Kaposi’s sarcoma-associated herpesvirus (human herpesvirus-8)

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Introduction

Discovered only 4 years ago (Chang et al., 1994), the eighth human herpesvirus (HHV-8), or Kaposi’s sarcoma-associated herpesvirus (KSHV), has rapidly become the focus of intensive research. Although still limited, the presently available data provide compelling evidence that KSHV/HHV-8 is the long-sought-after infectious cause of Kaposi’s sarcoma (KS) and that it is thus a new human tumour virus. In this review, I discuss the molecular and epidemiological evidence on which this conclusion is based.

Association with disease

KSHV is associated with three neoplastic disorders, KS, primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD). Two small fragments of the KSHV genome were initially discovered in KS tissue by representational difference analysis (Chang et al., 1994). Since then, many groups have reported that KSHV genomes can be detected, by Southern blot or PCR, in all epidemiological forms [human immunodeficiency virus (HIV)-associated, ‘classic’, endemic, post-transplant] and clinical stages (early patch/plaque, fully developed nodular) of KS (Chang et al., 1994, 1996a; Moore & Chang, 1995; Boshoff et al., 1995a; Buonaguro et al., 1996; Chuck et al., 1996; Dupin et al., 1995; Luppi et al., 1996a; Noel et al., 1996; Schalling et al., 1995; Su et al., 1995; Gaidano et al., 1996a; Dictor et al., 1996; Besnard et al., 1996; Lebbé et al., 1997a; Ziegler & Katongole-Mbidde, 1996). By nested PCR, KSHV can also be detected in non-involved tissue from KS patients, including skin, lymphoid tissue, prostate, semen and peripheral blood mononuclear cells (PBMC) (Whitby et al., 1995; Moore et al., 1996b; Lefrere et al., 1996; Humphrey et al., 1996; Gaidano et al., 1996a; Lebbé et al., 1995; Viviano et al., 1997; Monini et al., 1996a, b; Howard et al., 1997; Gupta et al., 1996; Corbellino et al., 1996a; Brambilla et al., 1996; Dictor et al., 1996; Smith et al., 1997). Detection of KSHV is possible in PBMC of 50–60% of patients with HIV-associated or ‘classic’ KS, but not, or only infrequently, in HIV-negative individuals from non-endemic areas (Whitby et al., 1995; Moore et al., 1996b; Lefrere et al., 1996; Brambilla et al., 1996; Humphrey et al., 1996; Bigoni et al., 1996; see also below). The odds ratios for having KS, if KSHV is detected in PBMC, are high in most studies (e.g. 6.5–147 for the results reported by Whitby et al., 1995; Moore et al., 1996b; Humphrey et al., 1996; see Olsen & Moore, 1998, for a detailed discussion of the strength of this association). In addition, detection of KSHV in PBMC of HIV-infected individuals predicts the subsequent appearance of KS lesions (Whitby et al., 1995; Moore et al., 1996b; Lefrere et al., 1996). The presence of antibodies to KSHV in non-endemic areas is also strongly associated with having KS, or being at increased risk of KS (see below).

KSHV genomes are also consistently found in PEL, also termed ‘body-cavity-associated lymphomas’ (BCBL) (Cesarman et al., 1995a; Nador et al., 1996; Gessain et al., 1997; Otsuki et al., 1996; Ansari et al., 1996; Carbone et al., 1996; Pastore et al., 1995; Komanduri et al., 1996; Gaidano et al., 1996a), a rare form of AIDS-related B-cell lymphoma which is characterized by malignant effusions in the pleural or abdominal cavity, immunoglobulin gene rearrangement, lack of most surface markers and, unlike Burkitt’s lymphoma, a lack of c-myc rearrangements (for a review see Jaffe, 1996). Most cases of PEL are dually infected with Epstein–Barr virus (EBV) and KSHV (Cesarman et al., 1995a, b; Gessain et al., 1997), but occasional cases of EBV-negative/KSHV-positive PEL have been reported (Renne et al., 1996a; Carbone et al., 1996; Cesarman et al., 1996a; Strauchen et al., 1996). A few cases of PEL have also been described in HIV-negative patients (Carbone et al., 1996; Strauchen et al., 1996; Hermette et al., 1996; Nador et al., 1995). Most PEL lymphoma cells harbour multiple (approx. 50–100) copies of KSHV as episomes (Cesarman et al., 1995a, b). It is possible that rare PEL cases may lack KSHV, but the classification of these cases is still controversial (Hermine et al., 1996; Cesarman et al., 1996c).

MCD is an atypical lymphoproliferative disorder which occurs in two histological variants, the hyaline-vascular variant and the plasma cell variant. Most cases of MCD in HIV-infected patients, in particular the plasma cell variant, contain detectable KSHV (Soulier et al., 1995; Gessain et al., 1996). In contrast, KSHV is much less frequently detected in MCD of HIV-negative patients (Soulier et al., 1995; Gessain et al., 1996; Barozzi et al., 1996). Thus, KSHV is strongly associated with KS and PEL, but less strongly associated with MCD.
Fig. 1. Genome of KSHV and other gammaherpesviruses. The organization of the KSHV genome is compared to that of EBV (a γ₁-herpesvirus) and the γ₂-herpesviruses (rhadinoviruses) HVS, EHV-2 and MHV-68 (Baer et al., 1984; Albrecht et al., 1992; Telford et al., 1995; Virgin et al., 1997). Conserved structural genes found in most gammaherpesviruses are shaded dark and grouped in blocks I–IV. Genes found in some, but not all, gammaherpesviruses are cross-hatched and genes which are unique to individual viruses are represented by an open symbol. The ‘non-conserved genes’ are located in groups A–F, indicated by grey shading. Small solid or hatched boxes and arrows depict short G+C-rich repeats and inverted palindromic areas. Solid circles indicate domains with known or presumed origin-like features, e.g. ori-Lyt₁, ori-Lyt₂ of EBV and ori and ori₉ domains in KSHV/HHV-8. The ori-P of EBV is represented by an open circle. This diagram, kindly provided by J. Nicholas, Baltimore (Nicholas et al., 1998) (with the permission of Oxford University Press), includes several potential KSHV genes in addition to those identified by Russo et al. (1996) and Neipel et al. (1997a) (see text).
KSHV has also been found in a few cases of angioimmunoblastic lymphadenopathy and some reactive lymphadenopathies (Luppi et al., 1996b; Gyulai et al., 1996a), but the implication of this observation is not clear.

In addition, the presence of KSHV in squamous cell carcinoma of transplant recipients, angiosarcoma and cutaneous T-cell lymphoma has also been suggested by some (Rady et al., 1995; Gyulai et al., 1996b; McDonagh et al., 1996; Sander et al., 1996), but could not be confirmed by others (Boshoff et al., 1995a, 1996; Uthman et al., 1996; Dictor et al., 1996; Chang et al., 1994; Jin et al., 1996; Tomita et al., 1996; Pawson et al., 1996) and an association of KSHV with these disorders appears unlikely. More recently, KSHV has been detected in bone marrow stromal cells of patients with multiple myeloma and benign monoclonal gammapathy (Rennie et al., 1997). However, the prevalence of KSHV antibodies among myeloma patients in the UK (McKenzie et al., 1997), France (Marcellin et al., 1997) and Italy (Whitby et al., 1997) is the same as in blood donors or patients with other non-Hodgkin’s disease lymphomas from the same countries, arguing against a consistent association of KSHV with multiple myeloma.

Classification, culture and morphology

Two small fragments of the KSHV genome were initially discovered by representational difference analysis and shown to be related to gammaherpesviruses (Chang et al., 1996). The subsequent sequence analysis (Moore et al., 1996a) of a larger genomic region containing blocks of structural genes found in all herpesviruses demonstrated that KSHV is a γ2-herpesvirus (rhadinovirus) and so far most closely related to herpesvirus saimiri (HVS), a lymphomagenic rhadinovirus of squirrel monkeys (Albrecht et al., 1992). More recently, closely related rhadinoviruses have also been detected in captive macaques (Rose et al., 1997; Desrosiers et al., 1997). The recent completion of the entire KSHV/HHV-8 genome sequence (Russo et al., 1996; Neipel et al., 1997a) showed, in addition, that the organization of the KSHV genome is very similar to that of other rhadinoviruses, in particular HVS and murine herpesvirus-68 (MHV-68), with which it shares a few genes not found in other rhadinoviruses (Albrecht et al., 1992; Efstathiou et al., 1990) (see below and Fig. 1).

To date it has been difficult to culture KSHV efficiently. Latently infected B-cell lymphoma cell lines have been established (Cesarman et al., 1995b; Arvanitakis et al., 1996; Gao et al., 1996b; Renne et al., 1996a; Said et al., 1996; Gaidano et al., 1996b) from PEL and from the peripheral blood of a PEL patient (Boshoff et al., 1998). Like the lymphoma cells, most PEL cell lines are dually infected with KSHV and EBV, but some PEL cell lines infected only with KSHV have been established (Renne et al., 1996a; Gao et al., 1996b; Said et al., 1996, Boshoff et al., 1998).

In culture, a small proportion of cells in these PEL-derived cell lines can spontaneously undergo lytic replication and this can be enhanced by treatment with phorbol esters or sodium butyrate (Miller et al., 1996, 1997; Lennette et al., 1996). Such treated cell lines can produce KSHV virions and have formed the basis of ultrastructural studies (Renne et al., 1996a; Said et al., 1996; Ablashi et al., 1997). As shown in Fig. 2, KSHV virions have morphological features typical of other herpesviruses, with a size of approximately 110 nm. Nucleocapsids with an electron-dense core are found in the nucleus of these induced lymphoma cells and enveloped virions are found in the cytoplasm (Renne et al., 1996a; Said et al., 1996). Similar particles have also been observed in KS lesions in a few cells with spindle-shaped morphology (Walter et al., 1984; Ioachim, 1995; Orenstein et al., 1997; Said et al., 1997).

However, serial propagation of KSHV in tissue culture has so far proved to be inefficient. Limited lytic replication and serial transmission have been achieved in 293 cells (Foreman et al., 1997a), but could only be detected by using PCR. Nevertheless it has been possible to culture KSHV directly from KS lesions (Foreman et al., 1997a) and saliva (Sieve et al., 1997) in this way, confirming the presence of KSHV virions in tumour samples.

It has also been possible to study the effect of herpesvirus DNA polymerase inhibitors on lytic KSHV replication in an tetradecanoyl phorbol acetate-induced PEL cell line (BcCl-1). Phosphonoacetic acid (‘foscarnet’), ganciclovir and cidovir, but not aciclovir, inhibit KSHV replication at pharmacological concentrations (Kedes & Ganem, 1997; Medveczky et al., 1997). Interestingly, foscarnet appeared to reduce the rate of new KS lesions appearing in AIDS patients in several studies (Jones et al., 1995; Glesby et al., 1996; Mocroft et al., 1996) and induced the regression of existing lesions in one small study (Morfeld & Torssander, 1994). Ganciclovir also had a preventative effect in two of these studies (Glesby et al., 1996; Mocroft et al., 1996), but aciclovir did not (Jones et al., 1995; Mocroft et al., 1996). However, foscarnet or ganciclovir do not always eliminate PCR-detectable KSHV from the PBMC of infected individuals (Humphrey et al., 1996), which would be consistent with the inability of herpesvirus DNA polymerase inhibitors to clear latent infections.

Genome organization and sequence variability

As shown in Fig. 1, the size and organization of the KSHV genome is very similar to that of other rhadinoviruses (Renne et al., 1996b; Russo et al., 1996; Neipel et al., 1997a; see legend to Fig. 1 for references to other herpesviruses). A long unique region of 140±5 kb is flanked by two terminal repeats consisting of several 801 bp repeat subunits of high (85±) G+C content (Russo et al., 1996; Neipel et al., 1997a, b). All the presently known open reading frames (ORFs) are encoded within the long unique region (Fig. 1). The nomenclature for individual viral genes corresponds to that previously adopted for HVS.
Genes that are unique to KSHV have been designated with a ‘K’ prefix (for KSHV) and numbered sequentially.

Like other herpesvirus genomes, that of KSHV contains genes encoding proteins which are required for replication and assembly of new virus progeny. In the genomes of gammaherpesviruses these ‘conserved’ genes are arranged in four or five blocks (gene blocks I–IV, IV* in Fig. 1) (Chee et al., 1990; Gompels et al., 1995; Albrecht et al., 1992; Russo et al., 1996; Neipel et al., 1997, b; Nicholas et al., 1997a; Virgin et al., 1997; Baer et al., 1984; Telford et al., 1995) and include major structural proteins (e.g. orf25, the major capsid protein), DNA synthetic enzymes (e.g. orf21, the thymidine kinase, and orf9, the DNA polymerase), glycoproteins [e.g. gB (orf8), gH (orf22), gM (orf38)], and a viral proteinase and assembly protein (orf17) (Moore et al., 1996a; Russo et al., 1996; Unal et al., 1997). orf2 and orf70, encoding dihydrofolate reductase and thymidylate synthase genes, respectively, are rearranged in KSHV relative to their positions in HVS (Russo et al., 1996). Within the ‘non-conserved’ gene blocks (A–E in Fig. 1) are genes also found in some other rhadinoviruses [e.g. orf72 (v-cyclin), orf73 (LANA), orf74 (vGCR)] and others, which are unique to KSHV (K1–K15) (Russo et al., 1996; Nicholas et al., 1997a, 1998; Neipel et al., 1997a, b).

‘Non-conserved’ gene block B contains several genes with homologies to mammalian proteins and appears more extensive than in other rhadinoviruses (Russo et al., 1996; Neipel et al., 1997a, b; Nicholas et al., 1997a, 1998; Fig. 1). Several growth factor homologues, including vIL-6 (orfK2), two macrophage inflammatory protein (MIP)-1α homologues (orfK4, orfK6) and an MIP-1β homologue (orfK4.1; vBCK), two homologues of an immediate early protein of bovine herpesvirus-4 (BHV-4) (IE1A, IE1B), as well as a thymidylate synthase (orf70) and a dihydrofolate reductase (orf2) are encoded in this region (Russo et al., 1996; Neipel et al., 1997a, b; Nicholas et al., 1997a, b, 1998).

The KSHV genome also contains two additional ‘non-conserved’ gene blocks which are not found in all rhadinoviruses. Block D2 contains two genes, K8 and K8.1 (Russo et al., 1996; Neipel et al., 1997a, b). The existence of the K8.1 glycoprotein has been confirmed experimentally by recom-
binant expression of the corresponding protein (Neipel et al., 1998; Chandran et al., 1998). Block D3 contains several genes which are unique to KSHV. Four of these have homologies to interferon regulatory factors (IRF) (Fig. 1; Russo et al., 1996; Neipel et al., 1997a; Nicholas et al., 1998).

Block E of several rhadinoviruses contains homologues of a D-type cyclin (orf72), the nuclear protein encoded by orf73 (the 'latency-associated nuclear antigen'), and a G-protein-coupled receptor homologue (vGCR; orf74) (Russo et al., 1996; Neipel et al., 1997a, Cesарман et al., 1996b; Guo et al., 1997; Rainbow et al., 1997; see below). In addition, block E of KSHV and HVS contains orfK13/orf71, encoding a protein with anti-apoptotic function (Thome et al., 1997; Bertin et al., 1997; see below), and KSHV block E also encodes K12 (see below). In EBV, block E contains the genes for EBNA2 and EBNA5, which are required for B-cell transformation, as well as an IL-10 homologue (Fig. 1).

Two putative origins of replication have been identified in the KSHV genome. As in the case of the two EBV origins of lytic replication (ori-Lyt), these are located in non-conserved gene blocks B and E adjacent to high G+C content tandem repeats (Fig. 1; Nicholas et al., 1998).

At present it appears that most of the KSHV genome is highly conserved in isolates or sequences obtained from KS biopsies or PEL cell lines and from different geographical regions. A comparison of a 20 kb genomic region obtained from a KS lesion and a PEL cell line showed only 0-1% nucleotide variation between the two isolates (Russo et al., 1996; Moore et al., 1996a). A nearly complete genomic sequence obtained from a KS biopsy (Neipel et al., 1997a) is also highly homologous to the complete genomic sequence from a PEL cell line (Russo et al., 1996), with the exception of the two ends of the virus genome (see below).

A limited degree of sequence variation has, however, been found in orf26 (Moore & Chang, 1995; Boshoff et al., 1995a; Collandre et al., 1995; Marchiolli et al., 1996; Zong et al., 1997). A combined analysis of several genomic regions in orf26 and orf75 yielded up to 15% overall nucleotide variation between isolates (Zong et al., 1997). First attempts have been made to exploit the limited degree of sequence variation in these regions for phylogenetic studies (Moore & Chang, 1995; Boshoff et al., 1995a; Zong et al., 1997), but detailed molecular epidemiological studies on the origin and transmission patterns of KSHV have not been possible.

In contrast, a comparison of several recently available complete or partial KSHV sequences suggests that orfK1 of KSHV, located at the ‘left’ end of the KSHV genome, may exhibit greater variability (presently up to 19% difference in the protein sequence) than the structural KSHV genes studied so far (Lagunoff & Ganem, 1997; Neipel et al., 1997a; Russo et al., 1996; Nicholas et al., 1998; Lee et al., 1998; P. M. Cook & T. F. Schulz, unpublished data; A. Davison, personal communication). It is likely that this genomic region will prove more informative for phylogenetic studies. Interestingly, in HVS, the highest degree of genomic variability, correlating with pathogenicity, has also been observed at the left end of the genome, where HVS genes required for the transformation of T-cells are encoded (Albrecht et al., 1992; see Meinl et al., 1995, for a review).

In addition, the ‘right’ end of the KSHV genome also appears to be highly divergent in some isolates. The prototypic HBL-6/BC-1 sequence (Russo et al., 1996) has so far been found much less frequently in KS biopsies or BCBL cell lines than a second sequence reported more recently (Nicholas, 1997c; Nicholas et al., 1998).

Function of non-conserved genes

Among the KSHV genes, which are unique to KSHV or only found in some other rhadinoviruses (Fig. 1), are several homologues of cellular proteins involved in the control of cell proliferation.

orfK1

This ORF encodes a 279–289 aa transmembrane glycoprotein with a cysteine-rich extracellular domain with some regional homology to immunoglobulin lambda light chains (Lagunoff & Ganem, 1997; Lee et al., 1998). There is significant sequence and length variation between isolates in its extracellular domain, whereas the cytoplasmic domain, which contains several tyrosine residues, and the membrane spanning region appear more conserved (Russo et al., 1996; Neipel et al., 1997a; Nicholas et al., 1998; Lagunoff & Ganem, 1997; Lee et al., 1998; A. Davison, personal communication; P. M. Cook & T. F. Schulz, unpublished data). There is some evidence (Lee et al., 1998) that sequence variability in the extracellular domain may affect multimerization of the K1 protein, but it is still unknown whether this has any impact on its function.

orfK1 has transforming properties when expressed in Rat1 fibroblasts (Lee et al., 1998). When recombined into a C strain of herpesvirus saimiri, from which its transforming gene STP has been deleted, orfK1 can immortalize common marmoset CD8 T-cells in vitro, and cause lymphoproliferative disease of a CD8+ phenotype in common marmosets in vivo (Lee et al., 1998). However, orfK1 is not expressed in latently infected BCBL cell lines (Lagunoff & Ganem, 1997; Sarid et al., 1998) and whether it is expressed in KS spindle (endothelial tumour) cells has not yet been established. Its role in the pathogenesis of KSHV-associated neoplasms thus remains to be established.

orfK2 (viL-6)

The protein encoded by orfK2 is 25% identical at the amino acid level to human IL-6 (Moore et al., 1996c; Nicholas et al., 1997b; Neipel et al., 1997c) and is a secreted cytokine which maintains proliferation of IL-6-dependent mouse and human myeloma cell lines (Moore et al., 1996c; Nicholas et al., 1997b;
Neipel et al., 1998). Similar to human IL-6, vIL-6 activates STAT1, STAT3 and Jak1 phosphorylation in HepG2 hepatoma cells (Molden et al., 1997). There may be subtle differences between human IL-6 and vIL-6 with regard to their use of the alpha (gp80) and beta (gp130) chains of the IL-6 receptor complex on human and murine cells (Molden et al., 1997; Nicholas et al., 1997b). vIL-6 is secreted by PEL cells infected by KSHV and expressed by CD20+ B-cells in lymphoid tissues from KSHV-infected patients, but not in KS tissues, suggesting that vIL-6 may contribute to the proliferation of haematopoietic but not endothelial cells (Moore et al., 1996c).

The expression of vIL-6 in infected B-cells is reminiscent of the induction of human IL-6 by EBV in infected B-cells (Tosato et al., 1990). However, whether or not vIL-6 contributes to the growth of B-cells other than plasma cells is presently unknown.

**ORFs K4, K4.1 and K6 (chemokine homologues)**

KSHV encodes three proteins with significant protein sequence similarity to cellular CC chemokines (Moore et al., 1996c; Russo et al., 1996; Neipel et al., 1997a; Nicholas et al., 1997b). The protein encoded by orfK6 (vMIP-I, vMIP-1A) has 37-9% amino acid identity with human MIP-1x and interacts with the CCR5 chemokine receptor to block the entry of CCR5-dependent primary HIV-1 strains (Moore et al., 1996c). The orfK4 protein (vMIP-II, vMIP-1B) is about 40% identical to human MIP-1x, inhibits CCR3-dependent entry of dual-tropic HIV-1 strains, and induces chemotaxis of human eosinophils by way of CCR3 (Boshoff et al., 1997). In another study (Kledal et al., 1997), vMIP-II was also reported to bind with high affinity to several chemokine receptors, including CCR1, CCR2, CXC4, CCR5 and the cytomegalovirus-encoded US28 receptor. The same group reported the inhibition of chemokine-mediated calcium mobilization and monocyte chemotaxis, as well as inhibition of HIV-1 entry (mainly CCR3-mediated, but also CXC4- and CCR5-mediated), by vMIP-II (Kledal et al., 1997). vMIP-I and vMIP-II are more homologous to each other than to any known cellular chemokine, suggesting that they may have arisen in the virus by genomic duplication (Moore et al., 1996c). Both vMIP-I and vMIP-II induce angiogenesis in the chorioallantoic assay, which could suggest a possible role in the neangiogenesis characteristic of KS lesions (Boshoff et al., 1997). In KS tumours, both vMIP-I and vMIP-II appear to be expressed only in a few lyrically infected cells, but not in the majority of persistently infected KS spindle cells (Stürzl et al., 1997b; Boshoff et al., 1997). In latently infected PEL cell lines there is some basal expression of vMIP-I and vMIP-II, which can be further induced by treatment with phorbol esters (Moore et al., 1996c). Whether vMIP-I and vMIP-II therefore play a paracrine role in the development of KS spindle cells, or whether their role is confined to promoting neangiogenesis or modulating the infiltration of macrophages and eosinophils, remains to be investigated.

A third viral gene, orf4.1, encodes a protein (vBCK) related to MIP-1β and macrophage chemoattractant protein (Neipel et al., 1997a, b; Nicholas et al., 1997a, b).

Adjacent to those for the chemokine homologues are the genes for two LAP/PhD zinc-finger proteins, IE-1A (orfK5) and IE1-B (orfK3) (Nicholas et al., 1997a).

**T1.1/nut-1 RNA**

Like some other herpesviruses, KSHV encodes a nuclear RNA which does not appear to encode any protein (Zhong et al., 1996; Sun et al., 1996). The T1.1/nut-1 RNA is polyadenylated, lacks a trimethylguanosine cap, is transcribed by RNA polymerase II and can associate with small ribonuclear proteins (Sun et al., 1996; Zhong & Ganem, 1997). In KS tumours it is expressed in a few tumour cells undergoing lytic replication (Staskus et al., 1997) and is inducible in PEL cell lines in a pattern corresponding to an immediate early or delayed early transcript (Sun et al., 1996; Zhong & Ganem, 1997). In this respect it differs from non-coding nuclear RNAs encoded by EBV (EBERs), HVS (HSURs) and herpes simplex virus (HSV) (LATs), which are all expressed during latency (and at reduced levels during lytic replication). T1.1/nut-1 contains regions which are highly homologous or complementary to U1 and U12 small nuclear RNA and may therefore be involved in the control of splicing (Sun et al., 1996; Zhong & Ganem, 1997).

**orf16 (Bcl-2)**

The KSHV-encoded Bcl-2 (orf16) shares 15–20% amino acid identity to cellular members of the Bcl-2 family, including Bcl-2, Bcl-XL, Bak, Bax and other viral Bcl-2 homologues such as EBV BHRF1 and HVS orf16 (Russo et al., 1996; Neipel et al., 1997a; Cheng et al., 1997; Sarid et al., 1997). Homology is concentrated in the characteristic BH (Bcl homology) 1 and BH2 domains, required for homo/heterodimerization, but poor in the BH3 domain (Cheng et al., 1997). Functional studies indicate that Bcl-2 prevents Bax-mediated toxicity or apoptosis in yeast, in transfected fibroblasts, and in Sindbis virus-infected cells (Cheng et al., 1997; Sarid et al., 1997). No homodimerization of vBcl-2 or heterodimer formation of vBcl-2 with human Bcl-2 family members (Bcl-2, Bcl-XL, Bak and Bax) has been observed in mammalian cells by co-immunoprecipitation (Cheng et al., 1997), whereas yeast two-hybrid experiments (Sarid et al., 1997) indicated heterodimer formation with human Bcl-2. vBcl-2, like its EBV homologue BHRF1 (Murray et al., 1996), appears to be primarily expressed during lytic virus replication (Cheng et al., 1997; Stürzl et al., 1997b). Although transcription of this gene can be detected in KS lesions by RT–PCR (Sarid et al., 1997), this is likely to have occurred in a subpopulation of cells undergoing active lytic virus replication (Staskus et al., 1997). Viral Bcl-2 homologues are commonly thought to be primarily active during lytic replication, during which time the cell may be particularly prone to apoptotic...
responses (Teodoro & Branton, 1997), and the expression pattern of KSHV vBcl-2 thus suggests that it may function to prolong the survival of lytically infected cells.

orfK9 (vIRF)

This ORF encodes the homologues of an IRF (Moore et al., 1996c). Members of the IRF family are involved in interferon signal transduction and act either as transcriptional activators or repressors induced or activated by class I interferon receptor signalling (reviewed in Taniguchi, 1995). Transfection of vIRF into 293 and HeLa cells inhibits interferon-induced gene transcription as measured in a reporter assay, and may thus mimic the role of IRF-2, a negative regulator of interferon-mediated signalling (Gao et al., 1997). vIRF appears not to be expressed in KS tissue, at least as judged by Northern blot (Gao et al., 1997). It is expressed at a low level in BCBL/PEL cell lines, where its expression is markedly enhanced after induction of the lytic cycle (Moore et al., 1996c). vIRF may therefore be mainly expressed during lytic replication and it is conceivable, although not proven, that it contributes to the survival of lytically infected cells, by opposing interferon-mediated host response mechanisms. However, transfection of vIRF/K9 into 3T3 cells results in transformation and tumour formation in nude mice (Gao et al., 1997), and an involvement of vIRF in the pathogenesis of KSHV-associated neoplasia is therefore still under investigation. Three other potential ORFs with homologies to mammalian IRFs are located in the same ‘non-conserved’ gene block, D2 (Fig. 1). Their functions are not yet known.

orfK12

This ORF is predicted to encode a small 60 aa hydrophobic protein, ‘kaposin’ (Zhong et al., 1996; Russo et al., 1996, Neipel et al., 1997a). It is translated from a 0.7 kb mRNA which is abundantly expressed in persistently infected PEL cell lines and KS spindle (endothelial tumour) cells (Renne et al., 1996a; Zhong et al., 1996; Staskus et al., 1997; Stürzl et al., 1997a). Its function is still unknown.

orfK13/orf71 (vFlip)

orfK13 has only little sequence homology to orf71 of HVS and was therefore regarded as a ‘unique’ KSHV gene by one group (Russo et al., 1996), but designated orf71 by another (Neipel et al., 1997a, b; Thome et al., 1997). KSHV K13, the HVS orf71 product, the corresponding E8 protein of equine herpesvirus-2 (E8-2) and the Molluscum contagiosum virus MC159 protein contain two domains with significant homology to mammalian ‘death effector domains’ (DEDs) (Thome et al., 1997; Bertin et al., 1997). DEDs are characteristic for the cytoplasmic adaptor molecule FADD (MORT1) and the N-terminal prodomain of FLICE (caspase 8). They mediate the interaction between these two proteins during apoptosis triggered by activation of the Fas/C95 or TNFR1 pathway. EHV-2 (E8) protein acts as a dominant negative inhibitor of Fas/CD95-mediated apoptotic signalling and hence has been given the name FLICE-inhibitor protein (vFLIP) (Thome et al., 1997; Bertin et al., 1997). No functional studies of KSHV K13/orf71 have yet been reported, but the presence of DEDs suggests a similar role. HVS orf71 is expressed during lytic replication and, like vBcl-2, has been hypothesized to protect lytically infected cells against apoptosis (Thome et al., 1997). KSHV K13/orf71 is encoded on a bi-cistronic 2.2 kb mRNA which also encodes orf72, the viral homologue of a D-type cyclin (see below) (Rainbow et al., 1997; Sarid et al., 1998; Kellam et al., 1997). Unlike vBcl-2, this mRNA is expressed in latently infected PEL cell lines and KS spindle cells (Stürzl et al., 1997b; Rainbow et al., 1997; Kellam et al., 1997; Sarid et al., 1998). It is therefore conceivable, but not yet established, that KSHV vFLIP (orfK13/orf71) may play a role in latently infected KS spindle and B-lymphoma cells.

orf72 (v-cyclin)

Interfering with the control of the G1 checkpoint of the cell cycle, mediated by D-type cyclins and the retinoblastoma protein pRb, is a common theme for many tumour viruses (for a review see Jansen-Dürr, 1996). Among rhadinoviruses (γ2-herpesviruses) some [HVS, KSHV, herpesvirus ateses (HVA)], but not others [alcephaline herpesvirus-1 (AHV-1), BHV-4, EHV-2] possess homologues to D-type cyclins (Casarman et al., 1996b; Nicholas et al., 1992; Albrecht et al., 1992; Telford et al., 1995; Lomonte et al., 1995; Neipel et al., 1997b) (Fig. 1). EBV, a γ1-herpesvirus, does not have a cyclin homologue, but induces the expression of the human D2 cyclin via its EBNA2 and EBNA-LP proteins (Sinclair et al., 1994, 1995).

The KSHV v-cyclin (orf72) has 27% amino acid identity with human cyclin D2 (Casarman et al., 1996b). The typical ‘cyclin box’ sequence of cellular cyclins, involved in their interaction with cyclin-dependent kinases (CDKs), is conserved in KSHV v-cyclin (Chang et al., 1996b). The KSHV v-cyclin interacts with CDK6 and mediates phosphorylation on authentic sites of the retinoblastoma protein pRb, the physiological target of D-type cyclin–CDK complexes (Chang et al., 1996b; Godden-Kent et al., 1997; Li et al., 1997; Swanton et al., 1997). In addition, the v-cyclin–CDK6 complex can induce phosphorylation of H1 histones, suggesting that it may be active in other phases of the cell cycle in addition to G1 (Godden-Kent et al., 1997; Li et al., 1997). Unlike the cellular D-type cyclin–CDK complexes, the complex of KSHV v-cyclin and CDK6 is not inhibited by the CDK inhibitors p16\(^{ink}\), p21\(^{cip1}\) and p27\(^{kip1}\) (Swanton et al., 1997). The functionality of KSHV v-cyclin has also been demonstrated by co-transfection with pRb into SAOS-2 cells which have homozygous deletions of both pRb and p53 (Chang et al., 1996b). In this assay, KSHV v-cyclin prevents the pRb-mediated senescence of SAOS-2 cells and induces continuous proliferation.
(Chang et al., 1996b). Furthermore, the expression of KSHV v-cyclin in quiescent fibroblasts stimulates cell-cycle progression from G₁ to S phase (Swanton et al., 1997). At present there is no evidence that the KSHV v-cyclin has transforming properties on its own.

KSHV v-cyclin is one of the few viral proteins presently known to be expressed in KS spindle (endothelial tumour) cells and latently infected PEL cell lines (Davis et al., 1997b; Stürzl et al., 1997b; Sarid et al., 1998).

orf73 (LNA)

Several rhadinoviruses (e.g. KSHV, AHV-1, HVS, BHV-4, MHV-68), but not all (e.g. EHV-2), contain a positional homologue of orf73 (Albrecht et al., 1992; Cesarman et al., 1996b; Russo et al., 1996; Neipel et al., 1997a; A. Ensser, personal communication). KSHV orf73 encodes the high molecular mass (224–234 kDa) latent nuclear protein, LNA (‘latent nuclear antigen’; Rainbow et al., 1997; Kellam et al., 1997), which had initially been identified with patient sera on Western blots of nuclear extracts of the KSHV/EBV dually infected HBL-6 cell line (Gao et al., 1996a). Staining of PEL cell lines with orf73/LNA-specific antisera (Rainbow et al., 1997), as well as transfection of orf73 expression vectors into mammalian cells (Rainbow et al., 1997; Kellam et al., 1997) produces a nuclear ‘speckled’ staining pattern characteristic of the previously described ‘latency-associated nuclear antigen’ (‘LANA’; Kedes et al., 1996; Gao et al., 1996b). The nuclear protein encoded by orf73, LNA, is therefore at least part of, if not identical to, LANA.

orf73 is also found in the HVS genome, but the sequence homology between the HVS and KSHV proteins is low. KSHV LNA/orf73 has a long acidic repeat region (the m.o.i. repeat) containing a leucine-zipper motif at its C-terminal end (Russo et al., 1996). This acidic repeat region, possibly combined with post-translational modifications, may account for its high mobility on polyacrylamide gels (Gao et al., 1996b; Rainbow et al., 1997). The length of the m.o.i. repeat varies from isolate to isolate, resulting in size variation of the orf73 protein among different isolates (Rainbow et al., 1997). The orf73 protein is translated from a 6 kb latent transcript which also includes the reading frames for orfK13 (vFLIP) and orf72 (v-cyclin) and is expressed in KS spindle cells (Rainbow et al., 1997). However, it is likely that the latter two proteins are translated from an alternatively spliced 2·2 kb mRNA which excludes orf73 (Sarid et al., 1998). In PEL cells and KS spindle cells the orf73 protein appears to be localized in intranuclear bodies (Gao et al., 1996b; Rainbow et al., 1997; Kellam et al., 1997), the identity of which has not yet been identified. Some of the features of orf73 protein (acidic nature, doublet pattern on polyacrylamide gels, localization in nuclear bodies) are reminiscent of other herpesvirus transactivator proteins (e.g. HSV ICP4, ICP0; cytomegalovirus IE1, IE2; EBNA-LP), but its function is still unknown.

orf74 (G-protein-coupled receptor, vGCR)

Proteins with significant similarity to GCRs are present in several γ herpesviruses (KSHV, HVS, HVA, AHV-1, EHV-2, MHV-68) (Albrecht et al., 1992; Cesarman et al., 1996b; Russo et al., 1996; Neipel et al., 1997a; A. Ensser, personal communication), but also in some betaherpesviruses (Neote et al., 1993; Gompels et al., 1995). In KSHV and HVS, the orf74 gene has high sequence homology to IL-8R (Albrecht et al., 1992; Cesarman et al., 1996b). Transient expression studies of the KSHV vGCR indicate that it binds to several CXC and CC chemokines and that it appears to be constitutively active (Arvanitakis et al., 1997). Transfection of the KSHV GCR into rat kidney fibroblast (NRK-49F) cells enhances their proliferation. vGCR has also been shown to transform cells to tumorigenicity in nude mice and to induce secretion of the vascular endothelial growth factor in these cells (Bais et al., 1998). These findings suggest that it may contribute to virus-induced tumour formation. However, vGCR was found to be expressed in a latently infected BCBL-1 cell line only after induction with TPA (Sarid et al., 1998). Expression of vGCR in KS lesions has been detected by RT–PCR (Cesarman et al., 1996b), but since both lytically and latently infected cells are found in these tumours (Staskus et al., 1997; Stürzl et al., 1997a; Orenstein et al., 1997), it is still unclear whether vGCR is expressed in the endothelial tumour (spindle) cells and whether it thus contributes to their proliferation.

Tropism and KSHV gene expression patterns in infected cells

KSHV has been detected in endothelial and spindle cells of KS lesions, in circulating endothelial cells, B-cells, CD8+ T-cells, macrophages and prostatic glandular epithelium (Boshoff et al., 1995b; Ambroziak et al., 1995; Li et al., 1996; Corbellino et al., 1996b; Harrington et al., 1996; Messi et al., 1996; Staskus et al., 1997; Stürzl et al., 1997a, b; Sirianni et al., 1997). PCR in situ hybridization experiments first showed that KSHV can infect the endothelial cells lining ectatic vascular spaces in KS lesions and spindle cells of fully developed, nodular KS lesions, but is not found in normal endothelial cells (Boshoff et al., 1995b; Li et al., 1996; Foreman et al., 1997b). More recently, in situ hybridization (Staskus et al., 1997; Stürzl et al., 1997a, b; Davis et al., 1997b) and LANA immunohistochemistry
(Rainbow et al., 1997) have confirmed KSHV gene expression in KS spindle cells within KS tumours.

The presence of circular KSHV genomes in BCBL cells (Russo et al., 1996) and KS tissue (Decker et al., 1996) suggests that the majority of cells in these tissues are latently (persistently) infected with KSHV. Only a limited repertoire of mRNA of as yet unknown coding potential located in the conserved gene block B have been identified immediately upstream of orf73 (Rainbow et al., 1996b; Zhong et al., 1997, Stürzl et al., 1997a, b; Davis et al., 1997b). This group of genes comprises the 0.7 kb mRNA for orfK12 (Renne et al., 1996b; Zhong et al., 1996; Staskus et al., 1997), the 2.2 kb mRNA for v-cyclin (orf72) and vFLIP (orfK13) (Stürzl et al., 1997b; Davis et al., 1997b). This group of genes includes a non-monocistronic unspliced and spliced mRNAs are encoded in the 'non-conserved' gene block E (Fig. 1; Nicholas et al., 1998). A putative enhancer/promoter has been identified immediately upstream of orf73/LANA (Nicholas et al., 1998; Sarid et al., 1998). From that of orfK12, the expression of these genes in BCBL cell lines is not upregulated by TPA or sodium butyrate treatment (Sarid et al., 1998).

A second class of genes is expressed in unstimulated PEL cell lines at a low level and can be further upregulated by phorbol esters or butyrate. This group of genes includes a non-translated nuclear RNA T1.1, vIRF (orfK9), MIP-1/MIP-1A, MIP-II/MIP-1B, vIL-6 (Moore et al., 1996c; Zhong et al., 1996). Several putative promoters for genes located in non-conserved gene block B have been identified and both monocistronic unspliced and spliced mRNAs are encoded in this region (Nicholas et al., 1998). Genes encoding structural proteins are not expressed in unstimulated PEL cells but are inducible and therefore grouped into a third class of 'lytic' genes. In KS tissue, only a small population of cells express inducible genes and these are believed to correspond to lytically infected cells (Staskus et al., 1997; Stürzl et al., 1997a, b). Some of these productively infected cells have the appearance of spindle cells (Staskus et al., 1997), and KSHV virions have been seen by EM in KS lesions (Walter et al., 1984; Ioachim, 1995; Orenstein et al., 1997). By immunohistochemistry, PEL cells and KSHV-infected B-cells in lymphatic tissue, but not KS spindle cells, express vIL-6, an inducible gene (Moore et al., 1996c). There are thus some well-documented differences in gene expression between KS spindle cells and KSHV infected B-cells and further differences are likely to be found in the future.

The expression of lytic KSHV genes has also been documented in monocytes/macrophages (Blasig et al., 1997). In addition, the presence of linear KSHV genomes in PBMC, suggesting the presence of productively infected cells, has also been reported (Decker et al., 1996).

Serological assays for KSHV

A variety of serological assays, detecting antibodies to both latent and lytic KSHV antigens, have been developed (Table 1). The most prominent latent serological antigen appears to be the high molecular mass (224–236 kDa) nuclear protein, LNA (Gao et al., 1996a), encoded by orf73 (Rainbow et al., 1997; Kellam et al., 1997). Antibodies to this latent nuclear antigen can be detected by Western blot (Gao et al., 1996a) or immunofluorescence assay (IFA) on unstimulated PEL cell lines (Kedes et al., 1996; Gao et al., 1996b; Simpson et al., 1996). LNA/orf73 appears to be the main component of the speckled nuclear immunofluorescence pattern ('LANA') obtained with serum from KSHV-infected individuals (Kedes et al., 1996; Gao et al., 1996b; Simpson et al., 1996; Rainbow et al., 1997; Kellam et al., 1997). Whether, as in the case of the EBV-encoded EBNA, other nuclear KSHV antigens contribute to 'LANA', is not yet clear (Rainbow et al., 1997; Kellam et al., 1997).

The sensitivity and specificity of LANA IFA and LNA Western blot are high, with antibodies being detected in 80–85% of patients with AIDS-associated KS, > 90% of those with 'classic' (HIV-negative) KS, and in only 0–3% of US or UK blood donors (Kedes et al., 1996; Gao et al., 1996a, b; Simpson et al., 1996; Lennette et al., 1996). There is no homologue of orf73/LNA in EBV, eliminating the possibility of antigenic cross-reactivity with this very prevalent gamma-herpesvirus.

To address the possibility that the distribution of antibodies to this latent antigen may underestimate the true prevalence of KSHV infection, intensive efforts have been made to devise assays based on 'lytic' (structural) viral proteins which avoid any cross-reactivity with homologous proteins in other herpesviruses, in particular EBV. Treatment of PEL-derived cell lines with phorbol esters or butyrate induces the expression of lytic viral genes (see above) and allows the detection of antibodies to such structural proteins by immunofluorescence (Miller et al., 1996, 1997; Lennette et al., 1996; Smith et al., 1997; Ablashi et al., 1997; O’Neill et al., 1997; Chandran et al., 1998). Depending on the experimental conditions used (serum dilution, counterstain with Evans’ blue to reduce background staining), these assays have been reported to detect antibodies in between 0% (Smith et al., 1997) and 25% (Lennette et al., 1996) of US blood donors. In view of the high homology of some KSHV structural proteins with their counterparts in EBV (e.g. the major capsid protein orf25, which is about 50% identical to EBV BcLF1; Moore et al., 1996a), and the documented example of serological cross-reactivity between KSHV major capsid protein and EBV (André et al., 1997), the specificity of these assays is still under investigation. Using low serum dilutions (< 1:40) may increase the potential for cross-reactivity (Smith et al., 1997).

Antibodies to several lytic antigens have also been detected by Western blot or radioimmunoprecipitation of chemically
Table 1. Detection of antibodies to KSHV by different serological assays

<table>
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<tr>
<th>Antigen</th>
<th>Assay*</th>
<th>Classic KS</th>
<th>AIDS KS</th>
<th>Homosexual men</th>
<th>Haemophilia</th>
<th>Blood donors</th>
<th>Other control groups</th>
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<td>'LANA' (latency-associated nuclear antigen)</td>
<td>IFA</td>
<td>85–94</td>
<td>71–88</td>
<td>18–30</td>
<td>0–3 (UK/USA)</td>
<td>0–3 (UK/USA)</td>
<td>12–21 (Greece)</td>
<td>Gao et al. (1996a, b)</td>
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<td>'LNA' (226/234 kDa nuclear protein)</td>
<td>WB</td>
<td>100</td>
<td>80</td>
<td>18</td>
<td>0 (USA)</td>
<td>62 (Uganda)</td>
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<td><strong>Structural (‘lytic’) antigens</strong></td>
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<td>40 kDa protein</td>
<td>WB</td>
<td>67</td>
<td>13</td>
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<td>vp19 (orf65)</td>
<td>ELISA/WB</td>
<td>86–94</td>
<td>75–84</td>
<td>31</td>
<td>2 (UK)</td>
<td>1:7–5 (UK/USA)</td>
<td>12 (Greece)</td>
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<td>vp23 (orf26)</td>
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<td>Undefined</td>
<td>IFA</td>
<td>90–100</td>
<td>90–100</td>
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<td>20–25 (USA)</td>
<td>32–100 (Africa)</td>
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<td>ELISA/IFA</td>
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* IFA, immunofluorescence assay; WB, Western blot.

induced PEL cell lines (Miller et al., 1996, 1997; Smith et al., 1997; Chandran et al., 1998). Prominent structural proteins, which are reactive with patient sera, include glycoproteins of 116, 110, 90, 70, 55 and 50 kDa, as well as other bands of 155, 105, 38, 40, 29 and 23 kDa (Miller et al., 1996, 1997; Smith et al., 1997; Chandran et al., 1998). KSHV virions can be purified from such chemically induced cell lines and have been used successfully in an ELISA to detect antibodies to KSHV (Whitman et al., 1997).

Several groups have generated defined recombinant structural antigens. A capsid-related protein, vp19, encoded by orf65, detects antibodies in 75–80% of AIDS KS and 85–90% of ‘classic’ KS sera, in approximately 1–3% of UK blood donors, but a slightly higher rate (5%) in US blood donors than ‘LANA’ immunofluorescence (Simpson et al., 1996). Assays based on a minor capsid protein, encoded by orf26 (a recombinant protein or peptides) have been reported to detect antibodies in approximately 40–60% of KS sera and 20% of blood donor sera (André et al., 1997; Davis et al., 1997a). More recently, a recombinant protein based on orfK1 has been reported to be highly reactive with patient sera but not with blood donor sera and may provide another recombinant
antigen with high sensitivity and specificity (Neipel et al., 1998; Chandran et al., 1998).

Epidemiology of KSHV

The detection of KSHV genomes by both PCR and serology has been used to define the epidemiology and transmission of KSHV. As a result of the different assays used (see above) and since only a relatively limited number of studies have been carried out so far, the exact prevalence rates are still uncertain. However, most groups agree that the prevalence of KSHV varies widely in different countries and geographical regions, being lower in Northern Europe and the US than in Southern Europe, and highest (so far) in several parts of Africa.

As outlined above, prevalence rates among UK or US blood donors for antibodies to LANA range from 0 to 3%, for antibodies to orf65/vp19 from 3 to 5%, and for antibodies to undefined structural antigens (IFA) from 0 to 25%. In Italy and Greece, 4–35% of control subjects or blood donors have been found to have antibodies to LANA, and approximately 10–25% have antibodies to orf65/vp19, with some regional variation within Italy (Simpson et al., 1996; Gao et al., 1996b; Calabrò et al., 1998; Whitby et al., 1998). In Africa, antibodies to LANA have been reported in 6–53% of tested sera (Lennette et al., 1996; Gao et al., 1996b; Simpson et al., 1996; Ariyoshi et al., 1998) with considerable regional variation. Antibody prevalences for orf65/vp19 range from 35% to approximately 60%, and for lytic IFA antigens from 34% to higher than 80% (Lennette et al., 1996; Gao et al., 1996b; Simpson et al., 1996; Ariyoshi et al., 1998; Mayama et al., 1998). Very limited studies from a few Central American and Caribbean countries found 0–12% prevalence of antibodies to LANA, and 10–29% of antibodies to lytic IFA antigens (Lennette et al., 1996; Goedert et al., 1997). In Thailand, KSHV appears also to be uncommon, with a 0–5% antibody prevalence rate for LANA and 2% for orf65/vp19 (Jaromanokul et al., 1998).

PCR studies on PBMC, samples from lymphoid tissue, and semen samples from healthy semen donors support this conclusion. With few exceptions (Decker et al., 1996), KSHV genomes have not been detected by PCR in PBMC samples from UK, US or French healthy control individuals (Whitby et al., 1995; Moore et al., 1996b; Lefrere et al., 1996). They have, however, been detected in the lymphoid tissue from approximately 10% of healthy Italian subjects (Bigoni et al., 1996; Viviano et al., 1997), in 10% of PBMC from HIV-negative healthy individuals in The Gambia (Ariyoshi et al., 1998), in 8% of febrile children in Zambia (Kasolo et al., 1997) and in 14% of control tumour tissues from Ugandan patients (Chang et al., 1996a). Similarly, semen samples from healthy US, UK, French and some Italian donors did not contain detectable KSHV (Howard et al., 1997; Lebbé et al., 1997b; Gupta et al., 1996; Corbellino et al., 1996a, b), with one exception (Lin et al., 1995), whereas KSHV was detected in 13–23% of Italian semen donors from areas where classic KS appears more frequently (Monini et al., 1996a, b; Viviano et al., 1997; reviewed in Blackbourn & Levy, 1997).

The higher prevalence of KSHV in some Mediterranean countries compared to Northern Europe and North America is broadly in agreement with the higher incidence of classic KS in some Southern European countries (Geddes et al., 1994, 1995; see also Beral, 1991). It appears that, within Italy, regions of higher KSHV seroprevalence, such as the South, Sicily, Sardinia and the lower end of the Po valley in Northeast Italy, are the same as those which have previously been noted to have a higher incidence of ‘classic’ KS (Calabrò et al., 1998; Whitby et al., 1998). However, while KSHV appears to be widespread in Africa (Lennette et al., 1996; Gao et al., 1996b; Simpson et al., 1996; Ariyoshi et al., 1998; Wilkinson et al., 1998), endemic KS is markedly more common in East and Central Africa than in the rest of the continent (Gompels & Kasolo, 1996). In The Gambia, West Africa, where KSHV seroprevalence in antenatal HIV-negative mothers is approximately 60%, endemic KS is rare and HIV-associated KS is virtually limited to individuals infected with HIV-1, but uncommon among those infected with HIV-2 (Ariyoshi et al., 1998). This observation would support a possible co-factor role for HIV-1, as postulated on the basis of the angiogenic properties of the HIV-1 Tat protein (Ensioli et al., 1994; Albini et al., 1996). Other explanations, such as the existence of different KSHV strains or other environmental co-factors, are also possible.

Among HIV-infected individuals from the US and Northern Europe, KSHV antibodies are found significantly more frequently in homosexual men (LANA, 22–35%; orf65/vp19, 31%; lytic IFA, 90%) than in other risk groups for HIV transmission, such as patients with haemophilia (LANA, 0–3%; orf65/vp19, 0%) or intravenous drug users (LANA, 0%; orf65/vp19, 5%; lytic IFA, 23%) (Kedes et al., 1996; Gao et al., 1996b; Simpson et al., 1996; Lennette et al., 1996). In Italy there is a similar difference between homosexual men (60%) and other HIV risk groups, although the prevalence in the latter is higher in Italy than in the UK or US, in keeping with the higher prevalence of KSHV in Italy (Calabrò et al., 1998; Whitby et al., 1998). This distribution of KSHV antibodies in HIV risk groups mirrors the previously reported high incidence of KS among HIV-infected homosexual men and the rarity of KS in HIV-infected patients with haemophilia or UK/US intravenous drug users (Beral et al., 1990; Beral, 1991; Peterman et al., 1993) and thus represents an important argument for KSHV being the infectious cause of KS.

Several studies have found evidence that KSHV can be sexually transmitted. Antibodies to KSHV antigens are more frequently detected in STD clinic attenders (Kedes et al., 1996, 1997; Simpson et al., 1996; Lennette et al., 1996). Among Danish homosexual men, the presence of antibodies and/or seroconversion to KSHV is associated with promiscuity, duration of homosexual activity, receptive anal intercourse,
and, in the early 1980s, sexual contact with US homosexual men (Melbye et al., 1998). Among US homosexual men, KSHV seroprevalence was strongly correlated with promiscuity and a history of STDs (Martin et al., 1998). Since these behavioural risk factors have previously been shown to increase the risk of KS developing in homosexual men (Beral et al., 1990) this observation also supports the notion that KSHV is indeed the 'KS agent'. The detection of KSHV in seminal fluid and prostates of KS patients (Monini et al., 1996a, b; Howard et al., 1997; Gupta et al., 1996; Corbellino et al., 1996a, b), and occasionally in healthy men (Monini et al., 1996a, b; Viviano et al., 1997; Staskus et al., 1997), provides a possible explanation for the sexual transmission observed in seroepidemiological studies.

However, antibodies to both LANA and orf65/vp19 have been detected in young children, aged 2–12 years old, from East and South Africa (Mayama et al., 1998; Wilkinson et al., 1998) and Italy (Calabrò et al., 1998). KSHV has also been detected by PCR in PBMC of 8% of young children in Zambia (Kasolo et al., 1997). Antibody rates increase with age in African children below the age of puberty (Mayama et al., 1998). In this study, the presence of antibodies to KSHV LANA and orf65 protein was associated with hepatitis B virus infection. This suggests that childhood infection with KSHV in Africa may follow a horizontal pattern and be facilitated by the same factors which determine the transmission of hepatitis B virus in childhood (Mayama et al., 1998). Non-sexual routes of transmission are therefore possible and may be more important in KSHV endemic areas (Mayama et al., 1998; Wilkinson et al., 1998). In contrast, at least as judged by PCR and antibodies to LANA and orf65/vp19, KSHV infection appears to occur mainly after puberty in US children (Blauvelt et al., 1997; Lennette et al., 1996). Some younger US children have been reported to have antibodies to undefined lytic IFA antigens (Lennette et al., 1996), the specificity of which remains to be established (see above). Occasional KSHV infection may, however, occur among children in countries of a high socio-economic living standard, and the case of a German HIV-negative 3-year-old boy with KSHV infection has recently been described (Kusenbach et al., 1997).

The mode of transmission in children is not yet known. KSHV has been detected in saliva by Boldough et al. (1995) and Koelle et al. (1997), but not other groups (Ambroziak et al., 1995; Whitby et al., 1995). KSHV from saliva samples could be propagated in short term culture on 293 cells, suggesting that KSHV in saliva can be infectious (Vieira et al., 1997). In one study (Koelle et al., 1997), the KSHV DNA copy number of KSHV in saliva was less than that for EBV, but comparable to values obtained with HHV-6. KSHV has also been detected in oropharyngeal tissues (DiAlberti et al., 1997). These observations suggest that, as for other herpesviruses, horizontal transmission, possibly via saliva, may be important for the spread of KSHV among prepubescent children in endemic areas. However, more detailed and larger studies are required to establish this firmly. A case of KSHV transmission by blood transfusion has been published (Blackbourn et al., 1997), but the significance of this is uncertain in view of the high prevalence of KSHV among Italian blood donors (Calabrò et al., 1998; Whitby et al., 1998) and the fact that blood transfusion does not appear to represent a risk for AIDS-associated KS (Beral et al., 1990). At present it appears that most patients with post-transplant KS were already infected with KSHV prior to transplantation and that transmission of KSHV by the graft is an exception (Parravincini et al., 1997). However, no detailed studies are available to assess the risk associated with transplanting an organ from a KSHV-positive donor into a KSHV-negative recipient.

**Is KSHV the cause of KS and PEL?**

The findings discussed in this review strongly suggest a causative role for KSHV in the pathogenesis of KS. From an epidemiological point of view, the association of KSHV with KS is extremely strong, both by PCR detection and serology. Odds ratios for the association between KS and the detection of KSHV in PBMC, or antibodies to KSHV, in patients vs HIV-negative control subjects are high (see above, and Olsen & Moore, 1997, for a discussion of odds ratios for individual published studies). Detection by PCR of KSHV genomes in PBMC and seroconversion to KSHV antigens precedes the appearance of KS lesions (Whitby et al., 1995; Moore et al., 1996b; Gao et al., 1996a; Melbye et al., 1998). As predicted for the 'KS agent', transmission of KSHV among homo/bisexual men occurs sexually and is associated with certain behavioural variables. The presence of KSHV antibodies in HIV-1-infected homosexual men predicts their subsequent progression to KS (Martin et al., 1998). In countries, and regions within countries, where KS occurs more frequently, KSHV is also more common; however, the converse is not always the case and this could point to the existence of co-factors contributing to KS development.

From a biological point of view, KSHV is closely related to other oncogenic rhadinoviruses and encodes several proteins with the potential to affect cellular growth control. KSHV establishes a persistent infection in KS spindle cells, the neoplastic population in KS lesions, but some infected cells can proceed to lytic infection. The viral homologue of D-type cyclins is expressed in persistently infected spindle cells and can interfere with cell-cycle control, and other latent viral proteins may also play a growth-promoting role. Finally, two inhibitors of herpesvirus DNA polymerases appear to reduce the appearance of KS lesions in AIDS patients. Taken together, these data strongly suggest that KSHV is a new human tumour virus and the cause of KS.

From a biological point of view a very similar argument can be made for PEL, as the virus also establishes a latent infection in these lymphoma cells, and, in notable contrast to KS, is maintained in tumour cell lines established from these lesions.
However, many PEL patients are also infected with EBV, and, because of the rarity of this condition, no epidemiological studies to quantify the strength of the association between KSHV and PEL have yet been carried out. Therefore, while biological arguments suggest that KSHV may well be the cause of PEL, the epidemiological case is not yet as strong as for KS.

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