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Kaposi's Sarcoma-associated Herpesvirus (KSHV/HHV8): Key Aspects of Epidemiology and Pathogenesis

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Abstract

The search for a transmissible infectious agent as the cause of Kaposi's sarcoma lead to the discovery in 1994 of Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus type 8 (HHV8)[1]. KSHV is the only human γ_2 herpesvirus (rhadinovirus) known so far, and is also associated with two other AIDS-related lymphoproliferative disorders: primary effusion lymphoma (PEL) and the plasma-cell variant of multicentric Castleman's disease (MCD). This review addresses key aspects of KSHV epidemiology, life cycle and pathogenesis, including the role played by key latent and lytic KSHV genes.

Key words

KSHV/HHV8. Kaposi's sarcoma. AIDS. Epidemiology. Tumor virology.

Introduction

Epidemiological studies had suggested the involvement of a transmissible agent in the pathogenesis of Kaposi's sarcoma (KS). However, this infectious agent was not discovered until 1994, when Chang, et al. analyzed KS tissue by Representational Difference Analysis (RDA), and identified DNA sequences that showed homology to two oncogenic γ herpesviruses, Epstein Barr virus (EBV) and herpesvirus saimiri (HVS) of squirrel monkeys[1]. Since then, the causal link between KSHV and KS has been well established[2]. The sequence of the complete viral genome has been obtained[3], and an association of KSHV with two other lymphoproliferative diseases, primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD), has been confirmed[4,5]. Establishment of persistently infected cell lines from PEL patients allowed the development of serological assays that demonstrated the epidemiological link between KSHV and KS, and showed that the virus was more common among risk groups, or in countries where KS had been shown to be more prevalent. These cells have also allowed the study of several aspects of the KSHV life cycle *in vitro*, the production of KSHV viral particles for electron microscopy (EM) and structural studies, and the identification of viral genes defining latency or lytic replication[6,7]. These studies have provided the basis to develop hypotheses on how KSHV, the latest of six human tumor viruses, causes disease. Some of these hypotheses involve novel concepts in the field of tumor virology.

Epidemiology

The distribution and transmission of KSHV and its association with disease have been studied by combining polymerase chain reaction (PCR) and serological assays. KSHV has been detected in all forms of KS, in PEL and in the plasma-cell variant of MCD[8]. Thus, KS only develops in KSHV-infected individuals.

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Conversely, KS is a rare event in immunocompetent individuals. Immunosuppression due to organ transplantation, HIV infection, or other unknown factors, promotes the emergence of Kaposi's sarcoma in a KSHV-infected individual. Likewise, the presence of KSHV DNA in effusion lymphoma with the phenotype of a late stage of B-cell differentiation is the defining feature of primary effusion lymphoma (PEL)[9]. PEL occurs mainly in AIDS patients, but it can also develop in HIV-uninfected individuals[9,10]. The plasma-cell variant of MCD is also frequently associated with KSHV, whereas other forms of MCD are not.

The geographic distribution of KSHV has been studied using serological assays measuring antibodies to a latent and a lytic KSHV antigen. The most commonly used serological assays detect either antibodies to the latency-associated nuclear antigen 1 (LANA-1) by immunofluorescence (IF), to a minor capsid protein (SCIP) encoded by open reading frame (*orf*) 65 by ELISA or Western blot, or to a virion glycoprotein encoded by *orf* K8.1[2,11,12]. In several studies, these assays have been used in combination, as neither of them are 100% sensitive or specific. Some uncertainty, therefore, remains as to the exact seroprevalence rates, in particular in low-prevalence areas, but there is now agreement on the overall distribution of KSHV in different countries and in those groups at risk for sexually transmitted diseases.

Geographical distribution of KSHV

KSHV seroprevalence in sub-Saharan Africa is in the order of 40-60% and 20-40% in South Africa. Interestingly, the HIV-negative variant of "classic" KS is more frequent in East Africa than in other parts of the continent, which may be due to the presence of other environmental co-factor(s)[2,11,13,14].

Low KSHV prevalence rates were observed in Asia, Northern Europe and the United States of America. However, in Mediterranean countries such as Italy, Greece and, to a lower extent Spain, prevalence rates are higher[2,11,15-17].

In South America, KSHV seroprevalence appears to be low in urban populations[18,19]. In contrast, isolated populations such as an Amerindian tribe in Brazil and the Noirs Marron, a population of African descent living in remote villages of French Guyana, have been reported to have much higher prevalence rates[20,21].

Many of these seroprevalence studies have noted an increase of KSHV prevalence with age in adults. This is likely to reflect ongoing transmission throughout adult life, but may also be due to increased viral replication after reactivation of a latent KSHV infection in elderly individuals.

Transmission

In KSHV-endemic countries, there is strong support for both mother-to-child and transmission among siblings[21-23]. Seroprevalence rises quickly in children aged more than two years[13]. Transmission by breast milk is therefore unlikely, in contrast to other herpesviruses (e.g. cytomegalovirus). In view of the secretion of KSHV in saliva, it has been proposed that transmission to children could occur via saliva; however, no direct evidence for this actually exists. In adult life there is evidence for sexual transmission both in endemic and Western countries. Most studies agree that there is evidence for sexual transmission in those at risk for sexually transmitted diseases, in particular commercial sex workers in KSHV-endemic countries, and homosexual men in Western countries. Both KSHV prevalence and incidence of AIDS KS are higher in HIV-infected homo/bisexual men than in the general population[24-26]. Several studies on prospective cohorts have observed that half of all HIV- and KSHV-infected homosexual men develop KS within a 5-10 year time span[24,25]. The risk of KSHV transmission rises with the number of sexual partners, and certain sexual behaviors seem to be linked to KSHV transmission. Since infectious KSHV has been found in saliva[27,28], but is not abundant in semen[29], and oral-genital contact has been identified as a risk factor[30], it has been suggested that saliva-mediated transmission may also be important for KSHV spread among homosexual men.

There are controversial results regarding parenteral transmission of KSHV. Transmission through blood transfusion, or among intravenous drug users, has been reported to occur, albeit infrequently[31,32], whereas another study that focused on the Amsterdam drug-user cohort did not observe parenteral transmission[33].

Transplant KS

The risk of post-transplant KS is increased by 40 to 80-fold due to KSHV infection[34-36]. Renal transplant patients have a relatively high risk of developing KS. The majority of these cases are due to the reactivation of the virus in the recipient, but transmission of KSHV from the donor's organ has also been observed[34,37].

Viral replication cycle

Latency

KSHV persists in a latent form in the majority of the infected cells. During latency, the KSHV genome replicates in the absence of virion production, and persists as extra-chromosomal episomal DNA circles, which are found in KS biopsies[38,39]. Latent replication is characterized by the expression of only a few genes: *orf*K12/"kaposin"; *orf*K13/vFLIP; *orf*72/v-cyclin; *orf*73/LANA-1; and in PEL and MCD B-lymphoma cells (but not in KS endothelial cells) *orf*K10.5/LANA-2[40-43, and references in 44].

Lytic replication

A proportion of virus-infected cells undergo lytic (productive) viral replication, resulting in the production and release of viral particles, allowing virus spread. Several data point to some lytic genes as key players on KSHV pathogenesis. First of all, drugs that

inhibit KSHV lytic replication lower the risk of developing KS. Moreover, increased KSHV replication is correlated with the development of MCD in immunocompromised patients. Finally, some lytic genes seem to have autocrine and paracrine effects, which may aggravate KSHV-mediated pathogenesis[45-47].

*Orf*50/RTA is the key transcriptional activator that triggers the entry into the lytic cycle[48-50]. Ectopic expression of RTA in PEL cells triggers the lytic cycle leading to the production of infectious virus[49, further references in 44]. The mechanism of action of RTA seems to involve both binding to specific DNA sequences and interaction with cellular transcription factors[51-53]. Other KSHV proteins, such as *orf*K8/K-bZIP, play roles in the regulation of the switch from latent to lytic cycle.

The patterns of KSHV gene expression following activation of the lytic cycle have been characterized by DNA arrays using PEL-derived cell lines. Lytic KSHV genes have been classified into primary, secondary, and tertiary lytic genes (or alternatively as alpha, beta, and gamma genes), depending on the transcription pattern prior and after induction of the lytic cycle with phorbol ester or Na-butyrate treatment[54,55]. Primary lytic genes play mainly regulatory roles, whereas those with secondary and tertiary kinetics of expression are involved in virion formation. A few cells in KS and MCD express tertiary lytic genes, implying that complete viral replication takes place in some infected cells[54,55].

The role of key KSHV genes in KSHV-mediated pathogenesis

KSHV contains genes that play roles in the inhibition of apoptosis, cell cycle control, transformation, and immune evasion. An extensive description of all KSHV genes involved in pathogenesis is beyond the scope of this review. In the next section we describe some of the key players in KSHV-mediated pathogenesis.

Persistence of the genome and transcriptional regulation: LANA-1

*Orf*73/LANA-1 is the only KSHV protein that can be detected by IF or IHC in practically all KSHV-infected cells[40,42] (Fig. 1). LANA-1 is responsible for persistence of the episomal genome, in a similar way as the EBNA-1 protein of EBV[56,57, more references in 44]. In order to perform this function, LANA-1 tethers the viral episome to host chromatin. It binds to chromatin via its aminoterminal region, and to the terminal repeat (TR) of the KSHV genome through its C-terminal region[58-60] (Fig. 2).

LANA-1 can also act both as a repressor and/or activator of transcription of cellular and viral promoters[60-62]. Binding of LANA-1 to p53 results in inhibition of p53-mediated apoptosis[63]. LANA-1 also binds to the retinoblastoma protein and in-

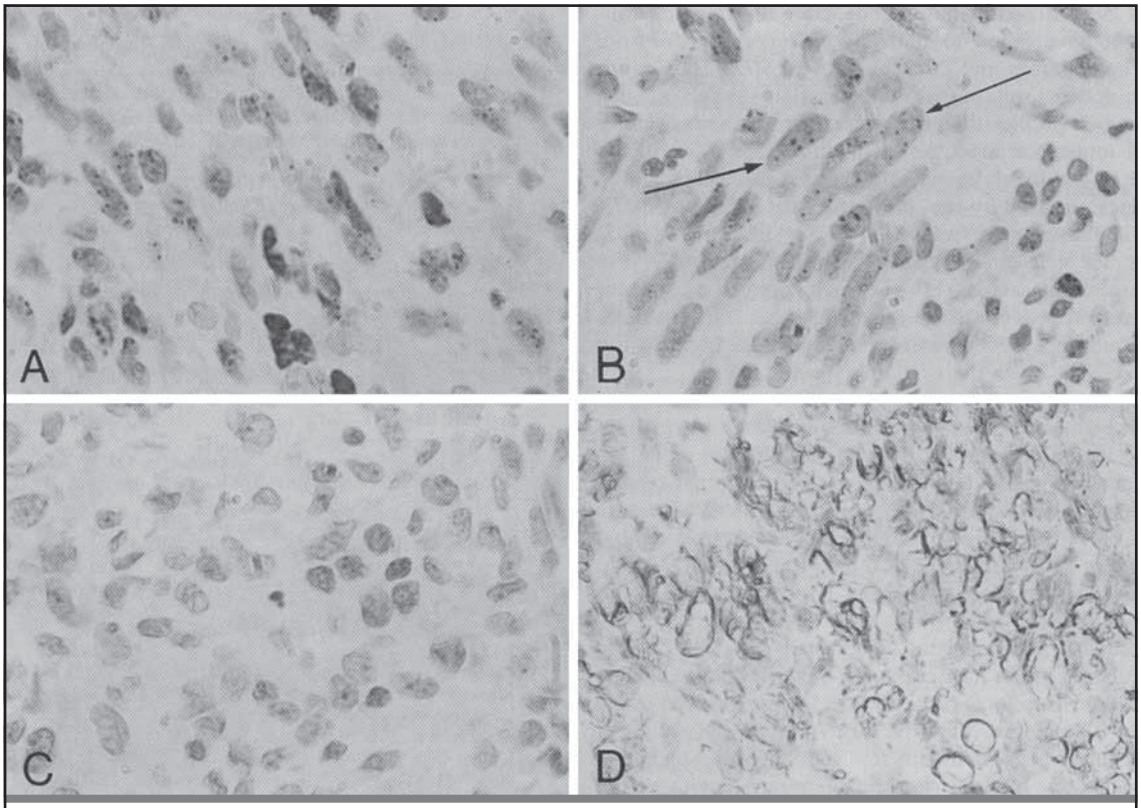

Figure 1. Detection of the latency-associated nuclear antigen-1 (LANA-1) and of orf65 in KSHV-infected cells. *The pictures show a nodular KS tissue stained with affinity-purified human antibodies to LANA-1 (A and B), to orf65 (C), and to the endothelial marker CD34 (D). The majority of the cells in panels A and B express LANA-1. The arrows in panel B point to the "dotted" or "speckled" pattern observed in some nucleus stained for LANA-1. Printed from [42] with permission from The Journal of Virology.*

duces E2F-dependent promoters[64]. It interacts through its C-terminus with several members of the *fsh* family of BET proteins, such as RING3, a cellular homeotic gene product and component of the "mediator complex", which is involved in transcriptional regulation[65] (Fig. 2).

Cell cycle control: LANA-1 and v-cyc

Interaction of LANA-1 with GSK-3β, a kinase involved in phosphorylation and consequent degradation of β-catenin by the proteasome, inhibits β-catenin degradation and increases β-catenin levels, leading to the activation of promoters containing Lef/Tcf-binding sites and subsequent entry into S-phase[66].

*Orf*72/v-cyc is another latent protein that regulates the cell cycle, promoting the progression of resting cells into S-phase[67-69]. v-cyc mediates phosphorylation, and thereby inactivation of pRb, through association with cdk6[67,68,70,71] (Fig. 2). Despite its homology with cellular D- and E-cyclins, KSHV v-cyc has some unique features. A main difference is that the CDK6/v-cyc complex is resistant to the cellular CDK inhibitors p16, p21, p27, and to p16INK4a[67,72]. Moreover, the viral and cellular homologues differ in their phosphorylation targets[68,71,73].

Inhibition of apoptosis: vBcl-2, K7, vIRF-1/-2, LANA-2, and vFLIP

KSHV *orf*16 encodes a viral homologue of human Bcl-2 (vBcl-2)[74]. vBcl-2 transcripts are detected in PEL cell lines[75], and protein expression has been observed for late stages of KS lesions[76]. vBcl-2 is thought to inhibit Bax-mediated apoptosis[75]. The product of *orf*K7, a lytic protein, inhibits apoptosis by bridging Bcl-2 and activated caspase-3, allowing Bcl-2 to inhibit caspase activity[77].

Viral interferon regulatory factor (vIRF) –1 and –2 inhibit interferon (IFN)-mediated apoptosis[78-81]. vIRF-1 transcripts are weakly expressed in KSHV-infected B-cells, whereas they are absent from spindle KS-cells[78]. vIRF-1 downregulates the expression of $p21^{WAF1/CIP1}$ and transforms NIH 3T3 cells[78]. It also inhibits the action of p53 and the retinoid-IFN-induced mortality-19 (GRIM19), a nuclear protein responsive to IFN/all-trans retinoic acid (RA) that increases caspase 9 activity and apoptosis[82-84]. Moreover, vIRF-1 and -2 inhibit IRF-1-induced expression of CD95L, an apoptosis-inducing ligand of CD95/Apo-1/Fas[81]. Another KSHV-encoded vIRF, K10.5/LANA-2, inhibits the activation of p53-dependent promoters and thereby p53-mediated apoptosis[43]. LANA-2 binding to p53 has not been shown *in vivo*. There-

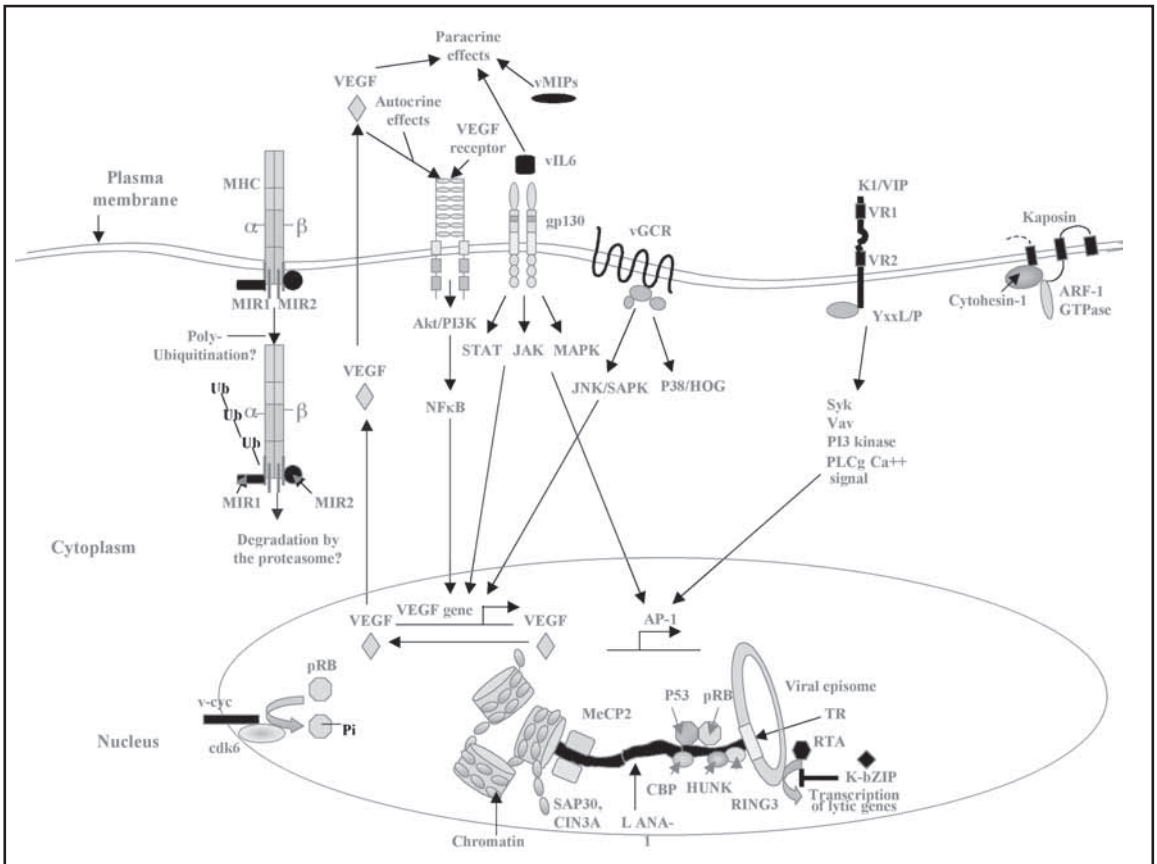

Figure 2. Schematic representation of some KSHV proteins (represented in black) involved in pathogenesis, and cellular proteins (represented in gray), and the pathways affected. *See text for details. Abbreviations: MHC: major histocompatibility complex; VEGF: vascular endothelial growth factor; PI3K: phosphatidylinositol 3-kinase; NFκB: nuclear factor kappaB; STAT: signal transducer and activator of transcription; JAK: Janus kinase; MAPK: mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; SAPK: stress-activated protein kinase; HOG: high-osmolarity glycerol response; PLCg: phospholipase C-gamma2; pRB: retinoblastoma protein; TR: terminal repeat; cdk6: cyclin-dependent kinase 6; Ub: ubiquitin.*

fore the mechanism of inhibition of p53-mediated apoptosis is still unclear. LANA-2 is expressed during latency only in B-cells of PEL and MCD but not in KS, suggesting a B-cell specific role for this protein[43].

Viral FLIP, (viral FLICE [Fas-associated death-domain-like IL-1 β-converting enzyme]-inhibitory proteins; v-FLIP) located in the same bicistronic RNA as v-cyc has been postulated to be a tumor progression factor, since it can block FAS-induced apoptosis through the interaction with apoptotic signals triggered by virus-specific T killer cells[85].

Angiogenesis: vGCR, vIL-6, vMIPs

The product of *orf*74, a "class I" gene, is a G-protein coupled receptor (vGCR), homologue of the human interleukin 8 (IL-8) receptor[86]. vGCR transforms murine cells and induces vascular endothelial growth factor (VEGF)-dependent angiogenesis, KS-like lesions in transgenic mice, or in animals inoculated with transfected cells, or in transgenic animals where endothelial cell-specific infection by a retroviral vector carrying the vGCR gene was carried out[45,46,87,88] (Fig. 2). vGCR modulates the transcription of angiogenesis-regulating genes, pro-inflammatory genes, and cytokines[89,90]. The fact that vGCR is only expressed in 10% of KS cells[91], and its ability to induce cellular cytokine secretion, suggest that vGCR has important autocrine and paracrine effects on KS[46] (Fig. 2). Expression of vGCR in PEL cells results in activation of p38 and ERK-2, increased transcription of KSHV lytic genes and higher production of vIL-6 and VEGF[92].

KSHV codes for a human IL6 (hIL6) homologue (vIL6). Many data support a role for vIL6 in the B-cell proliferation and plasmacytic differentiation seen in MCD and the pathogenesis of PEL. Like hIL6, vIL6 is able to support the growth of IL6-dependent B-cells *in vitro*[93]. However, vIL6 has a wider range of target cells than hIL6, probably due to the fact that vIL6 only requires one of the two cell surface IL6 receptor subunits (gp130) to stimulate cell growth, whereas hIL6 needs both gp130 and gp80[94,95] (Fig. 2). Since gp130 is more broadly expressed, this could account for the wider stimulatory properties of vIL6. Interferon-α (IFN-α) directly activates vIL6, which has antagonist effects on IFN-α signaling, allowing vIL6 to bypass IFN-α inhibitory effects, resulting in an autocrine dependence of tumor cells on the viral cytokine[96]. hIL6 cannot play such a role, since IFN-α downregulates the hIL6 receptor gp80 but not gp130, the receptor used by vIL6[96].

In vivo, vIL6-transfected fibroblasts induced tumors in mice, which were more extensively vascularized than those in control animals and showed high levels of VEGF, which correlated with the amount of vIL6 in these tumors[97]. VEGF is also detected in the malignant effusions of PEL patients, in PEL-derived cell lines, and a neutralizing antibody to VEGF blocked the formation of effusion lymphoma and bloody ascites in mice inoculated with PEL cell lines[98,99]. The formation of these malignant ascites, characteristic for this AIDS lymphoma, seems to be dependent on VEGF-induced stimulation of vascular permeability. The biochemical mechanism of vIL-6 action seems to involve the activation of STAT 3, JAK1 and the MAP kinase pathway[94,100] (Fig. 2).

KSHV encodes three chemokine homologues: *orf*K6/vMIP-I, *orf*4/vMIP-II and *orf*4.1/vMIP-III, which belong to the macrophage inflammatory protein family (MIP), hence their name, and play important roles in endorsing angiogenesis, chemotaxis and eosinophil migration. Thus, it has been shown that vMIP-I induces VEGF expression in PEL cell lines[101] (Fig. 2). vMIP-II binds to several chemokine receptors and behaves either as an agonist or as an antagonist[102,103], whereas vMIP-I is more selective, binding exclusively to and acting as an agonist of CCR8[104]. Both vMIP-I and -II act as chemo-attractant for monocytes and Th2 cells and not Th1, NK or dendritic cells[104-106]. This selective attraction may be responsible for the composition of the KS leukocyte infiltrate, where both CD4+ and CD8+ cells show a marked type-II cytokine profile[105]. It is possible that this strategy allows KSHV to switch the immune system from an antiviral type-I towards a type-II response[106].

Proteins with transforming and intracellular signaling activity: Kaposin, orfK1 and orf74

The K12/kaposin locus, located upstream of the LANA/v-cyc/v-FLIP group of genes, encodes several proteins among them one, Kaposin A, with transforming properties[107,108]. It has been reported that Kaposin A acts through direct interaction with cytohesin-1[109], a guanine nucleotide exchange factor for ARF GTPases and regulator of integrin-mediated cell adhesion (Fig. 2).

Two transmembrane proteins with immediate-early/early kinetics of expression (*orf* K1/VIP, *orf* 74/vGCR) cause tumors in transgenic mice, and activate several intracellular signal transduction pathways, e.g. Syk kinase and phospholipase C-gamma2 (PLCg) (Fig. 2) [for details and references see [44]]. The role of vGCR in angiogenesis is explained above.

K15

At the right-hand end of the KSHV genome, between *orf*75 and the KSHV terminal repeat (TR), is *orf*K15, a gene containing eight exons, which give rise to a family of alternatively spliced transcripts. The largest K15-derived protein is predicted to contain 12 transmembrane domains, and it has been shown to interact with TNF-receptor associated factors (TRAF's) and to be phosphorylated by Src kinases, resulting in the activation of NFkB and of two mitogen-activated kinase (MAPK) pathways[110,111]. The functional relevance of the interaction between K15 and TRAF's is currently unknown.

Interaction with the immune system: K3, K5, vIRF's and vMIP's

Two membrane proteins unique to KSHV, K3 and K5, also known as modulator of immune recogni-

tion (MIR) 1 and 2, respectively, downregulate MHC class-I molecules and protect virus-infected cells against NK cells or cytotoxic T-lymphocytes[112, and references in 44]. The mechanism of action of K3 and K5 seems to involve an increase in the rate of endocytosis and degradation of MHC class-I molecules, possibly via an ubiquitin/proteasome-dependent mechanism[113,114] (Fig. 2). Both proteins are expressed as early and late lytic genes following reactivation in PEL cell lines, whereas K3 transcripts were not detected in KS lesions[55,115-117].

The initial immune response against viral infection is regulated by IRF's through binding to IFN-stimulated response elements (ISRE's) in the promoters of IFN-responsive genes. KSHV vIRF's inhibit IFN-signal transduction, probably by impeding the formation of the transcriptional active complexes[118-120].

Summary and outlook

KSHV is associated with KS and two lymphoproliferative diseases in the AIDS setting, PEL and MCD. KSHV prevalence is high in Africa, in some Mediterranean countries, and within high-risk groups, whereas it is low in other geographical areas. There is still controversy regarding the route(s) of KSHV transmission, and the importance of environmental, immunological, and behavioral factors in disease susceptibility. However, it seems clear now that KSHV can be sexually transmitted, and that saliva plays a role in transmission.

KSHV may cause disease through a plethora of mechanisms. Many groups worldwide are investigating the importance of KSHV latent and lytic genes in pathogenesis, providing useful information for control of the virus. The use of KSHV bacterial artificial chromosome (BAC) and the information obtained with animal models based on KSHV-related agents, such as murine herpesvirus-68 (MHV-68), should provide new interesting data in the next few years.

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