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## Immune evasion by Kaposi's sarcoma-associated herpesvirus

Hye-Ra Lee<sup>1,†</sup>, Stacy Lee<sup>1</sup>, Preet M Chaudhary<sup>2</sup>, Parkash Gill<sup>3</sup>, and Jae U Jung<sup>1</sup>

<sup>1</sup> Department of Molecular Microbiology & Immunology, Keck School of Medicine, University of Southern California, Harlyne J Norris Cancer Research Tower, 1450 Biggy Street, Los Angeles, CA, 90033, USA

<sup>2</sup> Department of Medicine, Keck School of Medicine, University of Southern California, Harlyne J Norris Cancer Research Tower, 1450 Biggy Street, Los Angeles, CA, 90033, USA

<sup>3</sup> Department of Pathology, Keck School of Medicine, University of Southern California, Harlyne J Norris Cancer Research Tower, 1450 Biggy Street, Los Angeles, CA, 90033, USA

### Abstract

Persistent viral infections are often associated with serious diseases, primarily by altering functions of the host immune system. The hallmark of Kaposi's sarcoma-associated herpesvirus (KSHV) infection is the establishment of a life-long persistent infection, which leads to several clinical, epidemiological and infectious diseases, such as Kaposi's sarcoma, a plasmablastic variant of multicentric Castleman's disease, and primary effusion lymphoma. To sustain an efficient life-long persistency, KSHV dedicates a large portion of its genome to encoding immunomodulatory proteins that antagonize the immune system of its host. In this article, we highlight the strategies KSHV uses to evade, escape and survive its battle against the host's immune system.

### Keywords

immune evasion; immune modulatory protein; Kaposi's sarcoma; Kaposi's sarcoma-associated herpesvirus; primary effusion lymphoma

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Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), the most recent addition to the  $\gamma$ -herpesvirus family, was initially determined, from KS lesions of an AIDS patient, as an etiological agent in 1994 [1]. KSHV is also known as the causative agent of several B-cell malignancies, such as primary effusion lymphoma (PEL) and multicentric Castleman's disease [1–3]. The large KSHV DNA genome consists of 145 kb of unique sequences bounded by 40 kb of terminal tandem repeats, and is most similar in structure and gene content to Herpesvirus saimiri (HVS) of New World primates [4–6]. As is typical of other herpesviruses, KSHV establishes a life-long persistent infection in host cells by adopting one of two lifestyles known as latency and lytic replication. During lytic replication, the full repertoire of the viral genome is expressed in a highly orchestrated temporal order from

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<sup>†</sup>Author for correspondence: Tel.: +1 323 442 1713, Fax: +1 323 442 1721, hyelee@usc.edu.

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immediate-early ( $\alpha$ ), early ( $\beta$ ) and, finally, late ( $\gamma$ ) genes, thereby allowing viral replication and ultimately killing the infected cell. By contrast, viral gene expression is heavily restricted during latency, facilitating the maintenance of the viral genome and evading detection by the host.

The host immune system is constantly in a state of alert against pathogens, employing powerful antiviral mechanisms to deal with viruses on many levels as part of their innate and adaptive immune responses. The innate immune response is able to slow down viral replication and activate cytokines, which trigger the synthesis of antiviral proteins. The adaptive immune response neutralizes virus particles and destroys infected cells. Obligate intracellular parasites, such as KSHV, must cope with such immune responses in order to establish a persistent infection. Therefore, it is not surprising that viruses often resort to hijacking this cellular machinery to achieve a balance between activating the host defenses and escaping from these dangerous sentries. The viruses produce a wide range of immune modulator proteins and utilize a myriad of immune evasion mechanisms that exploit the host immune system to advance their own lifecycles. Due to the evolution of numerous immune-evasion mechanisms by KSHV, 25% of its proteins have been shown to regulate different aspects of the host immune system. In this article, we summarize the current state of research regarding KSHV-associated host innate and adaptive immune evasion strategies, and the implications these modulations have on the establishment and maintenance of a persistent infection.

## KSHV: replication cycle & gene products

Kaposi's sarcoma-associated herpesvirus is a member of the  $\gamma$ -herpesvirus subfamily, *Rhadinovirus* genus. Virus infection occurs when KSHV enters into the susceptible cell through membrane fusion by several enveloped glycoproteins [7–11]. Once the viral capsid is in the cellular cytoplasm, it is transported to the nucleus, upon which linear viral DNA rapidly circularizes to form a nuclear episome. Subsequently, it is decided which of the two known viral transcriptional phases of virus (latency or lytic phase) will arise. During latency, the viral genome is retained as a circular episome in the nucleus, expressing only a minimal number of the viral genes to facilitate the maintenance of viral genome as well as to escape host immune detection. To switch their lifecycle from latency to lytic replication, KSHV encodes ORF50 (replication and transcription activator [RTA]), a lytic master-switch protein that has been shown to be required and sufficient for the triggering of the lytic replication cycle, and hence the expression of the entire viral genome as well as the production of progeny virions [12–14].

As noted above, KSHV has a large dsDNA genome that consists of a 145-kb-long unique region encoding more than 80 genes, and a 40-kb terminal repeat region composed of highly GC-rich terminal repeat units in order to efficiently support such a highly regulated lifecycle [4,15,16]. Almost 50% of the KSHV genome is dedicated to modulating the environment within the host to promote its own survival, manipulating pathways such as cell cycle regulation, programmed cell-death, signal transduction and immune modulation. Moreover, several groups have collectively identified a total of 17 miRNAs that are transcribed from the latency-associated region of the KSHV genome, which includes several coding regions (latency-associated nuclear antigen [LANA], v-cyclin, v-FLIP and kaposin) and multiple overlapping transcripts [17–21]. Recent data and the fact that these KSHV miRNAs are involved in KSHV pathogenesis imply that they are essential for the control of KSHV replication and latency by regulating the expression of viral lytic genes and the host survival pathway [22–25]. In addition to miRNAs, KSHV encodes a noncoding RNA transcript, a 1077-nucleotide polyadenylated nuclear RNA [26]. It is highly expressed during the early

lytic phase and confines intronless RNA within the nucleus to block the assembly of export-competent messenger ribonucleoprotein particles [26,27].

## Innate immune evasion

The immune system is canonically divided into two major branches: innate and adaptive immunity. The innate response elicited by an invading pathogen involves the rapid recognition of general molecular patterns by non-immune cells or cells of the innate immune system, such as monocytes or macrophages, dendritic cells (DC), and natural killer (NK) cells. In the context of viral infections, the innate immune system acts as the first line of defense for sensing an infection, and responds by unleashing protective defense mechanisms. Hence, the first barrier to overcome for successful viral infection is the rapid innate immune response of the host, which are involved in several effector mechanisms, including complement cascade, type I IFNs, inflammatory cytokine, NK cell immunity, apoptosis, autophagy and Toll-like receptors (TLRs) pathway.

### Complement system

The complement system has long been considered to be a key arm of the innate immune system response to pathogens, but there is also growing evidence that the complement system participates in the adaptive immune response [28]. The complement system is composed of several dozen proteins that are either circulating in serum or are attached to cell surfaces. They orchestrate three distinct cascades, termed the 'classic', 'alternative' and 'lectin' pathways, which result in the activation of antimicrobial effector activities that range from the opsonization of foreign particles and the recruitment of phagocytes, to the lysis of infected cells. All three cascades converge by assembling a C3 convertase that can either initiate the opsonization of the foreign body or continue to activate C5 and thus propagate the cascade [29]. The complement regulatory proteins share a common structural motif, known as the short consensus repeat. The complement cascade is under tight cellular control by host inhibitor proteins, and thus, it is perhaps not surprising that viruses have either hijacked or co-opted some of these as an anticomplement defense system [30].

The ORF4 of KSHV encodes a lytic cycle protein that not only eases viral entry by acting as an attachment factor for binding heparin sulfate, but also mediates evasion from the host complement system by inhibiting the complement-mediated lysis of infected cells [31–34]. The protein, appropriately named 'KSHV complement control protein' (KCP), contains four short consensus repeats or complement control protein motifs at its N-terminus, and a C-terminal transmembrane anchor [31]. KCPs regulate the complement system by accelerating the decay of the classical C3 convertase and act as cofactors for the inactivation of C3b and C4b, components of the C3 and C5 convertases. However, the ability of KCPs to promote the decay of activated C3 convertase in the alternative pathway is modest, indicating that this pathway may not have a significant role in the clearance of the virus *in vivo*. Other  $\gamma$ -herpesviruses, including HVS, rhesus monkey rhadinovirus (RRV) and murine  $\gamma$ -herpesvirus 68 (MHV68), can also interfere with the complement system by encoding host complement control protein homologs that disrupt the progression of the complement cascade [31–34]. Taken together, this suggests that the evasion of the complement system is essential for viral fitness.

### Type I IFN-induced signaling

IFNs are signaling molecules that are essential for regulating the activation of immune cells during an antiviral response [35]. There are at least three distinct types of IFNs: type I (IFN- $\alpha/\beta$ ), type II (IFN- $\gamma$ ), and type III (IFN- $\lambda$ 1, - $\lambda$ 2 and - $\lambda$ 3, also known as IL-29), which are classified based on their amino acid sequences [36]. Specifically, the type I IFNs, IFN- $\alpha/\beta$ ,

represent a powerful and universal intracellular defense system against viruses of all sorts, which is best demonstrated in knockout mice that are unresponsive to IFN- $\alpha/\beta$  due to targeted deletions in their type I IFN receptor [37]. These mice quickly succumb to viral infections despite retaining an intact adaptive immune system [38]. Likewise, humans with genetic defect in STAT-1, a protein involved in the IFN signaling cascade, succumb to viral diseases at an early age [39]. All nucleated cells in the mammalian body are able to synthesize and secrete type I IFNs in response to virus infections. Secreted IFNs are then recognized by neighboring cells, causing them to express potent antiviral proteins [38,40]. In order to complete their lifecycle, viruses must find a way to modulate the host IFN-mediated immune response to work in their favor. Notably, a portion of the KSHV genome spanning a cluster of loci is dedicated to encoding four viral IFN regulatory factors (vIRFs; vIRF1–4), which are homologous to cellular IRFs [4,5]. They are all expressed during lytic reactivation [41,42], but vIRF1 and vIRF3 have also been detected in latently infected cells of different KS lesions and PELs [43–45]. The significance of this differential expression of vIRFs is not yet known, but these observations suggest that vIRFs may function redundantly, but act independently (depending on the nature of the cell type and stage of viral infection) to thereby elicit disparate and distinct mechanisms of IFN evasion [46,47].

The N-termini of the vIRFs exhibit significant levels of homology to the N-terminal DNA-binding domain of cellular IRFs, but lack several tryptophan residues essential for DNA binding such that vIRFs do not directly bind DNA [48–51]. Although they do not prevent cellular IRFs from binding to specific target DNA sequence, they are able to interact with cellular IRFs, other transcriptional factors and co-factors to obstruct various downstream cellular IRF functions [49–51]. vIRF1, however, seems to be an exception, as demonstrated in a recent study in which vIRF1 was shown to bind directly to a specific DNA sequence in the promoter region of a polycistronic transcript encoding K3, viral dihydrofolate reductase (ORF2) and viral IL-6 (vIL6), which may function to regulate viral gene expression [52].

Viral IFN regulatory factors (K9) contains a DNA-binding domain but does not compete with IRF1 for DNA binding, nor does it bind to or suppress IRF1 [50,53]. Rather, vIRF1 directly targets and regulate p300 activity. The association of vIRF1 with p300 interferes with the formation of CBP/p300 complex, as well as p300 HAT activity to prevent the IRF3-mediated transcriptional activity of IFN- $\alpha$  [54,55]. In contrast to this functionally relevant binding to p300, vIRF1 interaction with IRF7 does not block the transactivation activity of IRF7 [56]. Despite this range of anti-IFN activity, the time windows in which vIRF1 is able to block IFN responses in KSHV-infected cells are limited [53], suggesting that vIRF1 may have other functions during the multifarious stages of the virus lifecycle or that other vIRFs and viral immunomodulators are required to completely inhibit IFN responses. Indeed, full-length vIRF2 (which is translated from exons K11.1 and K11) inhibits the expression of IFN-inducible genes driven by IRF1, IRF3 and IFN-stimulating gene factor-3 (ISGF3), but not IRF7 [57]. Unlike vIRF1, it has recently been shown that vIRF2 accelerates the decay of activated IRF3 through caspase-3, thus functioning as a catalyst in the IRF3 degradation process, and concomitantly inhibiting IRF3 transactivation and host antiviral response [58]. Moreover, the first exon of vIRF2 (K11.1) prevents the activation of PKR [59], reducing protein synthesis and blocking IFN- $\alpha/\beta$  signaling [53,60]. In contrast to vIRF1, the specific mechanisms of vIRF2 activity have yet to be defined. Unlike vIRF1 and vIRF2, which mostly target IRF3-mediated IFN signaling, vIRF3 (also known as LANA2), specifically interacts with either the DNA binding domain or the central IRF association domain of IRF7. This interaction consequently leads to the suppression of IRF7 DNA binding activity and, therefore, the suppression of IFN-mediated immunity through the inhibition of IFN- $\alpha$  production [61]. It was recently reported that vIRF3 also interacts with IRF5, abrogating IRF5 binding to IFN-responsive promoter elements to thereby block IRF5-mediated promoter activation. Furthermore, vIRF3 was shown to

antagonize IRF5-mediated activation of the p21 promoter [62]. Consequently, vIRF3 is essential for the proliferation and survival of cultured PEL cells by releasing IRF5 from transcription complexes [62,63].

Taken together, the downregulation of the IFN regulatory pathway is a common characteristic of the three vIRFs whose functions have been well-studied because they target IRF3 and IRF7, key initial factors of host immune surveillance against viral infections. vIRF4, the most recently identified member of the vIRF family, has yet to be studied mechanistically, but it may also have the potential to affect IFN-mediated innate immunity. The apparent importance of the modulation of IRF3 and IRF7 activity by KSHV is made further evident by the fact that the latent protein LANA1 and the early protein K8 (K-bZIP) act to obstruct IRF3, while two immediate-early proteins, ORF45 and ORF50 (RTA), negatively regulate IRF7. First, LANA1 inhibits IFN- $\beta$  transcription and synthesis by competing with IRF3 to bind to the IFN- $\beta$  promoter, thus interfering with the formation of the IFN- $\beta$  enhanceosome and subsequently preventing the expression of the CREB-binding protein [64]. Additionally, K-bZIP (known as K8) also directly interacts with the IFN- $\beta$  promoter to impede its binding with IRF3, thus precluding efficient IFN- $\beta$  gene expression [65]. Alternatively, after reactivation, ORF45 interacts with IRF7, preventing its phosphorylation and blocking its translocation to the nucleus, thus downregulating type I IFN production [66]. RTA, another immediate early protein, augments ORF45 activity by promoting IRF7 ubiquitination, resulting in the induction of the proteasomal degradation of IRF7 [67]. The manipulation of the stability, function and modification of host IRF3 and IRF7 by many KSHV gene products provides a number of novel regulatory strategies for circumventing the innate immune defense system whilst enabling them to act at different stages of the viral cycle, with some proteins playing a role during the lytic replication cycle and others during latency. Ultimately, the inhibition of the IFN pathway by KSHV may be the virus' first line of protection against an innate immune reaction, enabling the successful establishment of viral persistence.

### Modulation of inflammatory signaling

Virus infection provokes the production of cytokines and chemokines, which regulate leukocyte trafficking and effector functions [68], thereby playing a key role in directing immune and inflammatory responses as well as angiogenesis. Hence, many viruses would benefit from antagonizing normal cytokine activities [69]. Chemokines can be divided into four subfamilies defined by the pattern and number of conserved cysteine residues located near their N-terminus, which are involved in disulfide bond formation: CC-, CXC-, CX<sub>3</sub>C- and C-family [69]. The induction of particular chemokines in combination with the differential expression of specific seven-transmembrane-domain G-protein-coupled chemokine receptors (GPCR) by certain leukocyte subsets determines which immune cells migrate to the site of infection during inflammation [69,70]. Chemokines interact with both their specific receptors to transduce signaling, and with cell surface glycosaminoglycans to form immobilized chemotactic chemokine gradients used by immune cells to travel across endothelial cell monolayers and into tissues via distinct binding sites [69,71]. In addition, cytokines can be either positive or negative regulators of the immune responses, and for this reason, some viruses encode their own cytokines (virokines) or chemokine receptors (viroceptors), or secrete chemokine-binding proteins, that are presumed to interfere with normal antiviral chemokine functions [72,73].

**vcCL-1, vcCL-2 & vcCL-3**—Kaposi's sarcoma-associated herpesvirus encodes three chemokine homologs produced during its lytic program: viral CC-chemokine ligand-1 (vcCL1; K6), vcCL2 (K4) and vcCL3 (K4.1), previously known as viral macrophage inflammatory protein (vMIP)-I, vMIP-II and vMIP-III, respectively. vcCL1 and vcCL3 are



specific agonists of host CC-chemokine receptor-8 (CCR8) or CCR4, which are present on both Th2 and CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells, and attract these cells to downregulate an immune response [74–77]. vCCL1, vCCL2 and vCCL3 appear to be involved in Th2 lymphocyte chemoattraction by specifically activating CCR3 and CCR8. Meanwhile, vCCL2 can also antagonize Th1 cells by inhibiting signaling mediated by many other classes of chemokine receptors with which it has a high affinity, including CCR1, CCR2, CCR5, CX<sub>3</sub>C-chemokine receptor-1 (CXCR1) and CXCR4 [78]. Consistent with these actions, vCCL2 inhibits signaling of these receptors for arrest of monocytes and Th1 cells in the presence of their authentic chemokine ligands, such as a RANTES or MIP1 $\alpha$  I, as well as promoting the arrest of eosinophils and Th2 cells [79,80]. Thus, vCCL2 not only blocks Th2 responses due to its agonist actions, but also impairs Th1 responses due to its antagonist activities. Apart from their functions in immune modulation, vCCLs have all been shown to promote angiogenic responses through the induction of VEGF and other angiogenic factors, such as viral GPCR (vGPCR) and vIL-6 in cultured PEL cells [81,82].

**vCD200**—Another negative regulator of inflammatory signaling is the production of viral K14, also known as viral cluster of differentiation 200 (vCD200), previously designated as viral OX2. K14 is an early lytic gene and exhibits a significant level of homology to cellular CD200, a broadly distributed cell surface leukocyte glycoprotein [83]. CD200 acts as a tissue-specific negative regulator of target cells by interacting with a specific receptor, CD200R, which has restricted expression in myeloid and T cells [84,85]. It was initially reported that vCD200 targeted and stimulated monocytes, macrophages and DCs to produce inflammatory cytokines, potentially to promote the cytokine-mediated proliferation of KSHV-infected cells [83]. On the other hand, it was recently reported that vCD200 directly binds to CD200R, inhibiting myeloid cell activation and reducing Th1-associated cytokine production [86,87]. Furthermore, vCD200 suppressed acute inflammatory responses in mice in which neutrophil-mediated inflammation was induced by carrageenan [88], indicating that vCD200 may contribute to immune dysregulation. Taken together, it seems likely that a crucial function of vCD200 is its role as an anti-inflammatory agent, although its primary target cell types within the myeloid compartment during KSHV infection *in vivo* are still uncertain.

**vIL-6**—K2, an early KSHV lytic gene, encodes vIL-6 and exhibits 25% amino acid identity, as well as structural and functional homology, with cellular IL-6 [89,90]. Both the cellular and vIL-6 molecules transduce signals through the common receptor subunit, gp130, to orchestrate inflammatory responses, angiogenesis and oncogenesis. Cellular IL-6 binds to the CD126 component, which then forms a complex with the gp130 transducer component of the receptor, causing activated gp130 dimerization. By contrast, vIL-6 seems to circumvent the normal cellular checkpoint of CD126 coupling with gp130 by directly binding to gp130 to initiate downstream signaling [91]. Consequently, because most human B cells lack CD126, vIL-6 may act on a much wider spectrum of B cells than its human counterpart [92]. As noted earlier, vIL-6 expression can be detected at various levels in all lytically infected cells, as well as in some latently infected B cells [41,93–96]. Knockdown of vIL-6 expression in PEL cells leads to markedly reduced cell growth [97]. It is suggested that vIL-6 may support the growth and survival of latently infected cells in an autocrine manner via intracrine signaling, ultimately contributing to the maintenance of latently infected cells and to virus-induced neoplasia. vIL-6 also acts as an angiogenic factor inducing VEGF to promote the recruitment of permissive cells, such as lymphocytes, monocytes and endothelial cells, to primary infection sites, thereby enhancing viral production [98]. The vIL-6 promoter itself possesses an IFN- $\alpha$  stimulated response element sequence, such that vIL-6 expression is actually induced by IFN- $\alpha$ , essentially functioning as a feedback loop that ensures blocking of IFN- $\alpha$  antiviral infection [99].

**vGPCR**—The KSHV vGPCR (or ORF74) is a *bona fide* signaling molecule, as signaling by vGPCR elaborates many mitogenic and angiogenic cytokines that are vital to the biology of KS and other KSHV-driven malignancies. vGPCR is transcribed as an early gene during lytic infection and is a viral homolog of the cellular angiogenic IL-8 receptor. Unlike its cellular homolog, vGPCR signaling is ligand-independent and constitutively active [100]. It constitutively activates a series of transcription factors AP-1, NF- $\kappa$ B and HIF-1 $\alpha$ , which, in turn, induce the expression of growth factors, pro-inflammatory cytokines and angiogenic factors such as VEGF [101]. Central to vGPCR-induced oncogenesis, it can also activate the MAPK, PI3k, Akt and mTOR cell survival pathways [102–104], and its oncogenic potential is demonstrated by the fact that it is sufficient to induce cellular growth transformation and VEGF-mediated angiogenesis in fibroblasts [101]. Interestingly, vGPCR is conserved in all  $\gamma$ 2-hepesviruses members, such as Epstein–Barr virus, MHV68, HVS and rhesus monkey rhadinovirus [105–107]. EBV vGPCR inhibits antiviral PKR activity, and the vGPCR of MHV-68 is required for efficient and productive viral replication in culture and *in vivo* [107–110]. Thus, all this collectively indicates that vGPCRs activate gene expression and help establish an intracellular condition that promotes viral replication, be it during *de novo* or reactivated lytic replication.

**vCKBP**—Some viruses encode a vCKBP, which does not share any sequence similarity with any known chemokines or chemokine receptors, but instead binds to chemokines with a high affinity and efficiently neutralizes their ability to establish a gradient along which immune cells can migrate [111,112]. Similar to poxviruses, cowpox virus and vaccinia virus (A41L), MHV-68 (M3) and  $\alpha$ herpesviruses (gG) encode different subfamilies of CKBP. They can all inhibit chemokine-mediated signal transduction by inhibiting chemokines from binding to their GPCR or glycosaminoglycan [113–115]. However, no vCKBP has yet been identified in KSHV.

### Modulation of apoptosis & autophagy

A common cellular response to pathogenic invasion is to undergo cell death executed by two programs to limit the infection: apoptosis and autophagy. Apoptosis (Greek for ‘self-killing’) is the best-described form of programmed cell death, but autophagy (Greek for ‘self-eating’, a lysosomal degradation and recycling of cytoplasmic constituents pathway essential for homeostasis) has also been reported to contribute to cell death [116–118]. Even though they are characterized by distinctive morphological and biochemical changes, both are tightly regulated, are highly conserved processes that are essential for homeostasis and development, and have been implicated in diseases [118,119]. Apoptosis is a cellular suicide mechanism and it has been well-documented as a host defense mechanism against viral infections. There are at least two pathways that lead to apoptosis: an extrinsic death-receptor-mediated and intrinsic mitochondria-dependent pathway. Indeed, apoptosis represents the predominant demise of virally infected cells. By contrast, cellular autophagy has been historically recognized as a survival program vital for the maintenance of homeostasis by removing damaged or superfluous organelles, and the protection of cells during times of stress by providing a temporary source of energy and macromolecule in essential cellular processes until normal conditions return. Given this primordial role of autophagy in removing harmful components in the cytoplasm, it is not surprising that autophagy may be used as an intrinsic cellular defense against viruses. As intracellular parasites, viruses are bound to encounter and interact with autophagy proteins in the course of an infection. Hence, viruses generally employ some gene products to disrupt and overcome both these altruistic processes because they are part of the overall host surveillance mechanism for blocking viral replication and dissemination. While these two pathways are seemingly independent, apoptosis and autophagy are not mutually exclusive; the two are substantially interconnected and coordinately regulated [118]. For instance,

several apoptosis signaling molecules (including TNF- $\alpha$ , TRAIL, FADD and p53) also mediate autophagy, while Atg5, an autophagy effector, triggers apoptosis through its interaction with FADD [120–127]. Furthermore, the inhibition of the class I PI3K, AKT and mTOR pathway suppresses both apoptosis and autophagy [123]. KSHV encodes proteins that target the crosstalk between apoptotic and autophagic signaling in the cell death pathway including: inhibition of death receptor signaling (as anti-apoptotic) or suppression of autophagy elongation by expressing viral FLICE/vFLIP; and negatively regulating both apoptosis and autophagy by binding specific effectors in these pathways with viral Bcl-2 (vBcl-2).

**vFLIP (K13)**—The KSHV-encoded anti-apoptotic protein, vFLIP (also known as K13), is a truncated homolog of the cellular FLIP (cFLIP) [42,128,129] and contains two death effector domains. Latent expression of vFLIP occurs via splicing of the LANA transcript from the tricistronic mRNA, and via the use of the IRES in vCyclin coding sequences [130–132]. The anti-apoptotic activity of vFLIP has been attributed to its inhibition of caspase-8 activation and more recently to its ability to induce the expression of anti-apoptotic proteins via activation of NF- $\kappa$ B, such as receptor-interacting protein, NF- $\kappa$ B-inducing kinase, I $\kappa$ B kinase (IKK) $\alpha$ , IKK $\beta$  and NF- $\kappa$ B essential modulator via the activation of NF- $\kappa$ B [133–136]. In support of this concept, vFLIP-expressing transgenic mice displayed constitutive NF- $\kappa$ B activation, ultimately leading to an increased incidence of lymphoma that was independent of its inhibition of Fas-induced apoptosis [137]. Furthermore, vFLIP likely contributes to the proinflammatory micro-environment because its expression was found to induce NF- $\kappa$ B-regulated cytokine expression and secretion [138,139]. The induction of NF- $\kappa$ B signaling has been linked to at least two aspects of KSHV-infected pathogenesis: viral latency and oncogenesis. On the one hand, NF- $\kappa$ B activation by vFLIP is critical for its inhibition of lytic replication via the AP-1 pathway [140,141]. On the other hand, enhanced NF- $\kappa$ B signaling may be an important component of the transforming and oncogenic potential of vFLIP, as demonstrated in Rat-1 fibroblast assays and tumors growth assays in nude mice [142]. Collectively, it may be stated that as a result of its strong activation of the NF- $\kappa$ B pathway, vFLIP plays a plethora of roles in viral infected cell survival, transformation, morphological change, inflammatory activation and latency control, thereby directly contributing to KSHV pathogenesis. More recently, Lee *et al.* revealed that vFLIP suppresses autophagy by preventing Atg3 from binding and processing LC3, a protein involved in the elongation step of autophagy [143]. Consequently, vFLIP not only possesses anti-apoptotic activities, but additionally serves as an anti-autophagy molecule through its inhibitory interaction with the E2-like enzyme, Atg3.

**vBcl-2**—Kaposi's sarcoma-associated herpesvirus ORF16 encodes a homolog of cellular Bcl-2, a pro- and anti-apoptotic molecule, which forms homo- or heterodimers to regulate the release of mitochondria apoptotic components [144]. KSHV Bcl-2 possesses Bcl-2 homology (BH) 1 and BH2 domains characteristic of this family of proteins, allowing the viral protein to tightly bind proapoptotic Bak and Bax peptides, and thereby inhibit apoptosis [145–147]. Interestingly, other  $\gamma$ -herpesviruses, including EBV, HVS and MHV68, encode a Bcl-2 homolog [148–150]. The Bcl-2 of MHV68, also known as M11, has been implicated in preventing Bax toxicity in yeast and blocking apoptosis in culture cells when apoptosis is induced by various stimuli [151,152]. Recent advances demonstrate that the vBcl-2 of  $\gamma$ -herpesviruses (including KSHV and MHV68) can also inhibit autophagy via a direct interaction with Beclin1, adding autophagy inhibition as an additional function through which vBcl-2 advances viral lifecycle and pathology [153,154]. In fact, MHV68 vBcl-2 exhibits an even higher binding affinity to Beclin1 and thus a more robust and persistent inhibition of Beclin1-mediated autophagy upon environmental stress, compared with its cellular counterpart [155]. Very recently, Xiaofei *et al.* demonstrated that



the inhibition of autophagy by vBcl-2 is important in maintaining latent infections, while the anti-apoptotic activity of vBcl-2 is largely involved in efficient viral reactivation from latency [156]. Overall, it can be postulated that vBcl-2 circumvents host immune responses to establish and/or maintain a persistent infection by concomitantly blocking both apoptosis and autophagy.

### Modulation of TLRs signaling

Toll-like receptors are evolutionarily conserved innate receptors expressed by mammalian hosts in various immune and non-immune cells, and play a crucial role in sensing infectious agents through the induction of inflammatory cytokines and type I IFNs. Currently, 11 human TLRs and 13 mouse TLRs have been identified, and each TLR has been demonstrated to recognize a particular pathogen-associated molecular pattern derived from various microorganisms, such as bacteria, viruses and fungi [157]. All TLRs contain 21–25 leucine-rich repeats in their extracellular domains, which recognize and bind to pathogen-associated molecular patterns in their N-terminal regions. The intracellular signaling domain, known as Toll/IL-1R (TIR) resides in the C-terminal region and is required for interaction with many different kinds of adaptor molecules to subsequently activate downstream signaling pathways that culminate in the induction of cytokines and IFNs through NF- $\kappa$ B and IRFs. TLRs are expressed in distinct cellular compartments: TLR1, TLR2, TLR4, TLR5 and TLR6 are expressed on the plasma membrane, whereas TLR3, TLR4, TLR7, TLR8 and TLR9 are expressed in intracellular vesicles (e.g., endosome and endoplasmic reticulum) [157–159]. It has hitherto been reported that TLR3, TLR4, TLR7, TLR8 and TLR9 are involved in the recognition of viruses by way of binding to RNA, DNA or viral glycoproteins [160,161]. A recent growing body of information is beginning to shed light on how KSHV modulates the TLR pathway. The first report showed that KSHV primary infection of human monocytes upregulates cytokine and chemokine activation in a TLR3-dependent manner, especially CXCL10 and IFN- $\beta$  [162]. In addition, infection of endothelial cells with KSHV suppresses TLR4 signaling by vGPCR-mediated ERK activation and vIRF1 [163]. Lastly, Gregory *et al.* screened several PEL cell lines with specific agonists of different TLRs. It was found that stimulation of TLR7 and TLR8 with a TLR8 ligand, single-stranded polyuridine (a synthetic single-stranded RNA homolog), strongly reactivated KSHV [164]. Taken together, there is evidence that KSHV has developed several different ways to escape TLR-mediated detection throughout its lifecycle. Moreover, a recent study demonstrates that another pathogen, vesicular stomatitis virus, induced KSHV reactivation in KSHV-infected PEL cells [164], thereby linking innate immune activation with viral reactivation from latency. Consequently, it has been suggested that either TLR agonists or oncolytic therapy may be a valuable treatment of KSHV-related neoplasms.

### Adaptive immune evasion

When the innate immune system confronts a pathogen, it becomes activated and prepares the adaptive arm of the immune system to respond appropriately. The adaptive immune system consists of two branches: the humoral immune response arm (production of antibodies by B cells) and the cellular immune response arm (activities carried out by cytotoxic CD4<sup>+</sup> and CD8<sup>+</sup> T cells). Both typically require antigen presentation in conjunction with MHC and a co-stimulatory signal for full activation. Accordingly, the antigen-processing machinery, particularly the MHC class I pathway has been targeted by many viruses, including KSHV.

## Humoral immune response

Antibodies (e.g., IgG, IgM and IgA) are produced by plasma cells, the final stage of B-cell development after B cells have recognized an antigen and have been stimulated by CD4<sup>+</sup> T cells and T-cell-derived cytokines. Unlike T cells, B cells can recognize antigen in its native form. In the lymph nodes, naive B cells recognize cognate antigen by their surface antibodies, become activated, switch from IgM to IgG production (class-switch), increase their immunoglobulin specificity and affinity, and differentiate into plasma or memory B cells as the cell continues to divide in the presence of cytokines. As a natural host defense mechanism, antibodies can directly neutralize viruses by sterically hindering the receptor–virus ligand interaction or by inducing conformational changes in viral receptor ligands. Other indirect effects caused by antibodies include the recruitment or activation of the innate immune effector system, such as antibody-dependent cell cytotoxicity, engulfment of antibody-coated (opsonized) viruses by phagocytes and complement activation. B cells are the likely cellular reservoir of KSHV infection [1,165]; therefore, KSHV seems to influence several aspects of B-cell biology through the modulation of the humoral immune response.

Recently, several groups have shown that a B-cell terminal differentiation factor, X-box-binding protein-1 (XBP-1), can effectively initiate KSHV reactivation by activating the RTA promoter, thus providing a link between B-cell development and KSHV pathogenesis [166–168]. XBP-1 has two major functions: it is triggered by a variety of different cellular stresses as part of the induction of unfolded protein response [169]; and participates in the terminal differentiation of B cells to plasma cells [170]. Interestingly, PEL cells predominantly express the inactive form of XBP-1, XBP-1u, however, when the active form, XBP-1s, is induced, the KSHV lytic cycle is activated by XBP-1s [171]. This raises the possibility that in KSHV-infected B cells, latency is maintained until plasma cell differentiation occurs.

The study of HIV-1 Vpu recently led to discovery of the IFN-induced transmembrane protein, tetherin (BST-2, CD317), as a novel component of host innate defense against enveloped viruses. To date, six viral proteins have been reported to counteract tetherin: HIV-1 Vpu, HIV-2 and SIV Env, SIV Nef, Ebola glycoprotein and KSHV K5 [172–178]. KSHV K5 induces the proteasomal degradation of tetherin during primary infection and upon reactivation from latency in endothelial cells. Furthermore, tetherin reduced KSHV release upon inhibition of K5 expression by siRNA, suggesting that tetherin is part of the IFN-induced innate immune response against KSHV [175].

## Cellular immune response

Besides effective host humoral immunity, the activation of cellular immunity is usually required for clearance of established infection. The recognition of viral peptides presented by MHC class I molecules on cytotoxic T lymphocytes (CTLs) is a key event in the elimination of cells producing abnormal or foreign proteins, specifically during a virus infection, and CTLs thus play a critical role in the control of a viral infection, especially as a long-term immune surveillance effector that can react more quickly against the same virus after a primary infection [179]. Hence CTL evasion is a prerequisite for the replication of persistent viruses, particularly in the case of herpesviruses, which must establish a persistent, latent infection and must then reactivate in immunologically primed hosts to shed infectious virions. Indeed, all herpesviruses probably implement some strategy of CTL evasion during their lytic cycle (reviewed in [180,181]). Two proteins encoded by KSHV K3 and K5 efficiently downregulate the expression of MHC class I molecules and a large number of supplementary membrane receptors on the surfaces of infected cells, thereby preventing antiviral CTL responses [182,183].

**Inhibition of MHC class I antigen presentation**—The K3 and K5 proteins, also known as modulator of immune recognition (MIR)1 and MIR2, are highly homologous and encode a RING-CH domain with a nonclassical C<sub>4</sub>HC<sub>3</sub> configuration of the cysteines and histidine residues [184,185], making them prototypes of a novel membrane-associated RING-CH (MARCH) family of ubiquitin E3 ligases. They interact with MHC class I molecules through transmembrane interactions, and trigger endocytosis and proteasomal degradation of MHC class I molecules by ubiquitinating its cytoplasmic tail, but without affecting their assembly or transport [186]. Interestingly, K3 downregulates the expression of both canonical and noncanonical MHC class I molecules in humans (HLA-A, -B, -C and -E), whereas K5 downregulates only HLA-A and -B alleles as substrate specificity is dictated by transmembrane interactions [182,183,187,188]. Additionally, vIRF1 has been suggested to interact with p300 to prevent basal transcription of MHC class I molecules. Thus, KSHV can also inhibit MHC class I transcription as another way of down-regulating MHC class I molecules on the cell surface of infected cells [189]. Notably, from the viral perspective, the virus must thwart both CTLs and NK cells because the downregulation of only MHC class I molecules renders infected cells sensitive to NK cell-mediated lysis. NK cells recognize infected cells in an antigen-independent manner, subsequently destroying such cells via their cytotoxic activities. Furthermore, they can rapidly produce large amounts of IFN- $\gamma$ , which induce diverse antiviral immune consequences upon receptor ligation, including the augmentation of antigen presentation and activation/polarization of CD4<sup>+</sup> T cells and macrophage [190]. Hence, K3 and K5 also target the IFN- $\gamma$  receptor-1 (IFN- $\gamma$ R1) by inducing its ubiquitination, endocytosis and degradation, ultimately resulting in the inhibition of IFN- $\gamma$ R1-mediated activation of transcription factors [191]. Overall, it is suggested that the downregulation of surface MHC class I molecules and IFN- $\gamma$ R1 by K3- and K5-mediated ubiquitylation and lysosomal degradation inhibits primary host immunity against viral infection.

**Inhibition of co-stimulation**—The generation of a robust adaptive immune response requires the engagement of T cells with professional antigen-presenting cells such as DCs, macrophages and B cells. To sufficiently activate T cells, co-stimulatory signals, such as the interaction between CD28 and its ligands, B7-1 and B7-2 on antigen-presenting cell surfaces [192,193], and between ICAM1 and lymphocyte function-associated antigen-1 are essential [194]. In addition to lowering the level of MHC class I molecules, K5 also downregulates other components of the immune synapse, such as ICAM (CD54) and B7-2 (CD86), two co-activating molecules for NK cell activation, on the B-cell surface by inducing their endocytosis and degradation [183,195].

Taken together, K3 seems to specifically target MHC class I molecules, whereas K5 shares this function in addition to targeting multiple receptors, including MHC class I, CD86 and ICAM, with the overall effect of preventing detection by CTL and NK cells. Even in a single gene overexpression system, the resultant downregulation is sufficient to confer resistance to immune cells in culture, and therefore may protect KSHV-infected cells undergoing lytic replication *in vivo*. However, the contribution of K3 and K5 to immune evasion by KSHV during latency is less clear. Their influence appears to be most important in the early stages of KSHV infection, when diminished detection by T cells can result in reduced antiviral cytokine responses and an impaired production of CTLs, thereby allowing the virus to establish a persistent infection.

## Conclusion

As previously described, KSHV harbors a surprising number of viral genes either pirated or modified from host genes, which contribute to immune evasion and set the foundation for a persistent viral infection and pathogenesis. Based on these, we briefly summarize the most

prominent immune evasion mechanisms utilized by unique KSHV viral proteins, homologs of cellular proteins which function in a coordinated manner to antagonize host innate and adaptive immunity (Figure 1 & Table 1). Given, KSHV's success at establishing a life-long persistence, it seems safe to say that these immunomodulation activities are quite effective. Furthermore, KSHV has multiple tropisms for different cell types, such that a certain subset of viral genes may help support virus survival in different cellular environments, securing its place as a parasite in the host. However, due to the lack of a permissive cell culture and an *in vivo* model system for KSHV, there remains the great task of integrating our observations regarding KSHV persistent infection with the sophisticated molecular mechanisms used by each viral immunomodulatory protein.

## Future perspective

Kaposi's sarcoma-associated herpesvirus is linked to several malignancies in the human population and accounts for a large proportion of cancer deaths in Africa where the AIDS pandemic continues to fuel the incidence of KSHV infection. Notwithstanding these pressing human health problems, attempts to study KSHV infection are greatly hampered by the lack of cell culture and animal models. Although numerous immune evasion mechanisms employed by KSHV seem to be well-understood, it is too early to translate the knowledge we have obtained from basic science research into developing more effective clinical management and therapies because we can only speculate on how latent and lytic genes function within the context of an intact host immune system. To address this serious necessity, a variety of animal models including macaques, NOD-SCID mice and humanized SCID mice were tested for KSHV infection, yet none of them led to the appearance of virus-associated diseases upon infection [196–199]. However, Mutlu *et al.* recently reported that engraftments of mouse bone marrow endothelial-lineage cells that had previously been transfected with a KSHVBac36 induce an angiogenic phenotype and a KS-like, KSHV-dependent tumorigenicity [200]. Despite this significant advance, this model, like others, fails to truly recapitulate the overall aspects of the KSHV lifecycle in a living subject. Fortunately, we have recently provided the first animal model of KSHV persistent infection established via a zoonotic transmission of KSHV into common marmosets (*Callithrix jacchus*), a New World primate [201]. In this study rKSHV.219 infection of the common marmoset was shown to result in sustained serological responses, latent infection of PBMCs, virus persistence and KS-like skin lesion development, making this primate model highly analogous to what is seen in human infection [201]. Thus, this model is expected to not only help advance future research efforts studying KSHV pathobiology, but also greatly improve our chances to design and develop more effective antiviral therapies and vaccines against KSHV.

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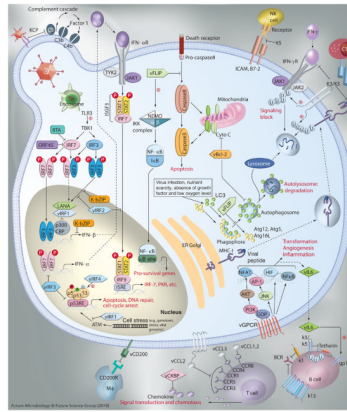
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**Figure 1. Kaposi's sarcoma-associated herpesvirus immune evasion strategies**  
 CCR: CC-chemokine receptor; GPCR: G-protein-coupled chemokine receptor; LANA: Latency-associated nuclear antigen; NK: Natural killer; RTA: Replication and transcription activator; TLR: Toll-like receptor.

**Table 1**

Immune evasion strategies of Kaposi's sarcoma-associated herpesvirus.

Strategy	Gene product	Function
<i>Innate immune evasion</i>		
Inhibit complement	ORF4 (KCP)	Inhibit complement activation
Inhibit IFN	ORF45	Prevent IRF7 activation
	ORF50 (RTA)	Promotes IRF7 degradation
	vIRF1	Inhibition of IRF3-mediated transcription
	vIRF2	Suppression of IRF1 and IRF3
	vIRF3	Inhibition of IRF7 DNA binding activity
	LANA	Competing with IRF3
	K8 (K-bZIP)	Impede IRF3 binding on IFN- $\beta$
Inhibit cytokines/chemokines	K6 (vCCL1)	CCR8 agonist and Th2 chemoattractant
	K4 (vCCL2)	CCR3 and CCR8 agonist, as well as a C-, CC-, CXC- and CX <sub>3</sub> C-chemokine antagonist
	K4.1 (vCCL3)	CCR4 agonist and Th2 chemoattractant
	K14 (vCD200)	Inhibition of myeloid cell activation, reduction of Th1-associated cytokine production
	K2 (vIL-6)	B-cell growth factor
	ORF74 (vGPCR)	Homolog of the cellular IL-8 receptor
Inhibit apoptosis and autophagy	K13 (vFLIP)	Inhibition of caspase-8 activity and prevention of Atg3 interaction
	ORF16 (vBcl-2)	Bind proapoptotic Bak and Bax proteins and direct bind with Beclin-1
<i>Adaptive immune evasion</i>		
Inhibit humoral immune response	K5	Induces tetherin degradation
Inhibit MHC class I antigen presentation	K3	Downregulates MHC class I molecules as well as CD1d
	K5	Downregulates HLA-A and -B as well as CD1d
Inhibit co-stimulation	K5	Downregulates ICAM1 and B7-2

KCP: KSHV complement control protein; LANA: Latency-associated nuclear antigen.