# Kaposi's Sarcoma and Kaposi's Sarcoma-associated Herpesvirus/Human Herpesvirus 8: An Overview

**Neil Renwick**[1] **, Thomas Schulz**[2] **, and Jaap Goudsmit**[1]

[1] *Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands*
[2] *Molecular Virology Group, Department of Medical Microbiology and Genitourinary Medicine, The University of Liverpool, L69 3GA Liverpool, United Kingdom*

Note: The text below refers to a more detailed "full text" version of this review which may be found on our Web site at `http://hiv-web.lanl.gov/`

## THE EPIDEMIOLOGY OF KAPOSI'S SARCOMA (KS)

Kaposi's sarcoma (KS) is a vascular tumor that was brought to the attention of the medical community over a century ago in a fascinating case series in which purple-coloured nodular skin lesions were observed on five elderly men, with widespread cutaneous and visceral involvement reported in one patient at autopsy (Kaposi, 1872 as cited in Ober, 1988). This 'Classic' variant of KS is rare and the majority of cases are found in elderly Mediterranean men and Jewish people born in Eastern Europe (Rothman, 1962). Large-scale epidemiological studies have highlighted three other variants of KS; 'African' or 'Endemic' KS in young black adults and children in equatorial Africa (Davies and Lothe, 1962; Oettle, 1962); 'Iatrogenic' or 'Posttransplant' KS in patients who have previously received immunosuppressive therapy or in those who are organ transplant recipients (Klepp *et al.*, 1978; Harwood *et al.*, 1979; Penn, 1979); and 'AIDS(-related)' or 'Epidemic' KS, first noted in immunocompromised homosexual men from New York and California (Friedman-Kien *et al.*, 1981). All four forms of KS have a predilection for males and the male:female ratio ranges from 2.3:1 in Iatrogenic KS to 106:1 in Epidemic KS and the clinical course of Epidemic/AIDS-KS is more aggressive (Friedman-Kien and Saltzman, 1990). The strikingly unusual pattern of KS distribution begs the question as to what features, if any, are common to the four epidemiological variants of KS.

A major advance in our comprehension of KS epidemiology came from studying the distribution of 13616 KS cases among 90990 persons with AIDS reported to the Centers for Disease Control, Atlanta, until March 31, 1989. Detection of KS in 21% of males who acquired HIV-1 through homosexual or bisexual contacts in comparison with <7% in all other HIV transmission groups such as heterosexuals of Caribbean, African and other origin, intravenous drug users, transfusion recipients and persons with haemophilia suggested that AIDS-KS might be a sexually transmitted infection (Beral *et al.*, 1990). Similar conclusions for the existence of a separate KS agent as the cause of Kaposi's sarcoma in the context of HIV-1 infection were drawn from a Canadian cohort study (Schechter *et al.*, 1991).

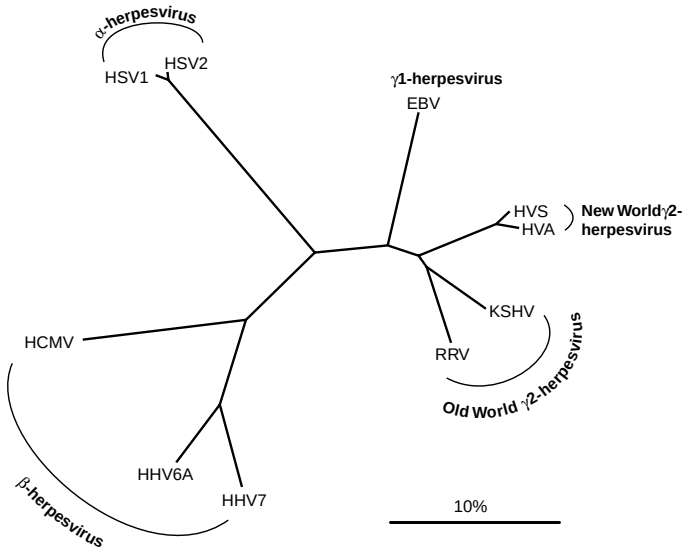
## KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV)

### Discovery and classification of KSHV in the rhadinovirus lineage of the gammaherpesvirinae

Given that there may be a separate KS agent, it was reasoned that the genomic difference between proposed infected (KS) and uninfected (skin) tissues from an individual patient with AIDS-KS should be that of the infectious agent. Two herpesvirus-like DNA sequences, named KS330Bam and KS631Bam, were discovered using a PCR-based technique, Representational Difference Analysis, that preferentially identifies these differences. It was possible to amplify the $KS330_{233}$ sequence by PCR from DNA extracted from 25/27 (93%) AIDS-KS tissues compared with 6/39 (15%) lymph nodes and lymphomas from AIDS patients without KS ($\chi^2 = 38.2$, $p < 10^{-6}$), suggesting that this Kaposi's sarcoma-associated herpesvirus (KSHV) might be the proposed KS agent (Chang *et al.*, 1994). The associations between KSHV (also known as Human Herpesvirus 8 (HHV8) for taxonomic purposes) and Kaposi's sarcoma among other diseases are discussed in more detail below.

Sequence analysis placed KSHV in the $gamma2$ (Rhadinovirus) lineage of the gammaherpesvirinae along with Herpesvirus Saimiri (HVS). The closest human relative is the $gamma1$ (Lymphocryptovirus) herpesvirus, Epstein-Barr Virus (EBV) (Moore *et al.*, 1996a). This finding is intriguing for the relationship that gammaherpesviruses have with lymphoproliferative disorders and cancer: HVS can induce malignant lymphomas in owl monkeys and marmosets (*Aotus* and *Saguinus sp*.) (Melendez *et al.*, 1969b) and EBV infection is linked to a fatal B-cell proliferation in young males with X-linked lymphoproliferative disease, posttransplant lymphomas, immunoblastic lymphomas in AIDS patients, Burkitt's Lymphoma, Nasopharyngeal Carcinoma and Hodgkin's Disease (reviewed in Rickinson and Kieff, 1996).

Rhadinoviruses are found in many species and several examples have been identified in non-human primates; HVS in *Saimiri sciureus* (Melendez *et al.*, 1968, 1969a), ateline herpesviruses (HVA) in *Ateles geoffroyi* (Melendez *et al.*, 1972), rhesus rhadinovirus (RRV) in macaques (Desrosiers *et al.*, 1997) and retroperitoneal fibromatosis herpesvirus in both *Macaca mulatta* (RFHVMm) and *Macaca nemestrina* (RFHVMn) (Rose *et al.*, 1997). Two distinct rhadinoviral sequences from African Green Monkeys have also been identified and these viruses are termed Chlorocebus rhadinovirus (ChRV) 1 and 2 (Greensill *et al.*, 2000). Phylogenetic analysis of DNA polymerase gene sequences from these primates show that there may be two distinct rhadinoviral lineages in Old World primates comprising respectively KSHV/RFHV/ChRV1 and RRV/ChRV2 (Fig.1).




DNA Neighbor-joining tree for a 2850-bp fragment from the DNA polymerase. Sequences were aligned with ClustalX (Thompson J. D. et al, *Nucleic Acids Res*. 1997 **25**(24):4876–82), gapstripped, and analyzed using the PHYLIP Neighbor program based on F84 model distances (PHYLIP version 3.5c; J. Felsenstein and the University of Washington).

Of these animal rhadinoviruses only the New World representatives (HVS, ateline herpesvirus) and RRV grow well in tissue culture and can therefore be used in animal models. HVS causes lymphomas in owl monkeys and marmosets (*Aotus* and *Saguinus sp*.; Melendez *et al.*, 1969b) and despite the close relationship to KSHV, RRV only causes benign lymphoproliferations in experimentally infected macaques (Wong *et al.*, 1999). RFHV has been detected in macaques suffering from retroperitoneal fibromatosis (RF) (Rose *et al.*, 1997). Currently this virus can not be grown in tissue culture and attempts to induce RF experimentally by inoculations with RFHV-containing macaque samples have not yet been successful (Bosch *et al.*,1999).

**The nucleotide sequence of KSHV provides insight into KS pathogenesis**

The genome of KSHV (based on two samples with a difference of 0.4%) has a 140.5-kb-long unique region (LUR) which is flanked by multiple 801bp terminal repeat sequences (Fig. 2). Within the LUR, 81 potential Open Reading Frames (ORFs) with more than 100 amino acids have been identified, and several additional spliced genes have since been added to this list. The overall G+C content in the LUR is 53.5% and 84.5% in the terminal repeat sequence. The numbering of KSHV ORFs is based on positional homologies with HVS due to substantial collinearity between these genomes whereas ORFs without positional homologues are numbered consecutively with a K prefix. These homologies are discussed in more detail in the full text version. The presence in the viral genome of open reading frames with significant homology to mammalian genes involved in cellular growth control suggests that 'molecular mimicry of cell cycle regulatory and signaling proteins is a prominent feature of this virus' (Russo *et al.*, 1996).

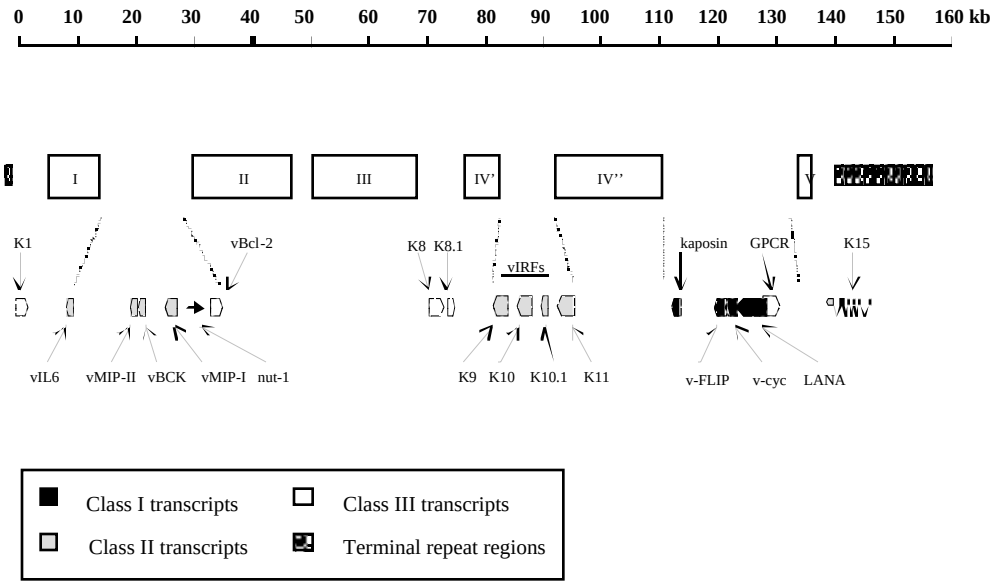



Figure 2: The KSHV genome is 140.5kb long and is flanked by multiple terminal repeat sequences which are depicted as hatched squares. Three classes of transcript are recognised (Sarid *et al.*, 1998); class I transcripts are constitutively expressed in PEL cells and these latent transcripts are coloured black, class II transcripts are expressed at low levels during latency but can be induced chemically and are shaded grey and class III transcripts are only present following chemical induction and are represented in white. It should be noted that the nut-1 transcript is not translated. Blocks of structural genes that are conserved between most gammaherpesviruses are labelled I-V. This diagram shows "non-conserved" genes that are discussed in the text and the map is not to scale.

**Tracing the origin of KSHV**

Comparison of protein sequences predicted from the genomes of Varicella-Zoster virus (VZV) and EBV suggests a common mammalian herpesviral ancestor (Davison and Taylor, 1987). Phylogenetic studies of alphaherpesvirinae genes and proteins using maximum parsimony and distance methods with evaluation by bootstrap analysis have shown that the molecular evolution of mammalian alphaherpesvirinae in the majority of cases parallels the evolution of their mammalian hosts (McGeogh and Cook, 1994). Based on the assumption of virus and host co-speciation over the same evolutionary timescale, similar phylogenetic analyses on common mammalian herpesviral ancestral genes such as uracil-DNA glycosylase imply that the differentiation of alpha, beta and gammaherpesvirinae occurred

between 180 and 220 million years ago and that major sublineages in the herpesvirinae were generated before the mammalian radiation of 60–80 million years ago with subsequent coevolution of virus and host (McGeogh *et al.*, 1995).

Analysis of the KSHV genome suggests that 42 genes are common to a set of genes that the mammalian herpesviral ancestor may have possessed. Eighteen genes are shared by the gammaherpesvirinae whereas 9 genes belong to the rhadinovirus lineage and 17 genes are specific to KSHV sublineage. There is also some preliminary phylogenetic evidence that the two Old World $gamma2$ herpesviruses KSHV and RRV separated from the New World HVS sublineage at the same time as the Old and New World primate separation approximately 35 million years ago and that KSHV and RRV sublineages separated with their hosts about 25 million years ago (McGeoch and Davison, 1999).

### Variation in ORFK1 sequences can be used to identify KSHV subtypes

The most variable region to date in the KSHV genome appears to be ORFK1 (refer full text version). Phylogenetic analyses using ORFK1 sequences obtained from different geographic regions, including 50 Classic and AIDS-KS and 10 Primary Effusion Lymphoma (PEL)/Body Cavity Based Lymphoma (BCBL) DNA samples, enabled the definition of four major subtypes, A–D (Nicholas *et al.*, 1998; Zong *et al.*, 1999). The ORFK1 sequences of these prototypes differed by 5.8–14.6% at the nucleotide level and 14–29% at the amino acid level and this sequence appears to be more reliable for examining strain variability. Interestingly almost 85% of the nucleotide differences led to amino acid subtitutions and the majority of these changes were concentrated in Variable Region 1 (aa 54–92) and Variable Region 2 (aa 199–227) for all subtypes with more extensive involvement of the amino terminus in subtype B. Minimal differences were seen in the putative transmembrane domain for all three subtypes whereas the amino acid sequence of the cytoplasmic tail for subtype A and C differed by 12/38 (32%) amino acids from subtype B. There is evidence that particular subtypes and variants are associated with particular geographic regions; A1, A4 and C3 variants were frequently seen in USA AIDS-KS cases whereas B subtypes predominated in African KS cases or people of African heritage and C subtypes were mostly found in Classic, Posttransplant and AIDS-KS cases from the Middle East and Asia. A rare D subtype was identified in patients of Pacific Island origin. This study states that if cospeciation of virus and host is assumed then the geographic distribution of KSHV may be the result of isolation and founder effects associated with the migration of human populations out of Africa over the past 35000–60000 years (Zong *et al.*, 1999).

Similar results were obtained by another study that determined complete (n=23) or near complete (n=25) ORFK1 sequences from 58 tumor and peripheral blood samples from patients with Classic KS, Posttransplant KS, AIDS-KS, lymphoproliferative disorders and asymptomatic KSHV infection (Cook *et al.*, 1999). Pair-wise comparisons of ORFK1 amino acid sequences from these samples varied from 0.4 to 44%. Phylogenetic analysis of 52 ORFK1 DNA and protein sequences (aa28–243) using distance, parsimony and maximum likelihood methods also distinguished A, B and C subtypes with the lowest bootstrap value of 87%. No samples from the proposed subtype D region were analysed in this study. This study narrows the definition of subtype variant however subtype B predominated in Africa while subtypes A and C were found more frequently in Europe. No correlation was noted between subtype and more aggressive KSHV-related disease or geographic regions (Cook *et al.*, 1999). Further evidence of strain variability has been provided and it has been noted that strains do not always correspond to the geographic origin of the sample (Meng *et al.*, 1999; Fouchard *et al.*, 2000).



## DETECTION OF KSHV AND SEROEPIDEMIOLOGY

Following the discovery of KSHV (Chang *et al.*, 1994), seroepidemiologic and nucleic acid-based assays were designed to study the geographic distribution of KSHV. These assays are discussed in greater detail on the website.

## Detection of KSHV

***Serological assays*** The demonstration of KSHV sequences in all forms of KS implies that antibodies to KSHV should be detectable in sera at the time of KS diagnosis and thereby serve as positive reference material (Olsen and Moore, 1998). Currently negative reference sera are chosen from a population that is at low risk for developing KS. Sensitivity and specificity data for assays can be calculated from these references and it is acknowledged that a low proportion of the negative group may have positive test results.

***Latent antigens*** Antibodies to a latent nuclear antigen (LNA or LANA) can be detected by Western Blot (WB) or immunofluoresence assays (IFA). Using AIDS-KS and Classic KS sera as positive reference material and blood donor sera from low prevalence countries such as the United States and the United Kingdom as a negative reference, both LANA WB and IFA formats have high sensitivity and specificity; KSHV antibodies are detected in 71–100% of AIDS-KS and Classic KS sera but only in 0–4% of blood donors (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996).

***Lytic antigens*** Lytic antigens have been identified to increase the sensitivity of KSHV immunoassays in order to estimate more accurately the prevalence of KSHV within populations and to avoid the problem that people may seroconvert to different antigens at different stages of KSHV infection (Goudsmit *et al.*, 2000). The reported sensitivity and specificity values for assays detecting antibodies to lytic antigens are generally high, but vary in different studies. Although these assays are suitable for seroepidemiology, they are not optimised for diagnostic use.

***PCR based studies*** The earliest study to detect KSHV by nested PCR in peripheral blood showed that 52% of patients with AIDS-KS were positive (Whitby *et al.*, 1995). Similarly, KSHV DNA was detected in 14–20% of semen samples from patients with AIDS-KS (Gupta *et al.*, 1996; Howard *et al.*, 1997). As all KS biopsies are infected with KSHV (Olsen and Moore, 1998), it seems that nested PCR may not be sensitive enough for more accurate determination of KSHV prevalence. The rate of KSHV detection (in PBMC) by PCR may also be dependent on the extent of clinical disease (Aluigi *et al.*, 1996, Brambilla *et al.*, 1996, Poggi *et al.*, 1997). Semiquantitative analysis of Southern blots showed higher levels of KSHV DNA in those patients with multicentric and visceral involvement than in localized disease (Mendez *et al.*, 1998). Despite these caveats, PCR studies have been invaluable in our understanding of the geographic and population distributions of KSHV, association of KSHV with disease, transmission of KSHV and the natural history of KSHV infection and are discussed alongside the seroepidemiology results in this review.

## Geographic distribution of KSHV

***Seroprevalence of KSHV*** Although there are a variety of KSHV serological tests and considerable inter-assay variability, it is possible to demonstrate that certain geographic regions have comparatively high or low KSHV seroprevalences (reviewed in Schulz, 1999). A more comprehensive discussion can be found in the full text version.

In Northern Europe, KSHV appears to be rare among the general population. In surveys in the UK, France, Switzerland, Denmark and Sweden, the prevalence ranges from 3–7%, also depending on the assay (Simpson *et al.*, 1996; Marcelin *et al.*, 1997; Melbye *et al.*, 1998; Regamey 1998a; Tedeschi *et al.*, 1999; Enbom *et al.*, 2000). KSHV seroprevalences rise dramatically in countries in Southern Europe. In Italy and Greece, prevalences of up to 35% are found among blood donors, and a marked regional variation has been noted (Calabro *et al.*, 1998; Whitby *et al.*, 1998). In blood donors in North America, there is also remarkable inter-study and inter-assay variation; antibodies to KSHV are found in 0–29% of the subjects sampled (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; 1997b; Lennette *et al.*, 1996; Simpson *et al.*, 1996; Chandran *et al.*, 1998; Chatlynne *et al.*, 1998; Rabkin *et al.*, 1998).

In Africa, antibodies to KSHV have been reported in 6–53% of tested samples such as HIV negative patients, antenatal mothers, hospitalized patients and unknown sources; regional variation is again noted (Gao *et al.*, 1996a,b; Lennette *et al.*, 1996; Simpson *et al.*, 1996; Ariyoshi *et al.*, 1998, Bestetti *et al.*, 1998; Mayama *et al.*, 1998; Olsen *et al.*, 1998; Wilkinson *et al.*, 1998). In one study, KSHV seroprevalence ranges from 32% in Zimbabwe to 100% for the Ivory Coast using a lytic IFA (Lennette *et al.*, 1996).

PCR studies have also shown that there is geographic variation in KSHV seroprevalence, but lack of sensitivity makes it difficult to determine seroprevalence rates (refer full text version).

## KSHV seroprevalence and disease association within populations at risk for the epidemiological variants of KS

### AIDS-related Kaposi's sarcoma

***KSHV seroprevalence in risk groups for HIV-1 transmission*** The hypothesis that AIDS-KS may be caused by a KS agent implies that the distribution of the agent should parallel the distribution of Kaposi's sarcoma among HIV-1 transmission groups such that KSHV seroprevalence is higher among homosexual/men than all other HIV-1 transmission groups (Beral *et al.*, 1990). Prevalences are considered first in terms of serological results and second in terms of PCR findings.

In the United States, KSHV antibodies have consistently been found to be more prevalent (22–35%) in HIV-infected homosexual men in comparison to other HIV transmission groups such as patients with haemophilia, transfusion recipients and injecting drug users (Gao *et al.*, 1996b: Kedes *et al.*, 1996; Lennette *et al.*, 1996). The gradient of KSHV seroprevalence was again seen in HIV transmission groups where antibodies were detected in 100% of homosexual men compared to 23% of injecting drug users and 21% of women (Lennette *et al.*, 1996).

Similar distribution patterns of KSHV have been found in HIV transmission groups in Europe. In Denmark, The Netherlands and the United Kingdom, KSHV antibodies are detected more frequently in homosexual men/bisexual men (30–39%) as opposed to <7% of those with haemophilia or injecting drug users (Simpson *et al.*, 1996; Melbye *et al.*, 1998; Renwick *et al.*, 1998). The pattern of distribution holds true even in Italy where in spite of a higher seroprevalence in the general population, 62% of homosexual men have antibodies to LANA or ORF65, compared to 11% in intravenous drug users and 17% in heterosexuals (Calabro *et al.*, 1998). This pattern is seen consistently in studies from Italy (Rezza *et al.*, 1998).

KSHV is only rarely detected in homosexual men unless they have KS when using nested PCR to detect KSHV DNA in PBMCs as seen in studies from the UK, US, France, Italy and Switzerland (Whitby *et al.*, 1995, Moore *et al.*, 1996b, Lebbe *et al.*, 1997, Dupon *et al.*, 1997; Quinlivan *et al.*, 1997). The detection of KSHV by PCR is not useful for determining KSHV prevalence and the presence of this virus in other cells and body fluids is discussed in greater detail in the full text version.

***Association of KSHV with AIDS-KS*** That KSHV is the causative agent of KS, among other diseases, is usefully considered in terms of criteria that describe a cause and effect relationship. Disease causation can be viewed in terms of the following features; (1) The prevalence of the disease should be higher in those exposed than not exposed to the proposed cause and conversely, exposure to this cause should be more common in those with the disease than those without the disease. In the same vein, the incidence of the disease should be higher in persons who are exposed than not exposed as shown in prospective studies. As the strength of association, as measured by an odds ratio or relative risk, between a causative agent and its disease is based on prevalence or incidence data in exposed and unexposed groups, the implication is that the stronger the association, the more likely that the relationship is causal (2) These associations should be observed at different places and times under varying circumstances (3) Exposure to the cause should precede the appearance of disease (4) In some instances, there should be a dose-response relationship between the severity of exposure and severity of outcome which in turn means that prevention or elimination of the risk factor or modification of the host response should produce identical trends in disease expression (5) The specificity of the relationship in that a single suspected cause produces a single effect provides weak supportive evidence for causation (6) Although not always necessary, additional support for a causal relationship comes from biological plausibility and the relationship should be consistent with the natural history of the disease (7) Experimental evidence should support the association (Hill, 1965; Evans *et al.*, 1978).

Studies that detect antibodies to LANA, ORF65 or vp40 using IFA, WB or EIA have all demonstrated high prevalences of KSHV antibodies in patients with AIDS-KS (range: 51.6–87.3%) compared with HIV-infected controls without KS (range:12.9–43.7%). As a result significant odds ratios (range:

3.7–18.7) have been found for the presence of KSHV antibodies between these cases and controls (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Miller *et al.*, 1996; Simpson *et al.*, 1996; Renwick *et al.*, 1998).

The detection of KSHV by PCR in PBMCs of AIDS-KS patients (range: 34.7–90.9%) and HIV-infected controls (range 0–18.7%) has similarly demonstrated a strong association between KSHV infection and KS with odds ratios varying from 2.3–440.0 (Whitby *et al.*, 1995; Moore *et al.*, 1996b; Lefrere *et al.*, 1996; Marchioli *et al.*, 1996; Humphrey *et al.*, 1996). This association remains strong even in a region of high KSHV seroprevalence in the general population such as The Gambia (Ariyoshi *et al.*, 1998). A strong association was not detected in the minority of studies (Collandre *et al.*, 1995; Decker *et al.*, 1996). The important temporal association between risk factor and disease was also demonstrated by PCR prior to the use of more sensitive serological assays (Whitby *et al.*, 1995).

Based on the Hill criteria for disease causation, results from prospective cohort studies in differing locations have provided the most convincing arguments to date that KSHV is the causative agent of KS within HIV-1 infected individuals (Gao *et al.*, 1996a; Martin *et al.*, 1998; Melbye *et al.*, 1998; Renwick *et al.*, 1998; O'Brien *et al.*, 1999; Rezza *et al.*, 1999). Not only are the associations between KSHV and KS consistently strong but also KSHV antibodies are usually detected before the appearance of clinical KS lesions. The timing of KSHV and HIV infections may be important as KSHV seroconversions that follow HIV-1 seroconversion appear to increase the risk for developing KS (HR=5.17: 95%CI 2.88–9.27) (Renwick *et al.*, 1998).

### KSHV seroprevalence in risk populations and association with Classic KS

KSHV seroprevalences range from 94.4–100% among persons with Classic KS and from 3.7–19.1% for matched or blood donor controls and odds ratios, when calculable, range from 130–257 (Gao *et al.*, 1996b; Simpson *et al.*, 1996; Calabro *et al.*, 1998). The full text version provides an in depth discussion on the parallels between incidence rates of Classic KS in particular countries and KSHV seroprevalences in blood donors from the same countries.

### KSHV seroprevalence in risk populations and association with Posttransplant KS

KS is also associated with receiving an organ transplant (Harwood *et al.*, 1979; Penn, 1979). Case-control studies from different geographic regions have again demonstrated a strong association between KSHV antibodies and Posttransplant KS. In Italy, 10/11 (91%) of transplantation recipients with KS were seropositive to antibodies to LANA and ORF65 prior to transplantation compared to 2/17 (11.7%) organ recipients who served as controls (OR=75; 95%CI: 4.7–3500) (Parravicini *et al.*, 1997). In Saudi Arabia, where KS is the commonest tumor to follow organ transplantation, 13/14 (92.9%) renal transplant recipients with KS were positive to p40 and sVCA immunoblot assays compared to 5/18 (27.7%) ($p < 0.001$) renal transplant recipients without KS (Qunibi *et al.*, 1998). In France, 17/25 (68%) of transplant recipients with antibodies to KSHV LANA or ORF65 pre- or post-transplantation developed KS compared to 1/33 (3%) KSHV seronegative transplant recipients ($p < 0.00001$) (Farge *et al.*, 1999). Independent risk factors for KS in this transplantation population included origin in Africa or the Middle East, use of antilymphocyte sera for induction and KSHV antibodies (OR=28.4; 95%CI: 4.9–279) (Farge *et al.*, 1999). In a separate study from France 16/166 (9.6%) transplant recipients were KSHV seropositive with LANA IFA (Frances *et al.*, 1999). Twelve of these sixteen (75%) of the KSHV seropositive patients survived past the first year and three patients developed Posttransplant KS wheras no such disease occured in the 150 KSHV seronegative patients. In Switzerland, KSHV seroprevalence in 220 recipients of renal transplants increased from 14/220 (6.4%) at the time of transplantation to 39/220 (17.7%) after one year of follow-up using ORF65 ELISA (Regamey *et al.*, 1998b). Two of the twenty five KSHV seroconverters in this study developed Posttransplant KS.

### KSHV seroprevalence in risk populations and association with African KS

Data on HIV negative KS patients in Africa are scarce. One patient was positive by LANA IFA and Western Blot in Uganda (Gao *et al.*, 1996b) and 28/28 (100%) serum samples from patients with Endemic/African KS were positive with both lytic and latent IFAs (Lennette *et al.*, 1996).

### The existence of a co-factor(s)

Despite high prevalences (13.8–24.1%) of KSHV in blood donors from Italy, population-based incidence rates of Classic KS are low at 0.7–3 per 100000. The predilection of KS for males does not seem to be explained by higher KSHV seroprevalences in men which at best are three fold higher than women in some but not all studies (Calabro *et al.*, 1998; Whitby *et al.*, 1998; Angeloni *et al.*, 1998). Similarly Endemic KS is more common in Central Africa and East Africa than in the rest of the continent, however KSHV seroprevalences are similar in West Africa and South Africa (Ariyoshi *et al.*, 1998; Wilkinson *et al.*, 1998) than in East Africa (Lennette *et al.*, 1996; Simpson *et al.*, 1996; Mayama *et al.*, 1998). In a study of 16 KS patients from West Africa where there are comparable amount of HIV-1 and HIV-2, 14 patients had HIV-1, 1 patient had HIV-2 and 1 patient was coinfected by HIV-1 and HIV-2 (Ariyoshi *et al.*, 1998). This study suggests that HIV-1 is a more effective co-factor than HIV-2 even when adjusted for CD4 count and that the role of HIV infection is not solely immunosuppression. Another unexplained aspect of KSHV epidemiology is that KS is as common as an AIDS-defining illness in HIV-infected drug users, patients with hemophilia and heterosexually infected women in KSHV endemic countries and countries where KSHV infection is rare suggesting that mode of transmission or the timing of infection plays a critical role (Casabona *et al.*, 1991). It is also possible that a more virulent strain of KSHV or co-factor is an important determinant of disease expression.

## TRANSMISSION OF KSHV

### Sexual transmission

Although the epidemiology of AIDS-KS suggests that KSHV is transmitted preferentially but not exclusively among HIV transmission groups, more formal evidence has been acquired using seroepidemiological results from several cross-sectional and prospective cohort studies on homosexual men.

In a cohort of Danish homosexual males, the presence of antibodies to KSHV was independently associated by multivariate analysis with the number of receptive anal contacts and with sex with men from the United States. Multivariate analysis of KSHV seroconversion over the follow up period to 1996 showed that KSHV seroconversion was independently associated with visits to homosexual communities in the United States and with HIV positive status. A decline in KSHV incidence in the early 1980s was attributed to changes in lifestyle (Melbye *et al.*, 1998).

Among participants and controls from the San Francisco Men's Health Study, no KSHV antibodies were detected among exclusively heterosexual men, whereas KSHV seroprevalence was 12.5% among men who reported mostly homosexual activity and 39.6% among exclusively homosexual men (Martin *et al.*, 1998). KSHV seropositivity was also strongly associated with base-line HIV infection. In addition the prevalence of KSHV increased linearly with the number of male intercourse partners in the preceding two years, the relative prevalences of KSHV were significantly raised among persons with a self-reported history of sexually transmitted disease and the highest prevalence of KSHV was found among men with more than five years of regular homosexual intercourse (Martin *et al.*, 1998). In the Amsterdam Cohort Studies (1984–1996) risk factors were examined for those who were KSHV positive at enrolment and also for those who seroconverted during the course of the study. The independent risk factors that were associated with KSHV seroconversion include oro-genital insertive or receptive sex with more than five partners in the past six months, as well as older age and preceding HIV infection (Dukers *et al.*, 2000).

Similar associations with sexual and in particular homosexual activity were reported from the Sydney HIV cohort (Grulich *et al.*, 1999), the San Francisco Young Men's Health Study (Blackbourn *et al.*, 1999), and from 2718 patients in a London sexually transmitted disease clinic (Smith *et al.*, 1999). There was also no evidence in the latter study for sexual transmission among heterosexuals.

There is considerable disagreement between studies on the precise mode of KSHV transmission between homosexual men however the mode of transmission must be either exclusively or a combination

of insertive or receptive oro-genital, oro-anal, oro-oral or ano-genital insertive or receptive sex. The detection of KSHV in various body fluids is discussed in the full text version.

Only 13/387 (3.4%) women from the San Francisco bay area had KSHV antibodies, and 12 of the 13 KSHV seropositives were also HIV seropositive (Kedes *et al.*, 1997a). In London, 13/169 (18.3%) women attending a sexually transmitted disease clinic were KSHV seropositive, and they were more likely to be African than born elsewhere (Whitby *et al.*, 1999). KSHV can be detected in cervicovaginal secretions in HIV-infected women (Calabro *et al.*, 1999; Whitby *et al.*, 1999). It is not clear why so few HIV-1 infected women get Kaposi's sarcoma.

**Childhood transmission**

Transmission before puberty appears to be rare in the United States (Blauvelt *et al*., 1997) but does occur in countries where KSHV is more widespread. Evidence for intrafamilial clustering has been seen in Italy (Angeloni *et al.*, 1998). The age distribution of KSHV antibodies in Ugandan children shows that the adult KSHV seroprevalence was reached well before puberty, but that KSHV was rare before the age of 2 (Mayama *et al.*, 1998). Correlation with hepatitis B infection suggests that KSHV is transmitted horizontally in conditions of close contact and crowding (Mayama *et al.*, 1998). In Cameroon there was a steady increase in seroprevalence from 27.5% at 4 years of age to 39% in the 12–14-year age groups and 48% above 15 years suggesting that KSHV infection occurs during childhood in Africa (Gessain *et al.*, 1999). In Egypt, the seroprevalence of antibodies exceeded 50% in children older than 6 years and the prevalence stabilized at ten years (Andreoni *et al.*, 1999). In South Africa, KSHV infection was common among children below the age of puberty, and increased above puberty (Wilkinson *et al.*, 1998).

It has been reported that that infections in children less than 10 are probably the result of mother-child transmission, although whether this occurred pre-, peri- or post-partum is not yet known (Bourboulia *et al.*, 1998). A subsequent study showed that the probability of mother-to-child transmission increased with increasing maternal antibody titer (Sitas *et al.*, 1999). In Zambia, 183 (48.4%) of 378 pregnant women were KSHV seropositive and 5 children with KS all had KSHV seropositive mothers (He *et al.*, 1998). In children less than 2 years, KSHV infection is however rare, even in endemic countries, arguing against transmission through breast milk (Goedert *et al.*, 1997; Mayama *et al.*, 1998; Gessain *et al.*, 1999; Lyall et al 1999).

**Parenteral transmission**

The presence of KSHV antibodies prior to transplantation in 10/11 (91%) of transplant recipients in the Italian study and in all 5 Posttransplant KS patients for whom pretransplant sera were available in the French study suggests that KS is mainly due to reactivation of KSHV in regions of KSHV endemicity (Parravicini *et al.*, 1997; Farge *et al.*, 1999, Frances *et al.*, 1999). There is some evidence that KSHV reactivates quickly after transplantation and that increased HHV8 DNA levels in PBLs is associated with KS development (Mendez *et al.*, 1999). KS remission seems to coincide with reduction or cessation of immunosuppression (Moosa *et al.*, 1998). Evidence is mixed on transmissibility of KSHV by blood transfusion (Blackbourn *et al.*, 1997, Lefrere *et al.*, 1997, Marcelin *et al.*, 1998, Mayama *et al.*, 1998, Regamey *et al.*, 1998b).

**KSHV and its association with diseases other than KS**

KSHV has been linked to several other diseases, among them a tumor named primary effusion lymphoma (Chang *et al.*, 1994; Cesarman *et al.*, 1995, 1996; Nador *et al.*, 1996) and Multicentric Castleman's Disease (MCD) (Soulier *et al.*, 1995). Constant detection of KSHV in these diseases is reasonable evidence of causation as they are too rare to be considered in terms of the Hill criteria. The KSHV literature is filled with reports on the association or lack of association between KSHV and an extensive list of dermatologic, haematologic, neurologic and oncologic disease, infectious disease syndromes and systemic disorders. The most controversial reports to date are those concerning sarcoidosis and multiple myeloma (Di Alberti *et al.*, 1997b; Rettig *et al.*, 1997).

## CONCLUSION

Several serological and nucleic acid based assays have been designed to detect KSHV and despite the limitations of imperfect reference standards and assay variability, it is possible to discern geographic regions and populations with comparatively high and low KSHV seroprevalences. Although KSHV DNA and/or antibodies are detected in the majority of patients with KS and strongly associated with all epidemiological variants of KS, it is clear that most KSHV-infected individuals do not develop KS. A co-factor appears to be required for this vascular tumor to be expressed clinically; HIV-1 is a necessary condition for AIDS-KS development. Immunosuppressive therapy plays a role in the development of Posttransplant KS, however the role of immune suppression, immune activation and/or co-factors remain to be established for Classic and Endemic KS.

Seroepidemiological studies have provided useful data on KSHV transmission. There is general agreement that KSHV is sexually transmitted among homosexual men although the precise mode of transmission is still hotly contested. Regardless of this disagreement, the public health message to practice safe sex is clear. In countries where KS is endemic, transmission of KSHV in childhood through close contact appears to be important. It is important to elucidate all modes of KSHV transmission as part of the drive to eliminate a preventable disease.

KSHV has been associated with a number of other diseases and these associations need to be explored. Little doubt remains around the relationship with Multicentric Castleman's Disease and Primary Effusion Lymphoma and these disease phenotypes may be dependent on tissue specific expression of KSHV genes. The availability of a cell culture system an/or an animal model will extend the studies that can be done on KS pathogenesis and also the evaluation of antiviral agents and pathogenesis-based therapies. The ultimate reward for studying a viral-induced tumor is that this disease should one day be eradicated.

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