a diagnosis of Kaposi's sarcoma. Similarly, two of five uninfected primary effusion lymphoma patients had Kaposi's sarcoma (Nador *et al.*, 1995, 1996; Said *et al.*, 1996b; Strauchen *et al.*, 1996).

Co-infection with EBV is common in primary effusion lymphomas; EBV monoclonality has been established in most cases (Komanduri *et al.*, 1996; Cesarman *et al.*, 1995a; Nador *et al.*, 1996). It is therefore of interest that several cell lines derived from these lymphomas, with genetic and immunological markers similar to those of the original lymphomas, were latently infected with KSHV/HHV8 but not EBV (Arvanitakis *et al.*, 1996; Renne *et al.*, 1996a; Said *et al.*, 1996a; Gao *et al.*, 1996b). KSHV/HHV8 has not been consistently detected in other lymphomas (see Table 9).

2.2.2 *Castleman's disease*

2.2.2.1 *Pathology and clinical presentation*

Castleman's disease, also referred to as angiofollicular or giant lymph node hyperplasia, is a rare, usually polyclonal, non-neoplastic disorder of unknown etiology (Castleman *et al.*, 1956). Two distinct histopathological variants with different clinical characteristics have been described: the hyaline vascular type and the plasma-cell type. The more common hyaline form presents primarily as a solitary mass, most frequently in

Table 8. Demographic, clinical and virological characteristics of patients with primary effusion lymphoma

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
46/M	+	HS	–	561	Abdominal	None	+	2	Clonal	Chadburn *et al.* (1993); Cesarman *et al.* (1995a)
31/M	+	HS	–	NR	Pleural	None	+	1	Clonal	Knowles *et al.* (1989); Cesarman *et al.* (1995a)
40/M	+	HS	+	NR	Pleural	Submandibular gland, lymph nodes	+	1	Clonal	Knowles *et al.* (1989); Cesarman *et al.* (1995a)
35/M	+	HS	–	NR	Abdominal	None	+	1	Clonal	Walts *et al.* (1990); Cesarman *et al.* (1995a)
38/M	+	HS	–	NR	Pericardial	None	+	2	Clonal	Cesarman *et al.* (1995a)
58/M	+	HS	–	NR	Pleural	None	+	1	Clonal	Cesarman *et al.* (1995a)
37/M	+	HS	+	109	Pericardial	None	+	1	ND	Nador *et al.* (1996)
42/M	+	HS	–	NR	Pleural	Oesophageal lymph node, lung	+	2	ND	Nador *et al.* (1996)
53/M	+	HS	–	84	Abdominal	None	+	2	Clonal	Ansari *et al.* (1996)
43/M	+	HS	–	34	Pleural	None	+	2	Clonal	Ansari *et al.* (1996)
44/M	+	HS	+	25	Pleural	None	+	2	Clonal	Ansari *et al.* (1996)
44/M	+	HS	+	33	Pleural	None	+	1	Clonal	Ansari *et al.* (1996)

Table 8 (contd)

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
54/M	+	HS	+	130	Pleural	None	+	1	Clonal	Ansari *et al.* (1996)
42/M	+	HS	+	NR	Pleural	None	+	1	Clonal	Gessain *et al.* (1997)
31/M	+	HS	–	NR	Peritoneal, pleural	Small intestine	+	EBER +	ND	Gessain *et al.* (1997)
35/M	+	HS, IVDU	–	58	Abdominal, pleural	None	+[a]	–		Komanduri *et al.* (1996)
40/M	+	HS	–	65	Abdominal	None	+	1	Polyclonal	Komanduri *et al.* (1996)
32/M	+	HS	–	91	Pleural	None	+	2	Clonal	Komanduri *et al.* (1996)
42/M	+	HS	–	181	Abdominal	Left atrial mass	+	1	Polyclonal	Komanduri *et al.* (1996)
31/M	+	HS	+	34	Pericardial	None	+	–		Komanduri *et al.* (1996)
32/M	+	HS	+	65	Pleural	None	+	–		Komanduri *et al.* (1996)
47/M	+	HS, IVDU	–	20	Abdominal, pleural	None	+[a]	1	Clonal	Komanduri *et al.* (1996)
40/M	+	HS	+	190	Abdominal	ND	+	–		Komanduri *et al.* (1996)
30/M	+	HS	–	NR	Pericardial	None	+[a]	1	ND	Walts *et al.* (1990); Cesarman *et al.* (1995a)
32/M	+	HS	+	NR	Pleural	None	+[a]	1	ND	Walts *et al.* (1990); Cesarman *et al.* (1995a)

Table 8 (contd)

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
85/M	–	–	–	288	Pleural	None	+	–		Nador *et al.* (1995, 1996)
78/M	–	–	–	NR	Abdominal	None	+	1	Clonal	Nador *et al.* (1996)
94/M	–	–	+	60	Pleural	Peritoneaum Pericardium	+	–		Strauchen *et al.* (1996)
85/F	–	–	+	NR	Pleural	None	+	–		Said *et al.* (1996b)
46/F	–	–	–	NR	Silicone breast implant	None	+	–		Said *et al.* (1996b)

M, male; F, female; NR, not reported; ND, not determined; HS, homosexual man; IVDU, intravenous drug user; EBER, Epstein-Barr virus-encoded RNA

[a] Only analysis by polymerase chain reaction

Table 9. Presence of HHV8 in lymphoid neoplasias

Histology	Positive/tested
Cases unrelated to AIDS	
Acute lymphoblastic leukaemia	0/44
Chronic lymphocytic leukaemia	0/61
Prolymphocytic leukaemia	0/10
Lymphoplasmacytoid lymphoma	0/3
Mantle-cell lymphoma	0/14
Follicular lymphoma	0/60
Monocytoid lymphoma	0/3
MALT lymphoma	0/16
Hairy cell leukaemia	0/18
Multiple myeloma and plasmacytoma[a]	0/28
Diffuse large-cell lymphoma	0/65
Small non-cleaved (including Burkitt's) lymphoma	0/57
Cutaneous T-cell lymphoma	0/9
Peripheral T-cell lymphoma	0/20
Anaplastic large-cell lymphoma	0/17
Lymphoblastic lymphoma	0/4
Adult T-cell leukaemia/lymphoma	0/13
Post-transplant lymphoproliferation	0/23
Hodgkin's disease	0/49
Primary effusion lymphoma	8/8
AIDS-related lymphomas	
Small non-cleaved lymphoma	0/42
Diffuse large-cell lymphoma	0/39
Anaplastic large-cell lymphoma	0/5
Peripheral T-cell lymphoma	0/1
Hodgkin's disease	0/14
Primary effusion lymphoma	34/35

From Chang *et al.* (1994), Pastore *et al.* (1995), Cesarman *et al.* (1995a), Karcher & Alkan (1995), Nador *et al.* (1995), Ansari *et al.* (1996), Arvanitakis *et al.* (1996), Carbone *et al.* (1996a,b), Gaidano *et al.* (1996b, 1997), Luppi *et al.* (1996b), Nador *et al.* (1996), Otsuki *et al.* (1996), Said *et al.* (1996a), Strauchen *et al.* (1996), Gessain *et al.* (1997)

MALT, mucosa-associated lymphoid tissue

[a] See also section 2.2.3 (multiple myeloma)

the mediastinum or retroperitoneum, is asymptomatic and is usually curable surgically. The rare plasma-cell type is typically characterized by generalized lymphadenopathy, immunological abnormalities and type B symptoms.

The systemic variety, also designated multicentric Castleman's disease, is primarily of the plasma-cell type, but the hyaline type has occasionally been reported in a multicentric clinical appearance (Herrada & Cabanillas, 1995; Shahidi *et al.*, 1995). Associated clinical findings are necessary to make the diagnosis of multicentric Castleman's disease, since the pathological features in lymph nodes can be nonspecific (Peterson & Frizzera, 1993; Shahidi *et al.*, 1995). EBV was reported to be present in 9 of 16 cases of localized and multicentric Castleman's disease (Barozzi *et al.*, 1996). Multicentric Castleman's disease has an aggressive clinical course with a poor prognosis, and such patients are at increased risk for Kaposi's sarcoma and lymphomas (Peterson & Frizzera, 1993). It has been suggested that some of the immunological changes observed in HIV-negative patients with multicentric Castleman's disease are similar to those in HIV-infected individuals (Lane *et al.*, 1985; Vuillier *et al.*, 1988; Birx *et al.*, 1990; Boyd & James, 1992; Ishiyama *et al.*, 1996).

2.2.2.2 *Descriptive epidemiology*

About 70% of patients with all forms of Castleman's disease are under 30 years of age, and men are affected more often than women. Patients with multicentric Castleman's disease often tend to be in their fifties or sixties and to have increased risks for non-Hodgkin's lymphoma and Kaposi's sarcoma (Peterson & Frizzera, 1993).

2.2.2.3 *Case reports and case series*

Few reports have addressed the presence of KSHV/HHV8 in Castleman's disease (Table 10). Soulier *et al.* (1995) detected KSHV/HHV8 in all of 14 HIV-positive lesions from French patients with multicentric Castleman's disease, comprising six plasma-cell type, seven mixed and one hyaline vascular type. Seven of the patients also had Kaposi's sarcoma in the same tissue sample, and an additional two at another site; 64% had Kaposi's sarcoma both in the same tissue and elsewhere. Of 17 HIV-negative multicentric Castleman's disease lesions, seven (three plasma cell, two mixed, two hyaline type) contained KSHV/HHV8. Kaposi's sarcoma was diagnosed in one of these subjects. Whereas the vast majority of cases among HIV-positive patients were found to contain the virus by Southern blot, only two of the seven cases in HIV-negative patients found to be positive by PCR were positive by Southern blot. To evaluate the significance of the positivity rate in the HIV-negative patients, reactive lymph nodes from 34 HIV-seronegative control patients were analysed; only one KSHV/HHV8-positive case was found.

Dupin *et al.* (1995b) reported the finding of KSHV/HHV8 in PMBC from two HIV-infected men with Castleman's disease, one of whom had Kaposi's sarcoma. [The authors did not specify whether these cases were multicentric.] Tirelli *et al.* (1996) found KSHV/HHV8 in lesions from an HIV-positive woman with multicentric Castleman's disease whose husband was diagnosed with Kaposi's sarcoma.

Gessain *et al.* (1996) detected KSHV/HHV8 by PCR in cryopreserved lymph node biopsy samples from one of three HIV-negative patients with multicentric Castleman's disease but in none of three with localized disease. Of the HIV-positive subjects, three of four homosexual men with multicentric Castleman's disease had KSHV/HHV8 in their

Table 10. Detection of KSHV/HHV8 in patients with Castleman's disease

Reference (country)	HIV-negative (KSHV/ HHV8 positive/total)			HIV-positive (KSHV/ HHV8 positive/total)			Method	Comments
	HV	PL	Mixed	HV	PL	Mixed		
Soulier *et al.* (1995) (France)	2/3	3/9	2/5	1/1	6/6	7/7	PCR, Southern blot	All MCD HIV-positive: 9/14 with KS HIV-negative: 1/17 with KS
Corbellino *et al.* (1996d) (Italy)	0/2	4/4					PCR, Southern blot	None with KS or lymphoma
Barozzi *et al.* (1996) (Italy)	1/11		0/5				PCR	Mixed: all MCD HV: all localized Paraffin-embedded biopsies
Gessain *et al.* (1996) (France)	0/1	1/5			3/4		PCR	HIV-positive: 3 HHV8-positive had KS in other organs HIV-negative: none had KS

HV, hyaline vascular type; PL, plasma-cell type; mixed, both HV and PL; MCD, multicentric Castleman's disease

lymph nodes. Kaposi's sarcoma was diagnosed in three HIV-positive and KSHV/HHV8-positive men but not in the KSHV/HHV8-positive but HIV-negative woman. Semi-quantitative PCR showed a high KSHV/HHV8 viral load in the lesions of patients with and without HIV infection.

In a group of Italian HIV-negative patients, Corbellino *et al.* (1996d) found high levels of KSHV/HHV8 by PCR and Southern blot hybridization in biopsy samples from all of four cases of plasma-cell type Castleman's disease but in none of two cases of the hyaline vascular type. [The authors did not specify whether these cases were multicentric.] Neither Kaposi's sarcoma nor lymphoma was diagnosed in any of the patients. None of 20 lymph node biopsy samples from 15 HIV-infected drug abusers with persistent lymphadenopathy or from five HIV-negative patients with reactive lymphadenitis contained KSHV/HHV8. Two of the four cases of plasma-cell Castleman's disease were EBV-positive by PCR.

In archival formalin-fixed, paraffin wax-embedded biopsy material from HIV-negative Italian patients with Castleman's disease, Barozzi *et al.* (1996) found KSHV/HHV8 in one of 11 patients with the localized hyaline vascular type and in none of five patients with multicentric disease. PBMC and saliva were positive for KSHV/HHV8-specific sequences in the KSHV/HHV8-positive patients, whereas serum, faeces and urine were negative.

2.2.3 *Multiple myeloma*

Rettig *et al.* (1997) demonstrated the presence of KSHV/HHV8 DNA by PCR and in-situ hybridization in the cultured bone-marrow dendritic cells of 15 patients with multiple myeloma but not in the myeloma cells (plasma cells). The authors also demonstrated by RT-PCR the expression of v-*IL-6* in three of three cultured myeloma bone-marrow dendritic cells, suggesting that KSHV/HHV8–v-IL-6 contributes to the mechanism whereby bone-marrow dendritic cells infected with KSHV/HHV8 promote myeloma growth. KSHV/HHV8 was not detected by PCR in 28 DNA samples from myeloma specimens (Cesarman *et al.*, 1995a; Pastore *et al.*, 1995; Gessain *et al.*, 1997) or bone-marrow samples (Rettig *et al.*, 1997) in previous studies; this was attributed by the authors to dilution of the sample with uninfected cells.

2.2.4 *Other lymphoproliferative disorders*

With the exception of primary effusion lymphoma, most large series of lymphoid malignancies, including a variety of immunophenotypic categories of B- and T-cell tumours, have not been shown to contain KSHV/HHV8 (Chang *et al.*, 1994; Cesarman *et al.*, 1995a; Pastore *et al.*, 1995; Luppi *et al.*, 1996b; Gessain *et al.*, 1997; see Table 9). Bigoni *et al.* (1996), however, found that with nested and semiquantitative PCR 7–9% of PBMC from non-Hodgkin's lymphoma patients and patients with Hodgkin's disease contained KSHV/HHV8.

2.3 Other tumours

In studies of angiosarcoma in HIV-negative individuals, Gyulai *et al.* (1996a,b) reported one KSHV/HHV8-positive case. McDonagh *et al.* (1996) found KSHV/HHV8 by PCR in seven cases in the United States; one case was also tested by Southern blotting. These findings were not confirmed by other investigators (Chang *et al.*, 1994; Boshoff *et al.*, 1995a,b; Dictor *et al.*, 1996; Jin *et al.*, 1996a,b).

Rady *et al.* (1995) reported the widespread presence of KSHV/HHV8 by PCR in various skin tumours from four immunosuppressed patients, but in studies in Austria, Germany, Sweden and the United Kingdom this association could not be confirmed (Adams *et al.*, 1995; Boshoff *et al.*, 1996; Dictor *et al.*, 1996; Uthman *et al.*, 1996).

3. Studies of Cancer in Animal Models

No animal model for KSHV/HHV8 has so far been reported; however, several closely related rhadinoviruses, e.g. HVS, *Herpesvirus ateles* (HVA) and murid herpesvirus 4 (MHV68), cause lymphoid malignancies or polyclonal proliferation. In terms of its tropism for B-cells, MHV68 is the most closely similar to KSHV/HHV8; however, angiogenic proliferation is not seen with these animal viruses. Whether any of them will provide in-vivo models for KSHV/HHV8 is unknown. Bovine herpesvirus 4 (BHV-4), also a gamma-2 herpesvirus, has not been associated with specific lymphoid disease but is included in this monograph.

Small DNA fragments of two new primate rhadinoviruses, retroperitoneal fibromatosis herpesvirus of *Macaca nemestrina* (RFHVMn) and retroperitoneal fibromatosis herpesvirus of *Macaca mulatta* (RFHVMm), have recently been obtained from two macaque species in one colony (Rose *et al.*, 1997) and these may be closely related to KSHV/HHV8. The animals are also infected with D-type simian retrovirus type 2 and develop retroperitoneal and subcutaneous fibrosis with progressive fibrovascular proliferation, reminiscent of Kaposi's sarcoma lesions (Stromberg *et al.*, 1984; Bryant *et al.*, 1986).

3.1 *Herpesvirus saimiri* (saimiriine herpesvirus 2)

3.1.1 *Description*

HVS is a non-human primate rhadinovirus; it was first isolated in 1968 from the kidneys of squirrel monkeys (*Saimiri sciureus*), New World primates common to South America (Meléndez *et al.*, 1968). The virus is indigenous to squirrel monkeys and is transmitted horizontally from mother to infant in saliva. There is no evidence that HVS can induce tumours in squirrel monkeys (Jung & Desrosiers, 1994); however, when newborn squirrel monkeys were removed from their mothers immediately after birth and bred in captivity, some also being immunosuppressed with ciclosporin, later inoculation of HVS provoked viraemia and the development of antibody but no illness (Fleckenstein

& Desrosiers, 1982). There are no published reports of the development of tumours in squirrel monkeys in the wild after exposure to HVS.

Squirrel monkeys originating from discrete geographical zones were all found to be infected with HVS. The virus can be recovered routinely from PBMC and other tissues and organs of squirrel monkeys either naturally or experimentally infected with HVS. There is no evidence of seasonal variation in HVS transmission; however, under crowded conditions more virus is shed from the throat (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994). Horizontal transmission of HVS by oropharyngeal secretions from a squirrel monkey to an owl monkey housed in the same isolation unit occurred experimentally (Barahona *et al.*, 1975).

HVS, like other gammaherpesviruses, remains latent in lymphocytes and can cause lymphomas, leukaemias and lymphoproliferative disorders in other species of New World primates — tamarins and marmosets (*Saguinus* and *Callithrix* species), owl monkeys (*Aotus trivirgatus*) and spider monkeys (*Ateles* species) — and in rabbits (*Oryctolagus cuniculus*) (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994).

3.1.2 *Host range, virus isolation and virus multiplication*

HVS was first isolated by chance when it was observed that cultured kidney cells derived from a healthy squirrel monkey degenerated spontaneously, with cytopathic effects reminiscent of herpesvirus-infected cultures (Meléndez *et al.*, 1968). Specifically, hexagonal enveloped virions were found of approximately 140 nm, with capsids of 101–108 nm and cores of 50–60 nm (Deinhardt *et al.*, 1974). HVS can replicate in various monkey and human cell cultures. Higher infectivity titres were obtained in kidney cells from owl monkeys ($\geq 10^{6.2}$/mL) than in those from African green or rhesus monkeys. This experiment also showed that the infection with HVS is highly productive, contrary to most other gammaherpesviral infections *in vitro* (Ablashi *et al.*, 1972).

The first evidence that HVS could infect human fibroblast cells was provided by Ablashi *et al.* (1971a), who showed that infected cells produce infectious virus; however, the infectivity titres obtained were lower than those in owl monkey kidney cells. Simmer *et al.* (1991) showed that HVS persists in T-lymphoblastoid cells of various primate species, including human T cells. The human B-cell line (Raji) containing episomal EBV DNA could be persistently infected with HVS. A human pancreatic carcinoma cell line of epithelial origin could also be infected with HVS, and the cells contained both episomal and linear HVS DNA and produced HVS. Oie *et al.* (1973) reported that the MEST cell line, which is a spontaneously transformed cell line of rhesus monkey embryo origin, showed cytopathic effects after infection with HVS and, after two subpassages, provided a continuous source of HVS. HVS has tropism for T lymphocytes and can immortalize $CD8^+$ and $CD4^+$ cells (Biesinger *et al.*, 1992).

Dahlberg *et al.* (1988) showed that replication of HVS in human cells is semipermissive because of a block in the synthesis of certain late proteins. The studies were conducted with human fibroblasts (HEp-2), other epithelial cells (KHOS) and human T cells and the use of polyclonal and monoclonal antibodies to HVS (Dahlberg *et al.*, 1985).

3.1.3 *Host response: antibody detection*

Klein *et al.* (1973) identified the early and late antigens in HVS-infected Vero cells (an African green monkey continuous cell line) using sera obtained from squirrel monkeys (*Saimiri sciureus*) and HVS-infected white-lipped tamarins (*Saguinus fuscicollis*) and owl monkeys (*Aotus trivirgatus*). Two strikingly different patterns of staining of early antigen were observed, both limited to the nucleus (one trabecular and the other punctate), very similar to EBV EA(R) and EA(D). HVS-infected owl monkeys and white-lipped tamarins behaved quite differently from squirrel monkeys in their early antigen responses, and took two to three times longer to develop antibodies. The early antigen titres lagged behind those of late antigen. Antibodies appeared later in owl monkeys than in white-lipped tamarins; in squirrel monkeys, the virus could be isolated at the same time or somewhat later than the appearance of antibody. In tamarins, HVS was isolated before or at the time of appearance of antibody, whereas in owl monkeys, the virus was usually isolated one to two weeks before antibody was detected.

3.1.4 *Human exposure*

Of 150 animal caretakers who were bitten several times while handling squirrel monkeys or HVS-infected owl monkeys or marmosets, 11 (7.3%) were seropositive (Ablashi *et al.*, 1988). Four of 100 control adult sera (4%) also contained antibody to HVS as tested by immunofluorescence assay. The antibody titre to late antigen in these sera was between 1:10 and 1:80, and intense nuclear fluorescence reactivity was observed in cells in most of the sera. When these sera were analysed by radioimmunoprecipitation, two from the exposed persons were exceptionally reactive and the others weakly reactive. The sera precipitated mainly HVS major capsid protein (160 kDa) but also recognized several other proteins. Unexpectedly, follow-up sera from the caretakers several months later showed no antibodies. Whether these results were due to antibody cross-reactivity with KSHV/HHV8 or another primate rhadinovirus to which these individuals might have been exposed is unclear. Several attempts to isolate HVS from PBMC of the caretakers were unsuccessful.

3.1.5 *Molecular aspects*

HVS has a linear double-stranded DNA genome of about 155 kb with a central unique light region of 112 kb (36% G + C) flanked by variable numbers of 1.4-kb tandem repeats of heavy DNA (72% G + C) (Bankier *et al.*, 1985; Cameron *et al.*, 1989; Albrecht *et al.*, 1992; Jung & Desrosiers, 1994). Earlier studies showed that DNA sequences at the left terminus of light DNA are required for in-vitro immortalization and for the oncogenic phenotypes (Desrosiers *et al.*, 1986). Most HVS genes have ORF sequences that are similar to those of the KSHV/HHV8 and EBV genomes, and most of these genes are arranged in collinear order. Nevertheless, HVS and EBV differ with respect to transformation and latent stage replication (Kung & Medveczky, 1996).

HVS strains have been classified into subgroups A, B and C on the basis of molecular analysis, biological properties and oncogenic and transforming potential. Mutational analysis demonstrates that most of the left ORF of strain 11 (subgroup A) is required to

immortalize common marmoset T lymphocytes, but not for replication of the virus. An ORF designated *STP-C488* (subgroup C) was more potent in transforming Rat-1 cells (Jung *et al.*, 1991). DNA sequencing of the left end of the light strand revealed that HVS contains a gene coding for dihydrofolate reductase (DHFR) with high sequence homology to human *DHFR*. The HVS *DHFR* gene differs from mammalian and avian genes in that it lacks introns, suggesting that it may have been acquired through a process involving reverse transcriptase. The *DHFR* gene is also not required for viral multiplication or for in-vitro transformation (Fleckenstein & Desrosiers, 1982).

Murthy *et al.* (1986) studied RNA derived from 0.0–6.7 map units (7.4 kb) of HVS light DNA by northern blot hybridization and nuclease protection analysis. Although several poly(A)-containing RNAs were found in this region in permissively infected monolayer cells *in vitro*, these RNAs could not be detected in lymphoblastoid tumour cell lines. Instead, transformed T cells expressed four small RNAs of approximately 73, 105, 110 and 135 nucleotides derived from this region; these RNAs were not detected during the course of lytic infection of monolayer cells. HVS and EBV also differ with regard to other genes relevant to transformation and latency. In HVS, there are no *EBNA-1* or *oriP*-like sequences in the region of the genome that corresponds to these sites in EBV (see monograph on Epstein-Barr virus; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994; Kung & Medveczky, 1996). Instead, HVS and other rhadinoviruses appear to have acquired from the host cells a block of genes at the left side of their genomes that is not found in lymphocryptoviruses. Many other viral genes of HVS have been identified which code for cytokines (Yao *et al.*, 1995), as has also been observed in KSHV/HHV8.

3.1.6 *Oncogenicity in non-human primates, rabbits and transgenic mice*

As indicated previously, HVS strains can be divided into A, B and C subgroups. HVS of subgroups A and C are highly oncogenic for a wide spectrum of New World primates, while HVS strains of subgroup B have limited tumour-inducing capacity. In rabbits, only subgroup C has oncogenic potential (Medveczky *et al.*, 1989).

HVS induces malignant lymphoma of the reticulum-cell type in cotton-topped tamarins (*Saguinus oedipus*) and white-lipped tamarins (*Saguinus fuscicollis*) (Meléndez *et al.*, 1969; Deinhardt *et al.*, 1974). Lymphocytic leukaemia was induced in owl monkeys (*Aotus trivirgatus*) inoculated with prototype strain of HVS (295C; Meléndez *et al.*, 1971). Lymphoproliferative disease was induced in cinnamon ringtail monkeys (*Cebus albifrons*) and African green monkeys (*Cercopithecus aethiops*; Meléndez *et al.*, 1972a).

Fleckenstein *et al.* (1978a) induced malignant lymphomas in 3/14 cotton-topped tamarins inoculated with isolated HVS DNA.

Five owl monkeys experimentally inoculated with HVS developed moderately well-differentiated malignant lymphomas with lymphogenous leukaemia within 57–178 days. HVS could be isolated from cells of the spleen, kidney, lung, tumour mass (lymph node), thymus and liver cultured from these organs. HVS could also be isolated from PBMC. In general, the leukocyte counts rose significantly above the baseline levels. Bone-marrow

samples showed eosinophilic hyperplasia and progressive infiltration by lymphocytes. Terminally, the bone marrow was intensely hypercellular, with 70% cells of the lymphogenous series (Ablashi *et al.*, 1971b). The tumour-bearing owl monkeys developed antibodies to HVS early and late antigens (Klein *et al.*, 1973) and membrane antigens (Prevost *et al.*, 1976).

Heat-inactivated HVS strain 295C induced malignant lymphoma in two owl monkeys, suggesting that HVS DNA, even though it may have been fragmented, can induce tumours (Ablashi *et al.*, 1973).

The common marmoset (*Callithrix jacchus*) did not develop tumours after inoculation with HVS, even though the virus was consistently present in the PBMC.

Daniel *et al.* (1974) induced lymphoid tumours in New Zealand white rabbits (*Oryctolagus cuniculus*) by intravenous inoculation of HVS, although not all of the inoculated animals developed tumours. Similar results were reported in an inbred strain III/J (Ablashi *et al.*, 1980). The clinical and pathological findings in these animals were nasal discharge, respiratory disorders leading to death, lymphocyte infiltrates in the nares, enlarged lymph nodes and generalized peripheral lymphoadenopathy. The animals developed antibody to HVS, and the virus could be isolated from lymph nodes, spleen and PBMC. All infected animals showed depressed cell-mediated immune response.

Kretschmer *et al.* (1996) showed that a previously identified ORF (*StpA*) which is necessary for oncogenicity in monkeys induces pleomorphic T-cell lymphomas in transgenic mice expressing StpA in a variety of organs.

3.1.7 *Transformation of mammalian cells* in vitro

Subgroup A and C strains of HVS efficiently transform marmoset PBMC to permanent autonomous growth *in vitro*, whereas cells transformed by subgroup B viruses are IL-2-dependent (Desrosiers *et al.*, 1986; Szomolanyi *et al.*, 1987).

Ablashi *et al.* (1985) established a T-cell line from the spleen of an HVS-infected New Zealand male rabbit which developed a well-differentiated lymphoma. This cell line was IL-2-dependent and was highly oncogenic in rabbits, since it produced well-differentiated lymphomas, and the animals died of lung complications shortly afterwards. The cells (7710 cell line) contained multiple copies of non-integrated circular HVS genome. As in other HVS-transformed non-producer cell lines, a large segment of light DNA was missing from the persistent circular viral DNA present in the 7710 cells.

Medveczky *et al.* (1993) showed that a collagen-like ORF-1 protein is expressed in permanent tumour-derived rabbit T cells and in transformed primate T cells infected *in vitro* with HVS group C strain 484-77. Antibody to ORF-1 protein was also found in rabbits bearing tumours, suggesting that this protein is expressed *in vivo*.

Pacheco-Castro *et al.* (1996) showed that HVS immortalized $\alpha\beta$ and $\gamma\delta$ human T-lineage cells derived from $CD34^+$ intrathymic precursors *in vitro*, and the $\gamma\delta$ lineage was IL-2-dependent.

3.2 *Herpesvirus ateles* (ateline herpesvirus 2)

3.2.1 *Description*

HVA is also a non-human primate rhadinovirus. Strain 810 was first isolated from a degenerating kidney-cell culture from a spider monkey (*Ateles geoffroyii*; Meléndez *et al.*, 1972b). Later, strain 73 of HVA was isolated from circulating lymphocytes of a spider monkey (*A. paniscus*) by co-cultivation with owl monkey kidney cells (Falk *et al.*, 1974). Immunological, biological and molecular analyses indicate that HVA is closely related to HVS but with homology to EBV. HVA is common in species of spider monkeys native to South America and does not cause disease in the host. HVA does not cross-react with other primate herpesviruses. The virus is latent in spider monkeys and can be isolated early from tissue and PBMC (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994).

3.2.2 *Host range, cytopathogenicity and viral multiplication*

Although HVA is a T-lymphotropic herpesvirus, it replicates efficiently in monolayer cell cultures of fibroblasts and epithelial cells of animal origin (Deinhardt *et al.*, 1974; Jung & Desrosiers, 1994). HVA in monolayer cultures induces cytopathic effects, with discrete, enlarged, round multinucleated giant cells. Owl monkey kidney cells are an excellent source of high-titre infectious virus. The host range of cells that can be infected with HVA is more distinct than that of HVS: HVA infects primary cultures of African green monkey, owl monkey, marmoset and squirrel monkey kidney cells. The cytopathic effects are slower (more than five days), and HVA titres are 2–3 logs lower than those of HVS. Rabbit kidney cells could not be infected by strain 73 but were infected by strain 810, which also infected cells of hamster heart origin. Lymphocytes from owl monkeys, marmosets and humans could be infected and transformed (Deinhardt *et al.*, 1974; Falk *et al.*, 1974; Ablashi *et al.*, 1976).

Luetzeler *et al.* (1979), using HVA-73, studied the ultrastructural morphogenesis of HVA in owl monkey kidney cells. The replicative cycle paralleled, in general, that of HVS, two morphologically distinct inclusion bodies appearing in the nuclei early in infection.

3.2.3 *Molecular analysis*

Little is known about the molecular biology of HVA. The structural organization of its genome is similar to that of HVS. There has been no sub-classification of its strains. Interestingly, 35% of the light strands of the DNA of HVA and HVS anneal with each other, but with considerable mismatching of base sequences. There is 10% homology between the heavy regions of HVS and HVA, with at least 13% divergence of their base pairs (Fleckenstein *et al.*, 1978b).

3.2.4 *Oncogenicity in non-human primates*

Three cotton-topped tamarins and two owl monkeys were inoculated intramuscularly with HVA strain 810. Two of the tamarins died 28 days after inoculation, and one was

killed when moribund at 40 days; all animals had malignant lymphomas, and generalized enlargement of the lymph nodes and splenomegaly were seen grossly. The two owl monkeys were killed 42 days after inoculation in moribund condition. No significant lesions were seen in one, while in the other focal interstitial collections of lymphoblasts, reticulum cells and eosinophils were present in the kidneys and lung and there was lymphocytic hyperplasia in most lymph nodes (Meléndez *et al.*, 1972b).

Hunt *et al.* (1972) inoculated HVA strain 810 into 12 cotton-topped tamarins. All animals died within about one month with malignant lymphomas of the lymphoblastic type. Leukaemia developed in 11 of the animals. One of four control tamarins in contact with the infected animals also developed lymphoma and leukaemia, indicating horizontal transmission.

Laufs and Meléndez (1973) inoculated partially purified HVA DNA or lymphoid cells derived from tumorous lymph nodes taken from experimentally infected cotton-topped tamarins immediately after death into owl monkeys (*Aotus trivirgatus*) and marmosets (*Callithrix jacchus*), two other New World monkey species, and African green monkeys (*Cercopithecus aethiops*). The two owl monkeys died after 20 and 28 days, respectively, of unknown causes. The two common marmosets died 36 and 104 days after inoculation with generalized malignant lymphoma, which resembled the disease observed in cotton-topped tamarins. The two adult African green monkeys inoculated with tumour cells or with partially purified HVA DNA survived, however, but the virus could not be isolated from their PBMC, indicating that this species of Old World primate is not susceptible to HVA infection.

Three viral isolates of HVA induced fatal malignant lymphomas in six cotton-topped tamarins and two white-lipped tamarins, while one isolate tested in squirrel monkeys did not cause overt disease (Falk *et al.*, 1974).

Two common marmosets infected with HVA strain 73 developed lymphomas of a variety of cell types within 27 days after inoculation. Some of the lymphoma cells were giant cells resembling the Sternberg-Reed cells observed in human Hodgkin's disease (Ablashi *et al.*, 1978).

3.3 Bovine herpesvirus 4 (Movar herpesvirus)

BHV-4 is one of four known bovine herpesviruses. It was first isolated by Bartha *et al.* (1966) under the name 'Movar' strain and has a worldwide distribution. It is the only known bovine gammaherpesvirus and is most probably a rhadinovirus, since it is similar to HVS (Bublot *et al.*, 1992). It has been isolated in a variety of clinical conditions as well as from healthy cattle (reviewed by Thiry *et al.*, 1989, 1990, 1992a,b). The group BHV-4 includes a large number of antigenically related isolates that are distinct from other bovine herpesviruses (Potgieter & Maré, 1974; Staczek, 1990). The role of BHV-4 as the etiological agent of a distinct disease entity is still questionable, although its role in the etiology of some diseases of the eye and respiratory and genital tracts has been suggested (Thiry *et al.*, 1989, 1990).

3.3.1 *Classification*

BHV-4 was initially known as bovine cytomegalovirus and was classified as a beta-herpesvirus because only its biological characteristics were taken into account (Storz *et al.*, 1984). Bublot *et al.* (1992) used molecular data to show that BHV-4 belongs to the Gammaherpesvirinae. Careful examination of the BHV-4 genome showed its close relationship to other gammaherpesviruses such as HVS, which allowed classification of BHV-4 into the rhadinovirus group (Lomonte *et al.*, 1996).

3.3.2 *Description*

The morphology of BHV-4 is typical of that of a herpesvirus. The nucleocapsid is icosahedral, with a dense core within the capsid which is made up of a regular arrangement of short tubular capsomeres (Todd & Storz, 1983). The diameter of the naked nucleocapsid is about 90–100 nm, while enveloped virions have a diameter ranging from 115 to 150 nm (Smith *et al.*, 1972; Munz *et al.*, 1974).

The BHV-4 virion contains double-stranded DNA of approximately 145 kb (Todd & Storz, 1983; Ehlers *et al.*, 1985). The genomic structure of BHV-4, typical of group B herpesviruses (Roizman, 1982), is similar to that of HVS, i.e. it has a unique coding sequence (light DNA) of approximately 110 kb flanked by a (G + C)-rich tandem repeat region of 2.65 kb (in BHV-4 VT strain) called polyrepetitive DNA (Ehlers *et al.*, 1985; Bublot *et al.*, 1990). RNA derived from the heavy strand of DNA has not been detected in infected cells (Chang & van Santen, 1992).

The overall genomic organization of BHV-4 VT strain was determined by sequencing 33 segments of the coding region (light strand) of its genome (Bublot *et al.*, 1992). Twenty-seven sequences showed homology to proteins present in either the three herpesvirus subfamilies or in only the two gammaherpesviruses, EBV and HVS. In the former case, the homology scores were always higher in comparison with EBV and HVS proteins than with the same proteins in alpha- or betaherpesviruses. Twenty-three of the sequenced regions of BHV-4 had homologous counterparts in both HVS and EBV genes. Nineteen of these regions belong to the five blocks of genes that are conserved among gamma- and alphaherpesviruses and/or betaherpesviruses. Five BHV-4 sequences were homologous to genes present only in the gammaherpesvirus genomes. The BHV-4 amino-acid sequences were more closely related to those of gammaherpesviruses than to those of alpha- or betaherpesviruses. Furthermore, the homology of most of these sequences was closer to the homologous products of HVS than to the equivalent proteins of EBV. Therefore, on the basis of the overall conservation of the sequences, BHV-4 is more closely related to HVS than to EBV. Six of the 33 BHV-4 sequences were homologous to neither HVS nor EBV genes nor to any other herpesvirus genes. All of these six sequences were located outside the conserved gene blocks. Genomic regions that were found to vary in size between BHV-4 isolates or strains (Bublot *et al.*, 1990, 1991a; Thiry *et al.*, 1992b) were also located outside these blocks (Bublot *et al.*, 1992).

The presence of five blocks of conserved genes was demonstrated in the BHV-4 genome, as in EBV and HVS. The length of the BHV-4 conserved gene blocks was estimated and found to be more closely related to that of HVS than that of EBV. Only

HVS block 3 was longer than those of BHV-4 and EBV (Lomonte *et al.*, 1996). This difference in size was due to the presence of a repeated region in the HVS gene 48 which causes an expansion of the acidic C-terminal domain of the protein (Albrecht *et al.*, 1992). The space between the BHV-4 gene blocks was also more like that of HVS than EBV, particularly between blocks 3 and 4 and blocks 4 and 5. Finally, as in the HVS genome, no large internal repeats were found between blocks 4 and 5 as is the case in the EBV genome.

No genes were found in region C, located between the second and the third conserved blocks. This region was less than 100 nucleotides long in the HVS and BHV-4 genomes (Lomonte *et al.*, 1996), whereas in the EBV genome this region contains the *BKRF1* gene coding for the EBNA-1 protein (Baer *et al.*, 1984) which is expressed in EBV-immortalized B lymphocytes (Middleton *et al.*, 1991).

A gene coding for viral fas-associated death domain protein interleukin-1β converting enzyme (FLICE)-inhibiting proteins, which prevent apoptosis, has been identified in BHV-4 DNA (Thome *et al.*, 1997).

Glycoprotein gB is a heterodimer and is a major component of the BHV-4 virion, unlike gBs of EBV (gp110) and murine gammaherpesvirus 68 (Lomonte *et al.*, 1997). This glycoprotein corresponds to the gp10/17 described by Dubuisson *et al.* (1989a). BHV-4 gB arises from a 142-kDa precursor which undergoes cleavage to give rise to two covalently linked glycopolypeptides of 128 and 56 kDa, corresponding to the previously identified gp10 and gp17, respectively. The resulting heterodimer has an apparent molecular mass of 210 kDa (Lomonte *et al.*, 1997).

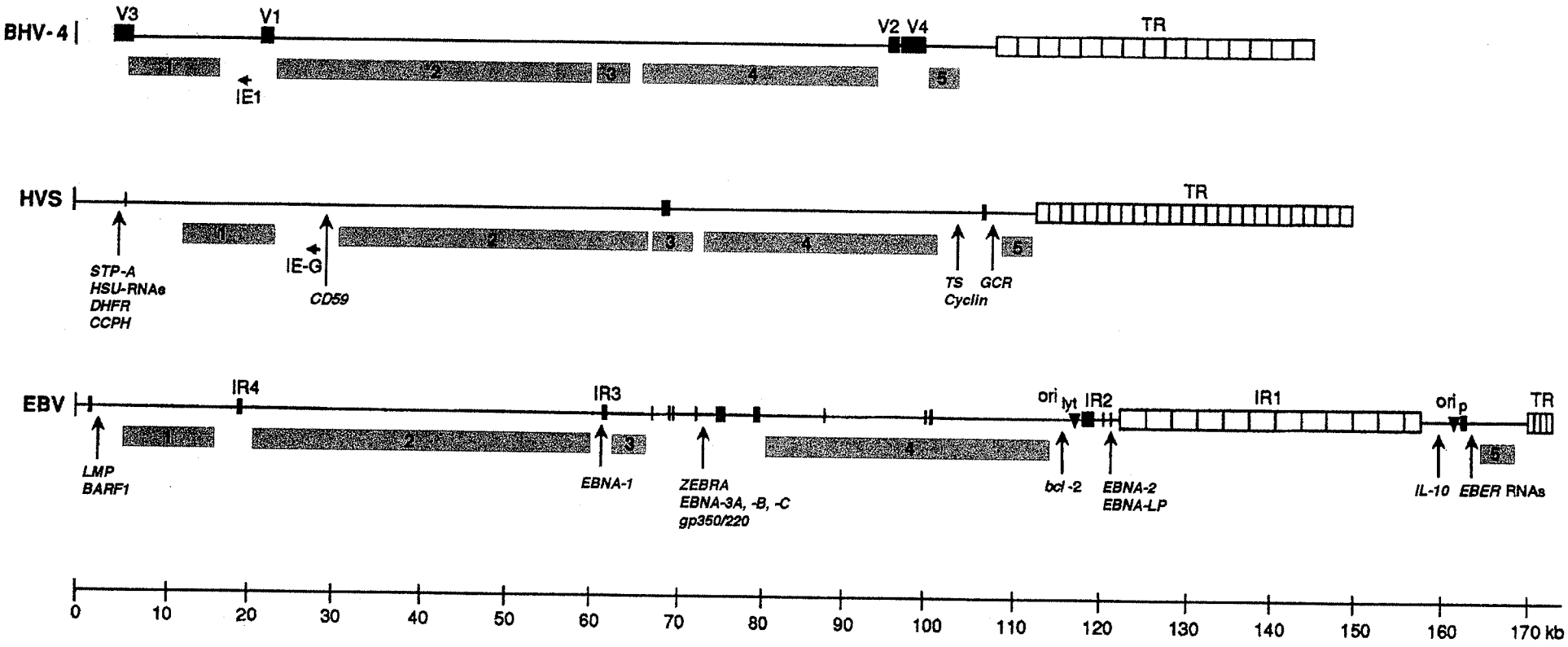
In summary, both genomic and protein analysis show the close homology of BHV-4 to HVS rather than to EBV (see Figure 7); therefore a possible relationship to KSHV/-HHV8 exists.

3.3.3 *Host range*

BHV-4 replicates in a variety of primary and established bovine cell cultures: primary kidney, testicle, lung, skin, spleen and thyroid, Madin Darby bovine kidney, Georgia bovine kidney, embryonic bovine kidney, embryonic bovine trachea, bovine bone marrow (BBM and FB4BM) and calf thymic lymphosarcoma cells (Bartha *et al.*, 1966; Luther *et al.*, 1971; Smith *et al.*, 1972; Parks & Kendrick, 1973; Rweyemamu & Loretu, 1973; Sass *et al.*, 1974; Theodoridis, 1978; Thiry *et al.*, 1981; Storz *et al.*, 1984; Theodoridis, 1985). BHV-4 has also been shown to replicate in cells of other animal species, including sheep, goats, pigs, dogs, rabbits, mink, horses, turkeys, geese, ferrets and potoroo (Luther *et al.*, 1971; Rweyemamu & Loretu, 1973; Kit *et al.*, 1986; Peterson & Goyal, 1988). Although the host range of BHV-4 *in vitro* is very broad, the virus does not replicate in cells of human (HeLa, Hep-2) or mouse (A31B77) origin or in chicken embryo fibroblasts (Luther *et al.*, 1971; Rweyemamu & Loretu, 1973). As in many other herpesviruses, the first receptor of BHV-4 is a heparin-like moiety on the cell surface, and its replication is cell cycle-dependent (Vanderplasschen *et al.*, 1993, 1995).

Of the ruminants, American bison *(Bison bison)* (Todd & Storz, 1983), African buffalo *(Syncerus caffer)* (Rossiter *et al.*, 1989), sheep (Van Opdenbosch *et al.*, 1986)

Figure 7. Order and spacing of gene blocks on the mature, linear genome of BHV-4 (V test strain), *Herpesvirus saimiri* (HVS strain; Albrecht *et al.*, 1992) and Epstein-Barr virus (EBV, B95-8 strain; Baer *et al.*, 1984)



Adapted from Bublot *et al.* (1992)

The five gene blocks that are conserved in gammaherpesviruses are indicated by the large shaded rectangles. Genes conserved in beta- and alphaherpesviruses are located in blocks 1, 2 and 4; blocks 3 and 5 contain only gammaherpesvirus-specific genes. The horizontal arrows indicate the positions of the major immediate-early gene of BHV-4 (*IE1*; van Santen, 1991) and HVS (*IE-G*). The vertical arrows indicate important genes located outside of the gene blocks and which are not conserved between EBV and HVS; the most commonly investigated genes are named: *STP-A*, saimiri transformation-associated protein; *HSU*-RNAs, *Herpesvirus saimiri* U-RNAs; *DHFR*, dihydrofolate reductase; *CCPH*, complement control protein homologue; *CD59*, cluster designation 59 homologue; *TS*, thymidylate synthase; *Cyclin*, cyclin family member homologue; *GCR*, G-coupled receptor homologue; *LMP*, latent membrane protein; *EBNA-2*, *-3A*, *-B*, *-C*, *-LP*, EBV nuclear antigen 2, 3A, 3B, 3C, leader protein; *bcl-2*, *bcl-2* proto-oncogene homologue; *IL-10*, interleukin 10 homologue; EBER, EBV-encoded RNA. EBV origins of replication are indicated by triangles and ori_{lyt} (origin of replication for the lytic cycle) and ori_p (plasmid origin of replication); terminal repeats (TR) and large internal repeats (IR) are represented by open rectangles; small internal repeated sequences are indicated by vertical lines or black rectangles. V1, V2, V3 and V4 are genomic regions which vary in size between BHV-4 isolates (Thiry *et al.*, 1992b). The orientation of the EBV genome is inverted relative to the conventional orientation (Baer *et al.*, 1984).

and goats (Moreno-Lopez *et al.*, 1989) undergo natural or experimental infection with BHV-4. European wild ruminants do not have antibodies to BHV-4 (Thiry *et al.*, 1988). The ability of BHV-4 to infect non-bovine ruminant species is understandable, considering the close phylogenetic relationships between these species.

BHV-4 can also infect species phylogenetically distant from ruminants: it has been isolated from a cat suffering from urolithiasis (Fabricant *et al.*, 1971). The latter isolate, designated 'feline herpesvirus type 2' or 'feline cell-associated herpesvirus', was characterized by restriction analysis (Kit *et al.*, 1986). Even if its role in feline disease is uncertain, the virus was undoubtedly isolated from cats and it has been shown to infect cats (Kruger *et al.*, 1990). The virus was a typical BHV-4 strain. Attempts to infect cats with the Movar 33/63 strain by conventional intranasal and intravenous routes were unsuccessful (Thiry *et al.*, 1991), perhaps due to a lack of adaptation of the virus to the cat rather than to strain specificity. The finding that this virus is a BHV-4 strain led to the disappearance of 'feline herpesvirus type 2' from the International Nomenclature.

The discovery that *Herpesvirus aotus* type 2, isolated from owl monkeys (*Aotus trivirgatus*), is also a strain of BHV-4 extends the range of species that are susceptible to BHV-4 (Bublot *et al.*, 1991b). This virus has been fully characterized; however, the genomic and protein differences from the prototype BHV-4 strains are compatible with the variations detected among BHV-4 strains (Bublot *et al.*, 1991b; Dubuisson *et al.*, 1991a). The antigenic relationship between *Herpesvirus aotus* type 2 and BHV-4 was proven by indirect fluorescence antibody testing with a broad panel of monoclonal antibodies raised against three BHV-4 glycoproteins and three other proteins and rabbit polyclonal sera specific for the two viruses (Bublot *et al.*, 1991b). The protein profile shows some variations between the two: glycoprotein gp6/gp10/gp17 of *Herpesvirus aotus* type 2 has a molecular mass of 160/131/52 kDa, with an extra band of 60 kDa which is probably a duplicate of the 52-kDa component; gp11 has a molecular mass of 109 kDa, while that of gp8 does not vary from those of BHV-4 isolates (Dubuisson *et al.*, 1991a). As a consequence, *Herpesvirus aotus* type 2 disappeared from the International Nomenclature of Herpesviruses (see Introduction, Table 2).

3.3.4 *Natural transmission*

BHV-4 is isolated in bovine species from both the anterior respiratory and genital tracts. Only respiratory infection has been studied experimentally, while other routes of inoculation, i.e. intravenous, intradermal and intratesticular, have been tested in cattle (Osorio & Reed, 1983; Dubuisson *et al.*, 1987, 1989b). Direct and indirect transmission of BHV-4 by means of infected materials is suggested.

3.3.5 *Evidence that bovine herpesvirus 4 causes disease*

After primary infection of cattle, BHV-4 replicates in mucosal cells and infects mononuclear cells, provoking generalized infection (Osorio & Reed, 1983). Viraemia is not always detected, but the virus can be re-isolated from many organs, including brain and spinal cord (Castrucci *et al.*, 1987; Dubuisson *et al.*, 1989b). BHV-4 may also infect the fetus (Kendrick *et al.*, 1976). Thereafter, BHV-4 establishes a latent infection. The spleen

is the main site of latency in rabbits (Osorio *et al.*, 1982, 1985); in cattle, the spleen is also the site where viral DNA is most frequently detected (Lopez *et al.*, 1996; Egyed *et al.*, 1996). In rabbits and cattle, non-B non-T cells and, presumably, monocytes and splenic macrophages are the main cells in which latent infection with BHV-4 is found (Osorio *et al.*, 1985; Lopez *et al.*, 1996); however, this issue is not definitely resolved, because BHV-4 can also replicate in lymphocytes (Egyed *et al.*, 1996). Latent virus can be reactivated by dexamethasone treatment, and re-excretion is shown to occur by the isolation of BHV-4 from peripheral blood leukocytes and nasal swabs (Krogman & McAdaragh, 1982; Dubuisson *et al.*, 1989b).

After viral entry, BHV-4 proteins are expressed in a cascade fashion, as for any herpesvirus. Trancription of two immediate-early genes (*IE1* and *IE2*) is initiated in infected cells (van Santen, 1991; Chang & van Santen, 1992; van Santen, 1993). IE1 and IE2 proteins share homology with IE110 of HSV and EBV R *trans*-activator, respectively (van Santen, 1991, 1993). IE2 was shown to *trans*-activate early (*E*) gene promoters, e.g. thymidine kinase gene (Zhang & van Santen, 1995) and late (*L*) gene (Bermudez-Cruz *et al.*, 1997). Expression of the *L* gene leads to abundant synthesis of a 1.7-kb RNA unique to BHV-4 (Bermudez-Cruz *et al.*, 1997). The precursor of the gB complex is expressed during the early phase and does not require DNA replication. Glycoproteins gp1, gp8 and gp11 are late proteins (Dubuisson *et al.*, 1991b,c, 1992a,b). BHV-4 DNA replication, and consequently the expression of late proteins, are dependent on the S phase of the cell cycle (Vanderplasschen *et al.*, 1995).

The contribution of BHV-4 to disease is speculative. BHV-4 has been designated as a passenger virus when isolated from ethmoidal tumours in Indian cattle (Moreno-Lopez *et al.*, 1989). The virus infects mononuclear blood cells, and this peculiarity allows it to be distributed to the whole body. Therefore, in primary infection and during reactivation of the latent state, BHV-4 can be reisolated from virtually all bovine tissues and organs. It is hard to discriminate between isolation by chance and a direct role of the virus in the observed lesions. BHV-4 has never been identified as a potential cause of tumours either *in vitro* or in cattle or rabbits *in vivo*.

3.3.6 *Isolates*

Two reference strains were isolated from clinical cases of conjunctivitis and respiratory disease: the Movar 33/63 strain in Europe (Bartha *et al.*, 1966) and the DN 599 strain in the United States (Mohanty *et al.*, 1971). Other strains were isolated from cases of respiratory disease (Smith *et al.*, 1972; Evermann *et al.*, 1984). In Africa, several herpesviral strains were isolated from people with a syndrome characterized by epididymitis and vaginitis (Maré & van Resenburg, 1961; Theodoridis, 1978, 1985), which are biologically similar to BHV-4 but have not yet been typed as BHV-4. The two Belgian reference strains, namely V. Test and LVR140, came from cases of orchitis and post-partum metritis, respectively (Thiry *et al.*, 1981; Wellemans *et al.*, 1984). Other BHV-4 strains were isolated from cases of genital disease in Italy (Castrucci *et al.*, 1986) and the United States (Parks & Kendrick, 1973; Evermann *et al.*, 1984). Reed *et al.* (1979) and Wellemans and Van Opdenbosch (1989) isolated BHV-4 from aborted

fetuses, and Reed *et al.* (1977) isolated BHV-4 from cases of mammary pustular dermatitis and ulcers of the udder. A few herpesviral strains biologically similar to BHV-4 were isolated from ruminal tumours (Kaminjolo *et al.*, 1972), tongue lesions (Rweyemamu & Loretu, 1973; Luini *et al.*, 1985) and diarrhoeal faeces (Eugster, 1978/-1979).

BHV-4 was also isolated or serologically suspected in cases of malignant catarrhal fever (Storz, 1968; Todd & Storz, 1983), lumpy skin disease (Alexander *et al.*, 1957; Rweyemamu & Loretu, 1973; House *et al.*, 1990), vesicular stomatitis (Evermann & Henry, 1989) and a variety of neoplasms such as lymphosarcoma (Potgieter & Maré, 1974), ocular squamous-cell carcinoma (Anson *et al.*, 1982) and T-cell lymphoma (Toho *et al.*, 1985).

BHV-4 is regularly isolated from healthy cattle. It has been recognized in renal-cell cultures (Luther *et al.*, 1971; Belák & Pálfi, 1974), in trigeminal ganglia (Homan & Easterday, 1981) and in triturated liver, lung and spleen (Krogman & McAdaragh, 1982).

3.4 Murid herpesvirus 4

Murine gammaherpesvirus 68 (MHV-68) is a naturally occurring rodent pathogen. Intranasal or intravenous administration of MHV-68 results in acute productive infection in B lymphocytes (Sarawar *et al.*, 1997) and lymphoproliferation (Sunil-Chandra *et al.*, 1992a,b, 1994; Sarawar *et al.*, 1997). The virus remains latent in B cells after primary infection (Sunil-Chandra *et* al., 1992b). Sequence analysis of its genome shows that it is most probably a rhadinovirus, more closely related to HVS and KSHV/HHV8 than to EBV (Mackett *et al.*, 1997).

3.5 Retroperitoneal fibromatosis herpesviruses

PCR with consensus gammaherpesvirus primers was used to detect small DNA fragments of two new, closely related rhadinoviruses in captive *Macaca nemestrina* and *M. mulatta* in one colony (Rose *et al.*, 1997). These animals were also infected with D-type simian retrovirus type 2 and suffered from retroperitoneal and subcutaneous fibrosis with progressive fibrovascular proliferation. This condition has some similarities to Kaposi's sarcoma (Tsai *et al.*, 1990; Rose *et al.*, 1997).

Earlier experiments had shown that cell cultures could be established from these lesions, which induced self-limited, transient spindle-cell proliferation, accompanied by pronounced vascularization when inoculated into nude mice (Tsai *et al.*, 1990, 1995).

The short sequences of these retroperitoneal fibromatosis herpesviruses presently available are derived from the polymerase gene and are about 70% identical at the nucleotide level (83–84% at the protein level) to the corresponding KSHV/HHV8 region (Rose *et al.*, 1997). If confirmed by a more extensive sequence analysis, these viruses could represent the closest relatives of KSHV/HHV so far. It may therefore prove to be a useful model for studying some aspects of Kaposi's sarcoma; however, nothing is yet known about the relative contributions of these new rhadinoviruses and simian retrovirus type 2 to the pathogenesis of retroperitoneal fibromatosis.

4. Other Data Relevant to an Evaluation of Carcinogenesis and its Mechanisms

4.1 Kaposi's sarcoma

4.1.1 *Cell biology*

4.1.1.1 *Origin of spindle cells*

Spindle cells surrounding slit-like spaces are characteristic of advanced Kaposi's sarcoma lesions. Endothelial cells (either vascular or lymphatic endothelium), cells from venous lymphatic junctions, fibroblasts, smooth-muscle cells, dermal dendrocytes and macrophages have all been proposed as possible progenitors of Kaposi's sarcoma spindle cells (reviewed by Roth *et al.*, 1992; Stürzl *et al.*, 1992a; Browning *et al.*, 1994; Kaaya *et al.*, 1995). Like normal lymphatic endothelial cells, spindle cells stain with the monoclonal antibody EN-4, which detects both vascular and lymphatic endothelium, but lack reactivity with the monoclonal antibody Pal-E, which reacts with blood-vessel but not lymphatic endothelial cells (Rappersberger *et al.*, 1990). Other markers for blood-vessel endothelium, such as OKM-5 and anti-factor VIII-related antigen (von Willebrand factor), stain Kaposi's sarcoma endothelial or spindle cells, although varying results have been reported by different laboratories. Studies with *Ulex europaeus* agglutinin 1, another marker for endothelial cells, have also produced contradictory results (Nadji *et al.*, 1981; Modlin *et al.*, 1983; Little *et al.*, 1986; Rappersberger *et al.*, 1990; further references in Roth *et al.*, 1992). Ultrastructural examination failed to show the presence of Weibel-Palade bodies, the storage vesicles for von Willebrand factor and therefore a characteristic feature of vascular endothelium, in spindle cells from Kaposi's sarcoma lesions (Rappersberger *et al.*, 1990). Staining with the monoclonal antibody BMA 120, which reacts with an antigen on endothelial cells, lends support to an endothelial origin of Kaposi's sarcoma cells (Roth *et al.*, 1988). Spindle cells and endothelia lining vascular spaces in Kaposi's sarcoma lesions express leukocyte adhesion molecule 1 and thrombomodulin, which are markers of lymphokine-activated endothelial cells (Zhang *et al.*, 1994). This observation further supports the notion that Kaposi's sarcoma spindle cells are of endothelial origin and are activated by growth factors (see below).

The staining of spindle cells with antibodies to CD14, CD68 and factor XIIIa, observed by some laboratories, has been interpreted as reflecting a link between these spindle cells and cells of the monocyte/macrophage lineage, possibly dermal dendrocytes (Nickoloff & Griffiths, 1989; Rappersberger *et al.*, 1990). These cells are distinct from Langerhans cells (Nickoloff & Griffiths, 1989). The staining of cultured Kaposi's sarcoma spindle cells with an antibody to smooth-muscle α actin (Weich *et al.*, 1991) and similar histochemical findings have been interpreted to suggest a relationship with smooth-muscle cells or myofibroblasts (reviewed by Roth *et al.*, 1992). These discrepant results suggest either that cells of different lineages can adopt a spindle-like morphology or that these markers are common to different cells of mesenchymal origin and Kaposi's sarcoma spindle cells derived from pluripotent mesenchymal progenitor cells. Currently,

CD34 (as detected by monoclonal antibody QBEND 10) is considered the best marker for Kaposi's sarcoma spindle cells (Russell Jones *et al.*, 1995).

Cells expressing markers characteristic for vascular or lymphatic endothelium from Kaposi's sarcoma lesions have been cultured in a number of laboratories (Delli Bovi *et al.*, 1986; Nakamura *et al.*, 1988; Roth *et al.*, 1988; Siegal *et al.*, 1990; Corbeil *et al.*, 1991; Herndier *et al.*, 1994), but cultures expressing smooth-muscle α actin (Albini *et al.*, 1988; Wittek *et al.*, 1991) and mixed populations (Siegal *et al.*, 1990; further references in Roth *et al.*, 1992) have also been reported. The lineage of these cultured cells has been defined by staining for similar markers as in studies *in situ*, notably vimentin and cytokeratin (to discriminate mesenchymal and epithelial cells, respectively), endothelial markers such as von Willebrand factor, Pal-E, OKM-5, BMA 120 (specific for blood-vessel endothelium), EN-4 and *Ulex europaeus* agglutinin 1 lectin (which reacts with blood-vessel and lymphatic endothelium), CD14 and factor XIIIa (for the monocyte/macrophage lineage), smooth-muscle α actin (smooth muscle and myofibroblasts) and others (reviewed by Roth *et al.*, 1992; Stürzl *et al.*, 1992a; Kaaya *et al.*, 1995). Spindle-shaped cells showing moderate expression of endothelial antigens have been cultured from peripheral blood of Kaposi's sarcoma patients (Browning *et al.*, 1994).

4.1.1.2 *Vascular lesions induced by Kaposi's sarcoma cell cultures in nude mice*

The various cell cultures established from Kaposi's sarcoma lesions differ in their ability to induce angiogenic lesions in nude mice. [The Working Group noted that the similarity of some of these lesions to Kaposi's sarcoma is controversial.] A cell line expressing endothelial markers, established by Siegal *et al.* (1990) and studied by Herndier *et al.* (1994), induced Kaposi's sarcoma-like tumours of human origin in nude mice. This cell line expressed the endothelial markers factor VIII, EN-4 and *Ulex europeaus* agglutinin 1 lectin. In addition, it produced high levels of urokinase plasminogen activator and plasminogen activator inhibitor 1 (Herndier *et al.*, 1994). Interestingly, plasminogen activator has been shown to be involved in the development of endothelial tumours in mice transgenic for the polyoma middle-T protein (Montesano *et al.*, 1990). A second cell line capable of causing tumours of human origin in nude mice has also been described (Lunardi-Iskandar *et al.*, 1995). The development of these cell lines suggests that a subpopulation of cells in Kaposi's sarcoma lesions may have progressed to a malignant phenotype; however, they do not contain KSHV/HHV8 DNA, and their relationship to spindle cells containing this virus (see section 4.5.1) is unclear.

A few other Kaposi's sarcoma cell cultures, also of an endothelial phenotype, are angiogenic *in vivo* but induce the growth of 'Kaposi's sarcoma-like' vascular lesions of murine origin when inoculated into nude mice (Nakamura *et al.*, 1988; Salahuddin *et al.*, 1988). Spindle-shaped cells grown from the peripheral blood of Kaposi's sarcoma patients have also been reported to induce angiogenesis in nude mice (Browning *et al.*, 1994). Although these cultures were not examined for KSHV/HHV8, similar cultures established more recently did contain viral DNA (Sirianni *et al.*, 1997).

Most other cell cultures, established by several laboratories (Roth *et al.*, 1988), were not angiogenic in nude mice (Delli Bovi *et al.*, 1986; Albini *et al.*, 1988; Roth *et al.*, 1988; Wittek *et al.*, 1991); furthermore, KSHV/HHV8 is either not present or is rapidly lost upon serial passage from such cultures (Ambroziak *et al.*, 1995; Lebbé *et al.*, 1995).

4.1.1.3 *Growth factors involved in proliferation of spindle cells*

The role of growth factors in the development of Kaposi's sarcoma has been studied in several laboratories, with inconsistent findings, probably because of the use of different cell types. The role of growth factors in the development of Kaposi's sarcoma *in vivo* and their interaction with KSHV/HHV8 are still unknown.

(*a*) *Fibroblast growth factors*

Basic fibroblast growth factor (FGF) is secreted by Kaposi's sarcoma cultures expressing endothelial cell markers and may promote the growth of these cells *in vitro* (Ensoli *et al.*, 1989). Other groups working with Kaposi's sarcoma cultures of either endothelial phenotype (Corbeil *et al.*, 1991) or mixed fibroblastoid/endothelial appearance (Werner *et al.*, 1989) also found FGF-like activity in supernatants of these cultures which stimulated the growth of normal fibroblasts and endothelial cells.

Members of the FGF family, including basic FGF and endothelial cell growth factor, are known to stimulate the growth of normal endothelial cells, and cultured Kaposi's sarcoma cells with endothelial characteristics have been shown to induce transient neoangiogenesis in nude mice (Nakamura *et al.*, 1988). The FGF family of cytokines may thus play a crucial role in the development of Kaposi's sarcoma. Expression of basic FGF and FGF5 has been shown to occur in spindle cells of Kaposi's sarcoma by in-situ hybridization (Xerri *et al.*, 1991). Acidic FGF and FGF6 are also expressed in these lesions (Li *et al.*, 1993), but the technique employed in this study (RT-PCR) did not permit identification of the cell type(s) that secrete(s) these two members of the FGF family. The importance of basic FGF in the development of experimental Kaposi's sarcoma-like lesions is further supported by the report that basic FGF-specific antisense oligonucleotide can inhibit the angiogenic effect of cultured Kaposi's sarcoma cells in nude mice (Ensoli *et al.*, 1994a).

(*b*) *Platelet-derived growth factor*

Both normal endothelial cells (Ensoli *et al.*, 1989; Roth *et al.*, 1989) and short-term cultures of Kaposi's sarcoma cells with endothelial characteristics (Ensoli *et al.*, 1989) produce platelet-derived growth factor (PDGF). Cultures that produce this factor thus do not require exogenous PDGF to promote proliferation (Ensoli *et al.*, 1989; Corbeil *et al.*, 1991). Short-term cultures were also shown to express mRNA for the receptors for PDGF-A and PDGF-B (Roth *et al.*, 1989; Werner *et al.*, 1990). Kaposi's sarcoma spindle cells express mRNA for the PDGF-β receptor *in vivo*, whereas mRNAs for PDGF-A and PDGF-B were expressed on some tumour cells located in the vicinity of slit-like spaces (Stürzl *et al.*, 1992b). PDGFs may therefore play a role in the pathogenesis of Kaposi's sarcoma.

(*c*) *Interleukin-1*

IL-1 has also been reported to be secreted by cultured Kaposi's sarcoma cells of the endothelial phenotype (Ensoli *et al.*, 1989; Corbeil *et al.*, 1991) and to have a potent stimulatory effect on these cells (Nakamura *et al.*, 1988).

(*d*) *Interleukin-6*

Cultured Kaposi's sarcoma cells of an endothelial phenotype secrete and proliferate in response to IL-6 (Miles *et al.*, 1990; Corbeil *et al.*, 1991). The expression of receptors for IL-6 on cultured cells has also been reported (Miles *et al.*, 1990), and Kaposi's sarcoma cells expressed IL-6 mRNA *in vivo* (Gillitzer & Berger, 1991). The former observation is of particular interest in the context of the IL-6 homologue encoded by KSHV/HHV8 (v-IL-6; see section 1.1); however, the v-IL-6 homologue is expressed only rarely in KSHV/HHV8-infected Kaposi's sarcoma spindle cells (Moore *et al.*, 1996b), suggesting that it does not play a major role in the pathogenesis of this disease.

(*e*) *Tumour necrosis factor* α

Tumour necrosis factor (TNF) α has a potent stimulatory effect on some Kaposi's sarcoma cell cultures (Nakamura *et al.*, 1988), but whether it is produced by cultures with endothelial characteristics is controversial (Ensoli *et al.*, 1989; Corbeil *et al.*, 1991). TNFα has been reported to be expressed by Kaposi's sarcoma cells in small amounts *in vivo* but was mainly found in epidermal cells adjacent to the tumour (Gillitzer & Berger, 1991), compatible with a possible paracrine role of this factor.

(*f*) *Miscellaneous growth factors*

Secretion of granulocyte–monocyte colony-stimulating factor and transforming growth factor β by Kaposi's sarcoma cell cultures with endothelial characteristics, but not by normal endothelial cells, has been reported (Ensoli *et al.*, 1989). The latter also promotes the growth of cultured Kaposi's sarcoma cells. In lesions, mature transforming growth factor β1 is found mainly in macrophage-like cells and not in spindle cells (Williams *et al.*, 1995). Hepatocyte growth factor (scatter factor) also promotes the growth of cultured Kaposi's sarcoma cells and may thus play a role in the pathogenesis of this disease (Naidu *et al.*, 1994).

4.1.1.4 *Role of HIV-1 Tat in promoting Kaposi's sarcoma lesions*

AIDS-associated Kaposi's sarcoma is clinically more aggressive than classic or endemic Kaposi's sarcoma, suggesting that HIV is a cofactor in the progression of this tumour. Experimental evidence suggests that the Tat protein of HIV-1 (see IARC, 1996) can enhance the growth of cultured 'endothelial' Kaposi's sarcoma cells (Ensoli *et al.*, 1990); the effect of Tat on other cell cultures was inconsistent (Roth *et al.*, 1992). Several cytokines, including TNF, IL-1 and interferon γ, can render normal endothelial and smooth-muscle cells susceptible to the growth-promoting effect of Tat (Barillari *et al.*, 1992), possibly by increasing the expression of integrin receptors which interact with Tat (Barillari *et al.*, 1993; Ensoli *et al.*, 1994a). Injection of Tat into nude mice

(Ensoli *et al.*, 1994b) or immunocompetent C57Bl mice (after incorporation into Matrigel; Albini *et al.*, 1994) induces angiogenesis, and this effect is potentiated by basic FGF (Ensoli *et al.*, 1994a,b) and heparin (Albini *et al.*, 1994, 1996b). Tat- and heparin-induced neoangiogenesis can be inhibited by the matrix metalloproteinase inhibitor TIMP-2 (Albini *et al.*, 1994), and Tat and basic FGF synergize to increase the expression of collagenase IV in nude mice (Ensoli *et al.*, 1994b). In addition, one group reported the emergence of Kaposi's sarcoma-like lesions in mice transgenic for HIV-1 *tat* (Vogel *et al.*, 1988); however, other lines of transgenic mice carrying the complete HIV-1 genome failed to develop similar lesions (Leonard *et al.*, 1988). Transgenic mice carrying the early region of BK virus included in a long terminal repeat-*tat* construct also develop 'Kaposi's sarcoma-like' lesions, in addition to other malignancies (Coralini *et al.*, 1993), and extracellular Tat released by tumour cell lines derived from these animals protects them from apoptosis under conditions of serum starvation (Campioni *et al.*, 1995). Tat can be released from HIV-infected cells and can act on HIV-uninfected cells. The growth promoting effect of extracellular Tat on cultured Kaposi's sarcoma cells and endothelial cells (Ensoli *et al.*, 1990; Barillari *et al.*, 1992) suggests that infection of cells not directly involved in the Kaposi's sarcoma lesion may be sufficient to trigger the sequence of events leading to the development of this tumour. In keeping with this interpretation, in *tat*-transgenic mice which did develop Kaposi's sarcoma-like lesions, expression of *tat* was not found in spindle cells but in neighbouring keratinocytes (Vogel *et al.*, 1988).

HIV-1 Tat has been reported to be detectable by histochemical techniques in Kaposi's sarcoma lesions in AIDS patients, probably originating from a few HIV-1-infected mononuclear cells (Ensoli *et al.*, 1994b). Thus, the angiogenic properties of Tat, alone or in concert with other growth factors, has been documented in a variety of experimental systems; however, the molecular basis for its angiogenic properties is still controversial. Tat has been reported to bind to $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins via an RGD sequence element in a manner similar to, and replaceable by, their physiological ligands fibronectin and vitronectin (Barillari *et al.*, 1993; Ensoli *et al.*, 1994b). Baboons infected with HIV-2, however, also developed Kaposi's sarcoma-like lesions, although HIV-2 Tat lacks an RGD domain (Barnett *et al.*, 1994). Tat binds with high affinity to the Flk-1/KDR receptor for vascular endothelial cell growth factor (Albini *et al.*, 1996c), and this interaction promotes angiogenesis. A basic heparin binding-like domain in *tat*, rather than the RGD domain, is thought to be involved in this interaction (Albini *et al.*, 1996b).

4.1.1.5 *Clonality of Kaposi's sarcoma lesions*

Individual Kaposi's sarcoma nodules in female patients have been shown, by studying X-inactivation markers, to contain monoclonal or oligoclonal cell populations (Rabkin *et al.*, 1995), and different nodules from the same patient have the same monoclonal origin (Rabkin *et al.*, 1997). Southern blotting of Kaposi's sarcoma DNA with KSHV/-HHV8 terminal-repeat probes showed one or a few bands, which is consistent with a monoclonal or oligoclonal expansion of virus-infected cells. Whether different lesions from the same patient have the same KSHV/HHV8 clonality pattern has not yet been

addressed by terminal-repeat hybridization, and it is not known whether this assay is sufficient to determine monoclonality (Russo *et al.*, 1996). Short-term cultures of biopsy samples from Kaposi's sarcomas have been found to contain chromosomal rearrangements, but no consistent pattern has emerged (Delli Bovi *et al.*, 1986). Two tumorigenic cell lines derived from Kaposi's sarcoma lesions have been reported to have a 3(p14) translocation in common (Popescu *et al.*, 1996); however, as neither is infected with KSHV/HHV8, the relationship of these findings to Kaposi's sarcoma spindle cells latently infected with the virus (see section 4.1.4) is unclear.

4.1.2 *Role of KSHV/HHV8 in development of Kaposi's sarcoma*

The consistent detection of KSHV/HHV8 in Kaposi's sarcoma biopsy samples and the epidemiological data discussed in Section 1 strongly support a causative role of KSHV/HHV8 in the pathogenesis of Kaposi's sarcoma. Although its precise role is not yet understood, the presently available data are compatible with the notion that KSHV/-HHV8 exerts a direct transforming effect on endothelial cells, the likely precursors of Kaposi's sarcoma spindle cells.

KSHV/HHV8 establishes a persistent infection in most Kaposi's sarcoma spindle cells, which involves the expression of at least three viral genes. All spindle cells, as well as the atypical endothelial cells of early lesions, express abundant mRNA for *ORF K12*, which encodes a putative small hydrophobic protein of unknown function (Zhong *et al.*, 1996; Stürzl *et al.*, 1997; Staskus *et al.*, 1997). Kaposi's sarcoma spindle cells also express the high-molecular-mass latent nuclear antigen (LNA) encoded by *ORF 73*, as shown by immunohistochemistry with affinity-purifed antibodies to this protein (Rainbow *et al.*, 1997). Expression of mRNA for the *ORF 72*-encoded D-type cyclin homologue has been demonstrated by in-situ hybridization (Stürzl *et al.*, 1997); however, as the mRNA encoding *ORF 73* extends through the *ORF 72/v-cyclin* gene, this result requires confirmation by immunohistochemical studies with specific antibodies. The *ORF K13* gene is also contained within the mRNAs encoding *ORF 72/v-cyclin* and *ORF 73/LNA* (Rainbow *et al.*, 1997) and may therefore be expressed in Kaposi's sarcoma spindle cells, but no immunohistochemical studies on the protein it expresses have yet been reported (see section 4.4.5). The KSHV/HHV8 homologue to *IL-6* (*ORF K2*) is expressed during latency in KSHV/HHV8-infected haematopoietic cells but is not generally expressed in Kaposi's sarcoma lesions (Moore *et al.*, 1996b).

A subpopulation (approximately 10%) of Kaposi's sarcoma spindle cells also expresses a polyadenylated nuclear *T1.1* RNA (Staskus *et al.*, 1997), which is abundant in primary effusion lymphoma cell lines induced into lytic replication (Renne *et al.*, 1996a; Zhong *et al.*, 1996). Expression of *T1.1* may therefore be indicative of lytic replication within Kaposi's sarcoma lesions, suggesting that a subpopulation of spindle cells can produce KSHV/HHV8 virions. Intranuclear herpesvirus-like particles or intranuclear inclusions characteristic of herpesviruses can be found in Kaposi's sarcoma tissues, indicating that the tumour is a source of productive infection (Walter *et al.*, 1984). Expression of *ORF 74*, encoding a functional chemokine receptor (Arvanitakis *et al.*, 1997), can

be found by RT-PCR, but it is not clear whether this gene is expressed during the latent or the lytic viral expression programme (Cesarman *et al.*, 1996a).

As discussed in section 1.1.6, v-cyclin can phosphorylate Rb and histone H1 and therefore dysregulate the cell cycle, but it has not so far been shown to have transforming properties of its own. The functions of *ORF 73/LNA* and *K12* are unknown; however, the expression of a limited set of genes in persistently infected cells is a familiar pattern with other (both oncogenic and non-oncogenic) herpesviruses and suggests that the role of KSHV/HHV8 in the pathogenesis of Kaposi's sarcoma is likely to be a direct one.

4.2 Primary effusion lymphomas

Primary effusion lymphomas have a unique constellation of features that distinguishes them from all other known lymphoproliferations: they are predominantly confined to 'body cavities' as an effusion; cells with cytomorphological features bridging those of large-cell immunoblastic and anaplastic large-cell lymphoma suggest that they represent mature B cells; a nil phenotype, although some primary effusion lymphomas with B- and/or T-cell markers have been described; clonal immunoglobulin gene rearrangements, further implying a B-cell phenotype; uniform lack of *c-myc* rearrangements and the presence of KSHV/HHV8 with or without EBV (see section 2.2.1). In at least one primary effusion lymphoma cell line tested, KSHV/HHV8 is clonal by terminal-repeat analysis (Russo *et al.*, 1996).

Most cases of primary effusion lymphoma have been described in severely immunocomprised individuals with HIV infection, in keeping with the notion that lack of immunosurveillance favours primary effusion lymphoma cell proliferation. Why these lymphomas persist predominantly as effusions, without lymph node or other lymphoid tissue involvement, is unclear, although a lack of 'homing markers' has been suggested (Karcher & Alkan, 1995).

Multiple, complex chromosomal abnormalities have been described in cases of primary effusion lymphoma; however, apart from a consistent absence of *c-myc* rearrangement, none of the described abnormalities is present in all cases (Ansari *et al.*, 1996).

In view of the frequent co-infection with EBV and KSHV in the cells of such tumours, these two viruses may act together to induce neoplastic transformation and/or the peculiar phenotypic features of these lymphomas. The presence of activation markers (similar in EBV-positive and EBV-negative KSHV/HHV8-positive cases) suggests that cell activation is secondary to viral infection (Ansari *et al.*, 1996; Nador *et al.*, 1996). Of note, all KSHV/HHV8-positive primary effusion lymphomas lack *c-myc* rearrangements. The few cases of 'effusion-based lymphomas' described that have *c-myc* rearrangements do not have a nil surface antigen phenotype, display different cytomorphological features and do not contain KSHV/HHV8 (see Table 8).

4.3 Multicentric Castleman's disease

Multicentric Castleman's disease is a polyclonal lymphoproliferation characterized by prominent vascularity in lymphoid tissue and associated systemic symptoms. There is a

strong association between multicentric Castleman's disease and Kaposi's sarcoma in patients with AIDS (see section 2.2.2).

Immunoregulatory abnormalities probably contribute to these lymphoproliferations. It was hypothesized that a virus acts as a cofactor, perhaps as a stimulus for cytokine production (Peterson & Frizzera, 1993). The association between multicentric Castleman's disease and the presence of IL-6 is notable. Thus, IL-6 is present at high levels in biopsy samples from patients with this disease, and PBMC from patients with multicentric disease secrete high levels of IL-6 (Yoshizaki *et al.*, 1989; Burger *et al.*, 1994). IL-6 has prominent actions on cells of the immune system, including stimulation of immunoglobulin synthesis by activated B cells and differentiation of cytotoxic T cells (Kikutani *et al.*, 1985). It also commits myeloid progenitors to differentiate into granulocytes and macrophages. IL-6 is synthesized in response to a number of stimuli, which include viruses and other cytokines such as IL-1 and TNF (Kishimoto, 1989). IL-6 also acts as an auto- and paracrine growth factor for multiple myeloma cells (Kawano *et al.*, 1988). Retroviral-mediated transfer of IL-6 into haematopoietic cells of mice results in a syndrome resembling multicentric Castleman's disease (Brandt *et al.*, 1990a,b). These mice develop anaemia, polyclonal hypergammaglobulinaemia, splenomegaly and peripheral lymphadenopathy, further supporting the hypothesis that dysregulated synthesis of IL-6 has a causative role in multicentric Castleman's disease.

KSHV/HHV8 is nearly always present in HIV-positive individuals with multicentric Castleman's disease; in immunocompetent hosts, the virus is present in about 40% of cases (see Table 10). The presence of HHV6 in two of five cases of multicentric Castleman's disease and EBV in two of five cases has also been reported (Barozzi *et al.*, 1996), but the significance of this finding is unknown.

4.4 Viral genes with cellular growth promoting or oncogenic potential

As discussed in Section 1 (Table 1), the KSHV/HHV8 genome contains multiple non-conserved viral genes, some of which are strikingly similar to human genes involved in cellular growth control. The specific viral genes discussed in this section are those with sequence similarity to known human oncogenes and/or are expressed during viral latency.

4.4.1 Open reading frame K1

The first gene at the left end of the KSHV/HHV8 genome (Figure 2), *ORF K1*, encodes a putative 289-amino acid, type I transmembrane protein, featuring a signal peptide, an extracellular domain with multiple cysteine residues, a typical transmembrane anchor domain and a short intracellular domain containing several potential tyrosine phosphorylation motifs (Russo *et al.*, 1996; Neipel *et al.*, 1997a). No experimental data are available to indicate that the K1 protein is phosphorylated, and neither its function nor its expression pattern in Kaposi's sarcoma-associated tumours has been described. The same genomic region is also highly variable between different strains of *Herpesvirus saimiri* (Jung *et al.*, 1991; Jung & Desrosiers, 1991; Biesinger *et al.*, 1995).

A comparison of deposited genomic sequences indicates that it may also be highly variable (Russo *et al.*, 1996; Lagunoff & Ganem, 1997; Neipel *et al.*, 1997a).

4.4.2 *Growth factor homologues*

Three KSHV/HHV8 genes have sequence similarity to members of the C-C-chemokine family (Russo *et al.*, 1996; Neipel *et al.*, 1997a; Nicholas *et al.*, 1997a,b). Two of these, encoded by *ORF 6* and *4* (v-MIP-I and v-MIP-II) are expressed at low levels in latently infected primary effusion lymphoma cells, and that expression increases during the lytic cycle of viral replication (Moore *et al.*, 1996b; Nicholas *et al.*, 1997a,b). v-MIP-I can interact with the C-CR5 co-receptor to inhibit the entry of some primary HIV strains (Moore *et al.*, 1996b).

The viral IL-6 homologue (v-IL-6), encoded by *ORF K2*, is 25% identical at the amino-acid level to human IL-6. Several groups have identified this protein (Moore *et al.*, 1996b; Russo *et al.*, 1996; Neipel *et al.*, 1997b; Nicholas *et al.*, 1997a,b) and shown that it supports the growth of the IL-6-dependent mouse myeloma cell line, B9 (Moore *et al.*, 1996b; Nicholas *et al.*, 1997b). It is expressed in latently infected lymphoma cell lines and primary effusion lymphoma tissue, but not generally in Kaposi's sarcoma tissue. Only a few scattered $CD20^+$ B cells expressing v-IL-6 were found in one of eight Kaposi's sarcoma specimens examined by immunohistochemistry (Moore *et al.*, 1996b). v-IL-6 activates the same Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway in HepG2 hepatoma cells as human IL-6; however, human IL-6 and v-IL-6 differ in their cellular IL-6 receptor interaction. Whereas human IL-6 requires both IL-6Rα and gp130 receptor protein for signal activation, v-IL-6 requires only gp130 (Molden *et al.*, 1997). Murine B9 plasmacytoid cell proliferation in response to v-IL-6 is inhibited by antibody to murine IL-6Rα, suggesting possible differences between human and mouse systems (Nicholas *et al.*, 1997b).

4.4.3 bcl-2 *homologue*

ORF 16 of KSHV/HHV8 encodes a homologue of the cellular anti-apoptotic protein bcl-2, with which it shares 16% sequence homology (Russo *et al.*, 1996; Cheng *et al.*, 1997; Neipel *et al.*, 1997a; Nicholas *et al.*, 1997a; Sarid *et al.*, 1997). The *BH1* and *BH2* domains, which are conserved in the bcl-2 family of proteins, are also found in the KSHV/HHV8 homologue. Functional studies indicate that bcl-2 prevents Bax-mediated toxicity or apoptosis in yeast, Sindbis virus-infected cells and transfected fibroblasts (Cheng *et al.*, 1997; Sarid *et al.*, 1997). There is contradictory evidence for the ability of v-*bcl-2* to heterodimerize with human *Bcl-2* or *Bax*: Sarid *et al.* (1997) found evidence of v-*bcl-2*–human *Bcl-2* interactions in a two-hybrid yeast system, but Cheng *et al.* (1997) found no specific interaction between the KSHV/HHV8 protein and other bcl-2-like proteins by co-immunoprecipitation. [This contradiction could be due to differences in the sensitivities or specificities of the assays used or to differences in the recombinant *bcl-2* constructs.] Both studies demonstrate, however, that the v-*bcl-2* has functional anti-apoptotic activity.

v-bcl-2 Transcripts can be induced in primary effusion lymphoma cell lines by 12-*O*-tetradecanoylphorbol 13-acetate and can be detected at low levels in Kaposi's sarcoma lesions and primary effusion lymphoma cell lines (Cheng *et al.*, 1997; Sarid *et al.*, 1997), similar to the proposed function of the EBV *bcl-2* homologue *BHRF1* (Roizman, 1993). [The function of *v-bcl-2* of KSHV/HHV8 may be to prolong the survival of lytically infected cells.] Since lytic virus replication is thought to be incompatible with survival of the cell (Roizman, 1993), expression of *BHRF1* may not play a direct role in EBV-mediated transformation.

4.4.4 *Viral interferon regulatory factor*

ORF K9 encodes the homologue of v-IRF (Moore *et al.*, 1996b; Russo *et al.*, 1996). This gene has low but significant homology to the IRF family of proteins responsible for interferon signal transduction. Two members of this pathway, IRF-1 and IRF-2, are antagonistic to each other in their effector functions (Taniguchi *et al.*, 1995a). IRF-1 positively regulates interferon signaling by binding to specific enhancer elements, called interferon-stimulated response elements, in the promoter regions of interferon-inducible genes. Activated transcription of interferon-stimulated genes results in phenotypic changes characteristic for the interferon-induced antiviral state, which include (but are not limited to) induction of CDKI p21, which can lead to shut-down of the cell cycle through inhibition of Rb (Taniguchi *et al.*, 1995a), and up-regulation of the major histocompatibility complex antigen (Taniguchi *et al.*, 1995b). *IRF-2* has oncogenic activities in NIH 3T3 cells which can be reversed by *IRF-1* overexpression (Harada *et al.*, 1993). Like KSHV/HHV8-encoded cytokine homologues, *v-IRF* is expressed in latently infected primary effusion lymphoma cell lines, and its expression is markedly enhanced after lytic cycle induction (Moore *et al.*, 1996b).

4.4.5 *Viral proteins that inhibit fas-associated death domain protein interleukin-1β converting enzyme (FLICE)*

ORF K13 encodes a small protein similar to the 'death effector' domains found in *ORF 71*-encoded proteins of three other rhadinoviruses, HVS, equine herpesvirus 2 and BHV-4 (Thome *et al.*, 1997); however, there is no significant, overall sequence similarity between KSHV/HHV8 *K13* and the *ORF 71* of other rhadinoviruses (Russo *et al.*, 1996). The protein of equine herpesvirus 2 encoded by *ORF 71* and a similar protein of molluscipoxvirus have been shown to act as dominant negative inhibitors of apoptotic signals by interfering with the interaction of fas-associated death domain protein (FADD) and tumour necrosis factor receptor-associated death domain protein (TRADD) and the assembly of the interleukin-1β-converting enzyme (ICE)-like protease complex and are therefore known as v-FLIPs (Bertin *et al.*, 1997; Thome *et al.*, 1997). No information on the function of the v-FLIP of KSHV/HHV8 is available, but conservation of the critical sequence elements suggests that it may also be able to inhibit this apoptotic pathway. Although the HVS v-FLIP may be expressed primarily during the lytic cycle of replication and not in tightly latent HVS-transformed human T-cells, the KSHV/HHV8 v-FLIP may be expressed in Kaposi's sarcoma spindle cells and primary effusion lym-

phoma cells, as its reading frame is included in mRNAs that encode the neighbouring ORF 72 (v-cyclin) and ORF 73 (LNA) proteins which are expressed in both Kaposi's sarcoma spindle and primary effusion lymphoma cells (Rainbow *et al.*, 1997). No direct evidence for its expression in endothelial tumour (spindle) or B-lymphoma cells is available.

4.4.6 *Viral cyclin*

ORF 72 of KSHV/HHV8, like that of HVS, encodes a protein that has about 30% amino-acid identity with human cellular cyclin D2 (Cesarman *et al.*, 1996a; Russo *et al.*, 1996; Li *et al.*, 1997). Cellular D-typed cyclins are implicated in the pathogenesis of several human malignancies (Sherr, 1995, 1996). *v-Cyclin* contains a region of high sequence similarity to the cyclin box domain of cellular cyclins, responsible for interactions with cyclin-dependent kinases (Chang *et al.*, 1996a; Godden-Kent *et al.*, 1997; Li *et al.*, 1997). The KSHV/HHV8 v-cyclin associates predominantly with cyclin-dependent kinase 6 and more weakly with kinase 4 (Godden-Kent *et al.*, 1997; Li *et al.*, 1997), and the cyclin-dependent kinase 6–v-cyclin complex can phosphorylate the physiological target of D-type cyclins, the retinoblastoma protein Rb (Chang *et al.*, 1996a; Godden-Kent *et al.*, 1997; Li *et al.*, 1997). Two-dimensional electrophoresis indicates that KSHV/HHV8 v-cyclin induces phosphorylation of Rb at authentic sites (Chang *et al.*, 1996a). The activation of cyclin-dependent kinase 6 activity induced by the v-cyclins of both HVS and KSHV/HHV8 is much more pronounced than that by human cyclin D2: the two have a broader specificity and can also phosphorylate histone H1 (Godden-Kent *et al.*, 1997; Li *et al.*, 1997). The activity of KSHV/HHV8 v-cyclin *in vivo* was demonstrated in the osteosarcoma cell line SAOS-2, which has homozygous deletions of both *Rb* alleles. When wild-type *Rb* is transfected into SAOS-2 cells, they stop replicating and take on a senescent phenotype characterized by an enlarged cytoplasm. Co-transfection of KSHV/HHV8 *v-cyclin* with *Rb* prevents entry of SAOS-2 into senescence, and the cells continue to proliferate (Chang *et al.*, 1996b).

KSHV/HHV8 *v-cyclin* is expressed in latently infected primary effusion lymphoma cell lines, in uncultured primary effusion lymphomas and in Kaposi's sarcoma tissue (Cesarman *et al.*, 1996a; Godden-Kent *et al.*, 1997). It is expressed in persistently infected cells in concert with K13/v-*FLIP* and ORF 73 (Rainbow *et al.*, 1997).

4.4.7 *Latency-associated nuclear antigen*

ORF 73 of KSHV/HHV8 encodes a large nuclear protein which is identical (Rainbow *et al.*, 1997) to the previously described high-molecular-mass (224–236 kDa) LNA (Gao *et al.*, 1996b) and a component of the LANA, defined by immunofluorescence (Gao *et al.*, 1996a; Kedes *et al.*, 1996). Its size varies between different virus isolates, due to varying lengths of the *moi* repeat region within this ORF (Russo *et al.*, 1996; Rainbow *et al.*, 1997). The *moi* region is translated as a long acidic repeat. *ORF 73*/LNA also encodes a leucine zipper region in the carboxy terminus of the protein (Russo *et al.*, 1996). Its apparent molecular mass (by sodium dodecyl sulfate polyacrylamide gel electrophoresis) is much higher than that predicted from its amino-acid sequence, pro-

bably because of the highly charged nature of the *moi* repeat region and possibly also as a result of post-translational modification. Post-translational modification may also explain the doublet nature of LNA on western blots (Gao *et al.*, 1996b; Rainbow *et al.*, 1997).

The function of ORF 73/LNA is not yet known. It is a latent protein, expressed in latently infected primary effusion lymphoma cell lines and Kaposi's sarcoma spindle cells (Gao *et al.*, 1996a,b; Rainbow *et al.*, 1997) and associated with subnuclear domains (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Rainbow *et al.*, 1997), the identity of which remains to be resolved. There is no evidence that ORF 73/LNA has transforming properties. [It is conceivable that, in analogy with *trans*-activating proteins of other herpesviruses, it is primarily involved in controlling transcription of other viral genes but could also affect the expression of cellular genes.]

4.4.8 *G Protein-coupled receptor homologue*

ORF 74 of both KSHV/HHV8 and HVS encodes a homologue of a G protein-coupled receptor with seven membrane-spanning domains (Cesarman *et al.*, 1996a; Arvanitakis *et al.*, 1997). This receptor has highest sequence homology to IL-8 chemokine receptors and is also related to the EBI-1 cellular protein induced by EBV infection (Birkenbach *et al.*, 1993). Studies of transient expression of viral G-protein-coupled receptor suggest that it is constitutively active and does not require ligand binding or is activated by a ligand commonly expressed into cell culture medium. The receptor is unusual in that it can bind chemokines belonging to both the C-X-C and C-C families. Transfection of the KSHV/HHV8 G protein-coupled receptor enhances proliferation of rat kidney fibroblasts (NRK-49F), suggesting that it may contribute to tumour-cell proliferation (Arvanitakis *et al.*, 1997). Its increased expression in primary effusion lymphoma cell lines treated with 12-*O*-tetradecanoylphorbol 13-acetate suggests, however, that it is expressed primarily during lytic replication. While its expression in Kaposi's sarcoma tissue has been documented by RT-PCR (Cesarman *et al.*, 1996b), it is not clear whether it is expressed in persistently infected Kaposi's sarcoma spindle cells or in the few cells in these lesions that are undergoing lytic viral replication.

4.5 Summary of potential roles of KSHV/HHV8 in tumorigenesis

4.5.1 *Kaposi's sarcoma*

KSHV/HHV8 establishes a persistent infection in most Kaposi's sarcoma spindle cells, characterized by a restricted pattern of gene expression (Zhong *et al.*, 1996). All spindle cells and the atypical endothelial cells of early Kaposi's sarcoma lesions express abundant mRNA for *ORF K12*, which encodes a small hydrophobic protein of unknown function (Zhong *et al.*, 1996; Stürtzl *et al.*, 1997; Staskus *et al.*, 1997). Kaposi's sarcoma spindle cells also express the high-molecular-mass LANA encoded by *ORF 73*, as shown by immunohistochemistry with affinity-purified antibodies to this protein. Expression of mRNA for the *ORF 72*-encoded D-type-cyclin homologue has been demonstrated, but as the mRNA encoded *ORF 73/LANA* extends through the *ORF 72/v-cyclin* gene, this result

should be confirmed by immunohistochemical studies with v-cyclin. The *K13/v-FLIP* gene is also contained within the mRNAs encoding *ORF 72/v-cyclin* and *ORF 73/LANA* (Rainbow *et al.*, 1997) and may therefore also be expressed in Kaposi's sarcoma spindle cells, but no immunohistochemical studies on this protein have been reported. The KSHV/HHV8 homologue of IL-6 (*ORF K2*) is expressed during latency in KSHV/-HHV8-infected haematopoietic cells but is not generally expressed in Kaposi's sarcoma lesions (Moore *et al.*, 1996b). Expression of *ORF 74*, which encodes a functional chemokine receptor (Arvanitakis *et al.*, 1997), can be found by RT-PCR (Cesarman *et al.*, 1996c). KSHV/HHV8 virions have been seen in a subpopulation of Kaposi's sarcoma spindle cells and infiltrating haematopoietic cells (Orenstein *et al.*, 1997).

These findings indicate that all or nearly all endothelial tumour (spindle) cells in Kaposi's sarcoma lesions are infected with KSHV/HHV8 (Boshoff *et al.*, 1995b). There is not yet enough evidence to conclude that the gene expression programme of KSHV/-HHV8 (i.e. lytic and latent gene expression programmes) is similar to that of other herpesviruses. A set of viral genes that is expressed in primary effusion lymphoma cells (*ORF K12*, *ORF K13*, *ORF 72*, *ORF 73*) is not up-regulated by chemical treatment and could therefore represent latent genes that are also expressed in Kaposi's sarcoma spindle cells. Since a minority of these cells appears to undergo full lytic replication, it is too early to conclude whether KSHV/HHV8 establishes a latent infection in most spindle cells; however, it is also unclear whether a latent expression pattern is required for the expansion of spindle cells. The presently available evidence is insufficient to conclude whether a limited lytic expression programme is compatible with virus-mediated cellular proliferation.

4.5.2 *Primary effusion lymphoma*

Ninety percent of the described cases of primary effusion lymphoma contain both KSHV/HHV8 and EBV (see Section 1), and the cells contain multiple episomal copies of KSHV/HHV8 (Cesarman *et al.*, 1995a,b; Nador *et al.*, 1996). KSHV/HHV8 gene expression in primary effusion lymphoma has been studied mainly in derived cell lines, and the results may not fully reflect the gene expression pattern *in vivo*. It is, however, largely similar to that in Kaposi's sarcoma lesions (Zhong *et al.*, 1996; see section 4.5.1). In addition, of the genes investigated, *v-bcl-2*, *v-MIP-I*, *v-MIP-II*, *v-IRF*, *v-IL-6* and *v-GCR* are expressed at low levels in primary effusion lymphoma cell lines, and their expression can be up-regulated by treatment with phorbol esters and/or sodium butyrate. The untranslated *T1.1* RNA (see Section 1) is strongly expressed in unstimulated primary effusion lymphoma cell lines but can be further up-regulated by chemical treatment. Unlike Kaposi's sarcoma, primary effusion lymphoma cells strongly express v-IL-6 protein, as shown by immunohistochemistry of fresh primary effusion lymphoma cells (Moore *et al.*, 1996b).

In cell lines concomitantly infected with EBV and KSHV/HHV8, the EBV is monoclonal and its expression is limited to EBNA-1 and (in one case) LMP-2 (Moore *et al.*, 1996a; Nador *et al.*, 1996).

Despite attempts by several groups, there is currently no evidence that KSHV/HHV8 can transform or immortalize lymphocytes *in vitro*.

Given the low level of expression of several KSHV/HHV8 genes shown to dysregulate the cell cycle and/or prevent apoptosis, it is at least conceivable, although unproven, that these viral genes play an important role in the development of primary effusion lymphoma.

4.5.3 *Multicentric Castleman's disease*

Unlike Kaposi's sarcoma and primary effusion lymphoma, multicentric Castleman's disease is not universally associated with KSHV/HHV8 infection, although infection with this virus is very common in HIV-associated cases (see Section 2). Multicentric Castleman's disease is a polyclonal disorder and, like Kaposi's sarcoma, characterized by prominent angiogenesis. No published data are available on KSHV/HHV8 gene expression in these lesions. Given the polyclonal nature of the disease and the inconsistent association with KSHV/HHV8, it is likely that multicentric Castleman's disease has a multifactorial etiology and that the role of KSHV/HHV8 is indirect, conceivably mediated by virus-encoded cytokines.

4.6 Antiviral agents

Specific antiviral therapy has been evaluated in only a few studies and a case series (Morfeldt & Torssander, 1994; Jones *et al.*, 1995; Glesby *et al.*, 1996; Mocroft *et al.*, 1996). All of the currently available antiherpesvirus drugs that have been evaluated belong to the class of DNA polymerase inhibitors, which are active against lytic but not latent herpesviral infection. Morfeldt and Torssander (1994) described clinical regression of AIDS-associated Kaposi's sarcoma lesions in three of five patients after administration of high doses of phosphonoformic acid (foscarnet), but no untreated control patients were available for comparison. Jones *et al.* (1995) examined follow-up data on 20 228 HIV-positive persons and AIDS patients enrolled in a study of adult diseases, of whom 1033 (5%) developed Kaposi's sarcoma. Proportional hazards analysis was used to demonstrate that the risk of patients receiving foscarnet for developing Kaposi's sarcoma after a median of 14 months' follow-up was 30% of that of patients who did not receive herpesviral therapy, after taking into account CD4 count, age, sex, race, route of exposure to HIV, other opportunistic illnesses and antiretroviral therapy ($p = 0.001$). Similar analysis for gancyclovir (odds ratio, 1.0; $p = 0.8$) and acyclovir (odds ratio, 1.4; $p < 0.001$) showed either no effect or an increased risk for disease with therapy. A similar analysis of 935 participants in a multicentre study of AIDS showed a non-significant preventive effect against Kaposi's sarcoma for forscarnet (RR, 0.40; 95% CI, 0.05–3.10; $p = 0.38$) and gancyclovir (RR, 0.56; 95% CI, 0.22–1.44; $p = 0.23$) but not for acyclovir (Glesby *et al.*, 1996). Mocroft *et al.* (1996) followed 3688 patients with HIV infection or AIDS for a median of 4.2 years, of whom 598 (16%) developed Kaposi's sarcoma. After adjustment for sex, route of exposure to HIV, age, antiretroviral treatment, prophylaxis for *Pneumocystis carinii* pneumonia, opportunistic infections and CD4 count, use of foscarnet (relative hazard, 0.38; 95% CI, 0.15–0.95; $p = 0.038$) and

gancyclovir (relative hazard, 0.39; 95% CI, 0.19–0.84; p = 0.015) but not acyclovir (relative hazard, 1.1; 95% CI, 0.88–1.4; p = 0.40) were associated with a decreased risk of developing Kaposi's sarcoma.

Foscarnet has some direct antiretroviral activity (Sandstrom *et al.*, 1985) and may therefore have activity against Kaposi's sarcoma, but other opportunistic tumours and infections (aside from susceptibility to herpesviral infections) are not known to respond to foscarnet therapy.

Kedes and Ganem (1997) demonstrated that gancyclovir, foscarnet and cidofovir at pharmacological concentrations inhibit virion induction *in vitro* in the primary effusion lymphoma cell line BCBL-1, whereas acyclovir does not. An anti-retroviral protease inhibitor and ritonavir analog, A 77003, did not act on KSHV/HHV8 replication.

5. Summary of Data Reported and Evaluation

5.1 Virus–host interactions

Kaposi's sarcoma herpesvirus/human herpesvirus 8 (KSHV/HHV8) is a gamma-2 herpesvirus (a rhadinovirus) with a 165-kb genome. Its closest relatives are *Herpesvirus saimiri* (HVS), a tumorigenic rhadinovirus of New World primates, and a group of recently identified rhadinoviruses in Old World monkeys. It contains blocks of conserved herpesvirus genes that encode mainly structural proteins. In addition, several genes similar in sequence to other viral and cellular oncogenes and growth controlling factors are present in the KSHV/HHV8 genome. These include homologues of interleukin 6, the antiapoptotic protein bcl-2, a D-type cyclin and a chemokine receptor, some of which are known to be functional.

KSHV/HHV8 has been found in B cells, macrophages and dendritic cells *in vivo*. It establishes a persistent infection in endothelial Kaposi's sarcoma spindle cells and in primary effusion lymphoma cells, which involves a disease-specific pattern of expression, with at least four (in Kaposi's sarcoma) or seven (in primary effusion lymphoma cells) viral genes. Lytic replication occurs in a subpopulation of infected spindle and haematopoietic cells.

KSHV/HHV8 DNA is readily detected in Kaposi's sarcoma lesions, primary effusion lymphoma cells and some lymphoid tissue from patients with multicentric Castleman's disease by Southern blotting or polymerase chain reaction (PCR). In contrast, only small amounts of viral DNA are generally present in non-neoplastic tissue from KSHV/HHV8-infected individuals, in particular in peripheral blood mononuclear cells and semen, requiring the use of sensitive PCR techniques for detection. Serological methods have been developed for the detection of antibodies to a latent nuclear protein and to defined and undefined structural antigens, including immunofluorescence assays, enzyme-linked immunosorbent assays and western blotting. Serological and PCR testing of peripheral blood mononuclear cells and semen shows that infection with KSHV/HHV8 is uncommon among the general populations of northern Europe and the United States, but more common in some Mediterranean countries and frequent in parts of Africa; however,

precise estimates of prevalence rates, especially in non-endemic areas, are still not available. There is some evidence that KSHV/HHV8 is sexually transmitted, but other routes of transmission are likely and probably account for a high prevalence in parts of southern Europe and Africa.

5.2 Human carcinogenicity

DNA analysis has consistently demonstrated the presence of KSHV/HHV8 at high (> 90%) rates in Kaposi's sarcoma lesions and at a generally low rate in neoplastic and non-neoplastic tissues from control patients. The load of viral DNA is higher in tissue from Kaposi's sarcomas than in unaffected tissues from the same patients. When mononuclear cells from Kaposi's sarcoma patients and controls were examined by PCR, KSHV/HHV8 was detected in significantly more cases (up to 50%) than controls. Despite differences in sensitivity, in specificity and in the antigens examined, all of the available serological studies are consistent in showing high rates of antibody-positivity in Kaposi's sarcoma patients and lower rates of seropositivity among various controls. Studies among HIV-1-positive and -negative populations at different risks for Kaposi's sarcoma indicate that seroprevalence is generally in accordance with the risk for developing the disease. The limited number of longitudinal analyses based on either detection of KSHV/HHV8 DNA by PCR or the presence of antibodies to KSHV/HHV8 suggest that KSHV/HHV8 infection precedes the development of Kaposi's sarcoma in the majority of cases.

Thus, the strength of association between infection with this virus and Kaposi's sarcoma is high, as measured by PCR, Southern blotting and serology, with odds ratio greater than 10 being found in most studies involving large numbers of cases and well-defined controls. This association is found in studies with various designs and for all epidemiological types of Kaposi's sarcoma.

Primary effusion lymphoma has been recognized as a new disease entity only since the identification of KSHV/HHV8. It has a characteristic morphology and cell surface phenotype, and all of the cases reported in the literature that showed these characteristics have been found to contain KSHV/HHV8 DNA, sometimes at high copy numbers. The vast majority of cases also contain clonal EBV. Owing to the rarity of this malignancy, no epidemiological studies are yet available.

Multicentric Castelman's disease is a rare and usually polyclonal lymphoproliferative disorder. In studies based on very few cases, KSHV/HHV8 has been found in a substantial proportion of HIV-positive patients with this disorder, and a high proportion of these patients also had Kaposi's sarcoma; a much smaller proportion of HIV-negative cases of multicentric Castleman's disease showed KSHV/HHV8 DNA.

KSHV/HHV8 has occasionally been reported to be present in other tumours, but the results are inconsistent. Whereas some of these discrepant results probably reflect the marked geographical differences in KSHV/HHV8 prevalence and the fact that the virus can be detected at several body sites and in samples from some KSHV/HHV8-infected but healthy individuals, other reports are more difficult to explain and remain controversial.

5.3 Animal models

KSHV/HHV8 has not yet been tested for tumorigenicity in experimental animals; however, studies of related viruses have proved informative.

Herpesvirus saimiri (HVS) and *Herpesvirus ateles* (HVA) do not induce disease in their natural hosts, the two New World monkeys, squirrel monkeys and spider monkeys, but they induce tumours and/or lymphoproliferation in a variety of heterologous non-human primates. The natural host populations become infected early in life, perhaps through horizontal transmission, and maintain the virus in latency throughout their lives. Both HVS and HVA are T-lymphotropic viruses and readily transform and immortalize human and simian T cells. As they are gamma-2 herpesviruses (rhadinoviruses), HVS and HVA are more closely related to KSHV/HHV8 than to EBV. Lymphoid cell lines derived either by transformation *in vitro* or from tumour tissues contain viral DNA, express viral proteins and release variable amounts of virus and viral genome copies per cell. HVS induces lymphoid tumours in New Zealand white rabbbits.

There is evidence of gamma-2 herpesviruses in Old World monkeys, which may play a role in retroperitoneal fibromatosis, a condition with some similarities to Kaposi's sarcoma.

Bovine herpesvirus type 4 is another gamma-2 herpesvirus which includes a large number of antigenically related isolates distinct from other bovine herpesviruses. This virus has not been established as the etiological agent of a distinct disease entity, but its role in the etiology of some diseases of the genital tract has been suggested. It has never been identified as a potential cause of tumours.

Murid herpesvirus 4 is a B-lymphotropic gamma-2 herpesvirus that causes B-cell proliferation in mice. As it is a gamma-2 herpesvirus, it could serve as a model for KSHV/HHV8 infection.

5.4 Molecular mechanisms of carcinogenesis

The role of KSHV/HHV8 in the pathogenesis of Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease is still poorly understood. The virus is present in the endothelial tumour (spindle) cells of Kaposi's sarcoma lesions and in primary effusion lymphoma cells. The latter are of monoclonal origin, and there is evidence to suggest that Kaposi's sarcoma lesions are also monoclonal. The viral homologue of D-type cyclins, which can disrupt cell cycle control, is expressed in both these tumour types, as are some other proteins of as yet unknown function. In the case of primary effusion lymphoma, several growth factors, a growth factor regulatory protein and a growth factor receptor are also expressed. Some KSHV/HHV8-infected Kaposi's sarcoma spindle cells undergo lytic replication. It is therefore at present unclear whether, as in EBV, a latent programme of gene expression is required for cellular transformation, with lytic infection of spindle cells representing an abortive pathway. Some viral genes whose expression can be upregulated during lytic infection (e.g. several growth factors and a growth factor regulatory protein) may contribute to virus-mediated expansion of Kaposi's sarcoma spindle cells. There is a striking correspondence between genes

encoded by KSHV/HHV8 and human genes involved in the control of cell growth, which are induced after EBV infection. This suggests that the two viruses may use different strategies to modify the same cellular regulatory and signalling pathways. Similar considerations apply to the role of KSHV/HHV8 in the pathogenesis of primary effusion lymphoma, the cells of which can also undergo lytic infection *in vitro*.

KSHV/HHV8 is not always found in multicentric Castleman's disease, especially in HIV-negative cases. There are no published data on which cell type in these lesions harbours KSHV/HHV8; however, KSHV/HHV8 probably plays an indirect role in this disorder, conceivably involving cytokines such as viral interleukin-6, since cellular interleukin-6 has been implicated in its pathogenesis.

5.5 Evaluation

There is compelling but as yet limited evidence for a role of KSHV/HHV8 in the causation of Kaposi's sarcoma.

KSHV/HHV8 *is probably carcinogenic to humans (Group 2A).*

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