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).

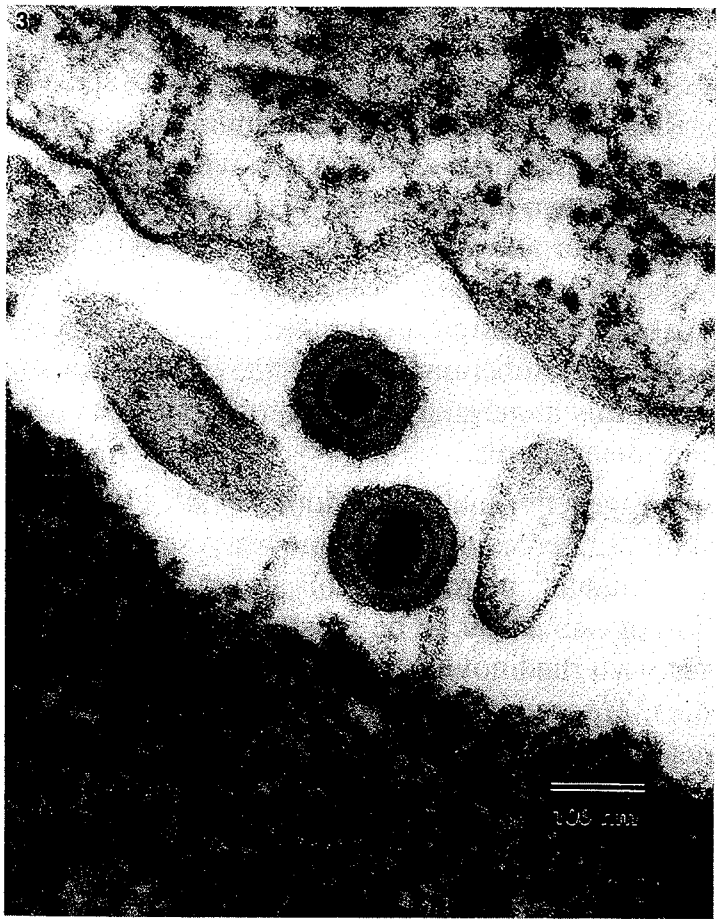
1.1.2 *Structure*

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).

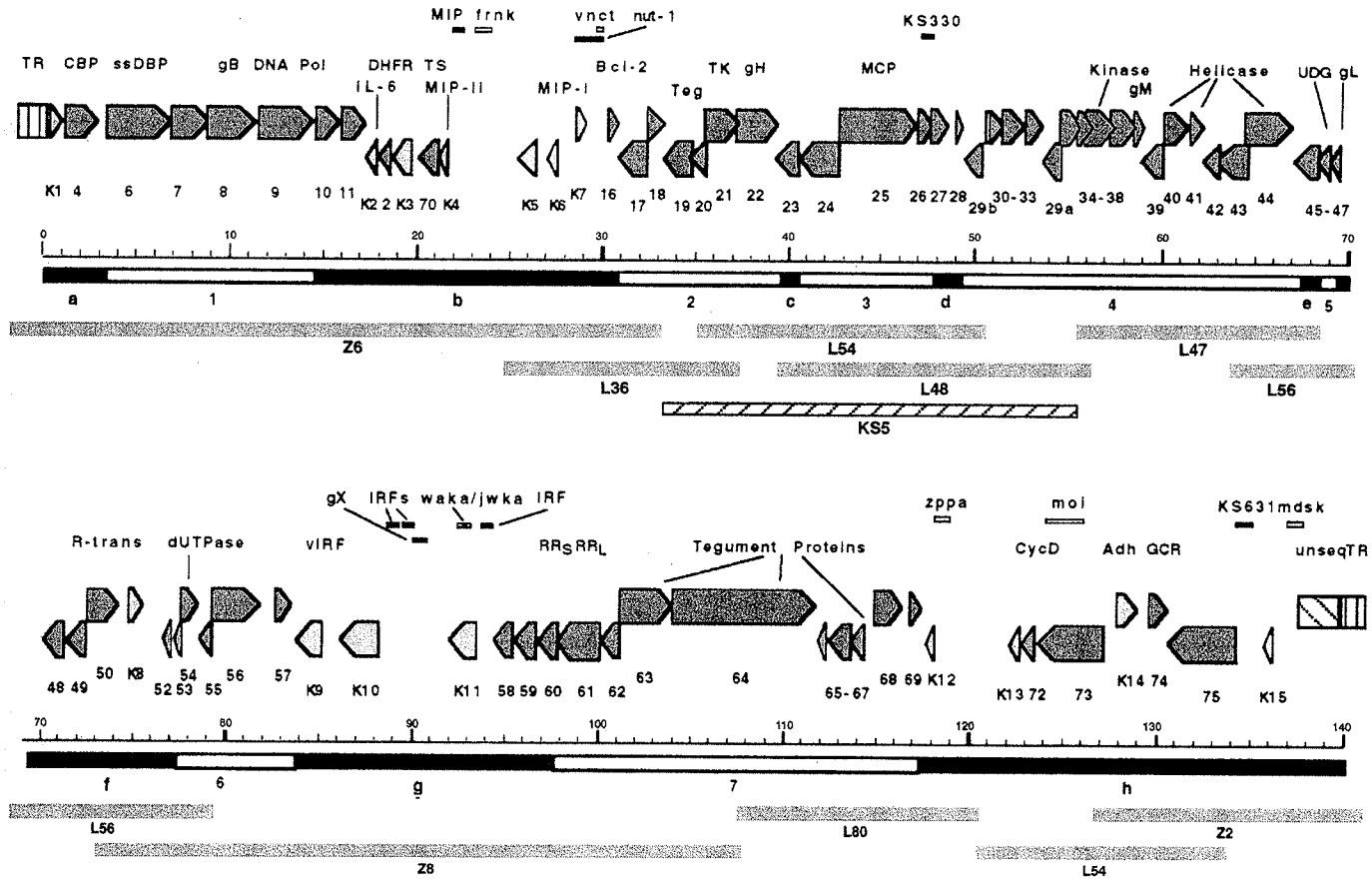
Figure 1. Electron microscopic view of KSHV/HHV8 capsid structure surrounded by a lipid envelope with an electron-dense central core representing viral DNA



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.

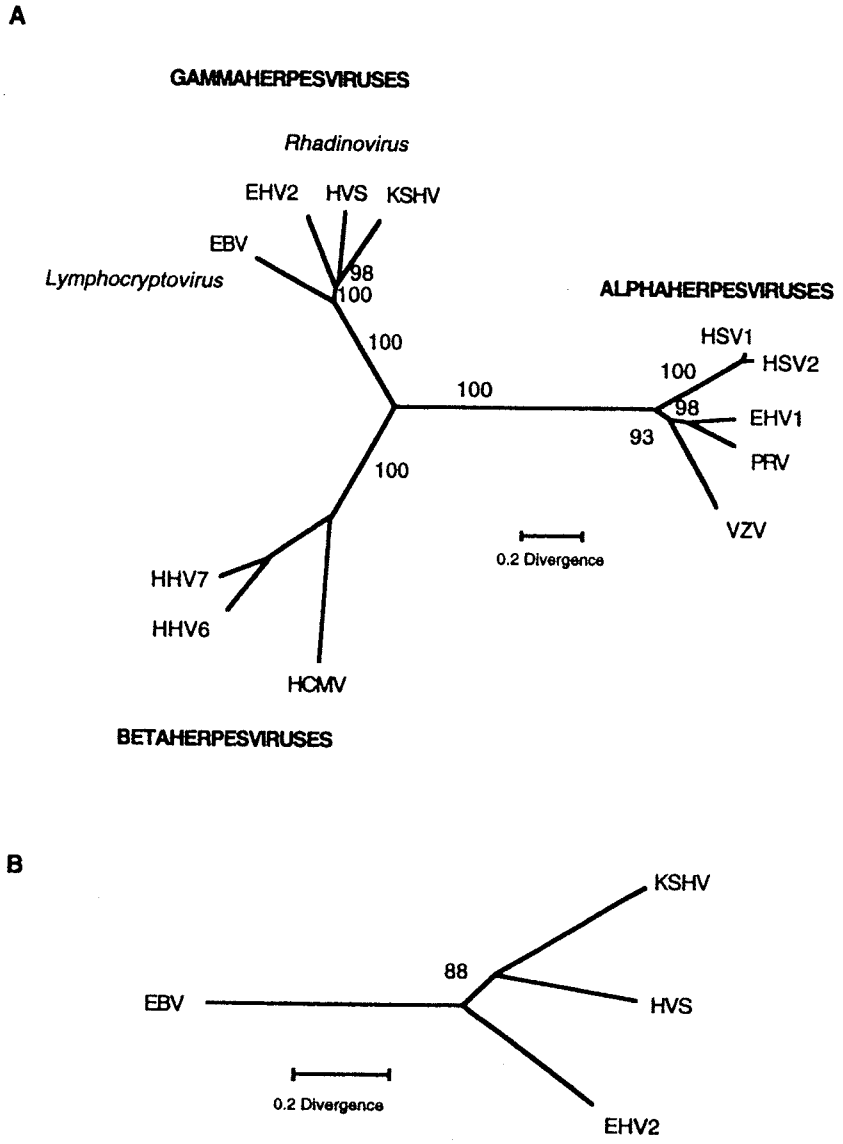
1.1.5 *Related non-human viruses*

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.