[Frontiers in Bioscience 7, e185-203, May 1, 2002]

KAPOSI'S SARCOMA ASSOCIATED HERPESVIRUS IMMUNE EVASION STRATEGIES

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1. ABSTRACT

The battle between viruses and their hosts is beautiful in its complexity. The interplay between viral proteins and the immune system has taught researchers much about not just the virus, but also the molecular mechanisms underlying the immune response. With additional evasion strategies constantly being described, this avenue of research is still rich with potential discoveries. In this review, we examine a number of proteins encoded by Kaposi's sarcoma herpesvirus (HHV-8) and detail how they aid the virus in escape from immune system elimination. We include, where possible, examples from other homologous viral systems.

2. INTRODUCTION

Prior to the beginning of the acquired immunodeficiency syndrome plague, Kaposi's sarcoma (KS) was a little studied disease. Virus pathology was rarely observed outside of two groups, selected African populations and elderly men of southern European or Mediterranean decent (1, 2). During the early 1980s, KS began appearing at a high rate amongst gay men in the United States. Epidemiological studies demonstrated that the increased incidence of KS in this previously unaffected group was linked with infection and immunosuppression by human immunodeficiency virus (3, 4). Several years of dedicated epidemiological and molecular biology research by a number of groups led to the identification of a new virus, Kaposi's sarcoma-associated herpesvirus (KSHV) or HHV8, as the likely etiological agent of KS (5-9). KSHV has now been linked, not only with KS, but also with pleural effusion lymphomas (PEL) (6) and multicentric Castleman's disease (10). Worldwide, classical KS has a prevalence rate around 2.4%, with the United States having an incidence rate around 0.3%. The more aggressive endemic KS, seen primarily in Africa, can account for nearly half of the reported cancers within some regions (extensively reviewed in 2).

KSHV is classified as a gamma-herpesvirus based on genome co-linearity and homology with several other members of this sub-group including herpesvirus saimiri (HVS) and rhesus monkey rhadinovirus (5, 11-14). It has a large, ~165kb, double stranded DNA genome which has been found, *in vivo*, in a wide variety of cell types including B cells, endothelial cells, epithelial cells

and macrophages (6, 7, 13-17). *In vitro*, while HHV8 has been shown to latently infect a wide variety of cells, no tissue culture system has been found to support high level, lytic replication. This limitation has made it difficult to study individual gene functions in the context of the virus. Similarly, no permissive animal model exists, making a number of aspects of the viral life cycle unclear. Much effort is being put into the development of better cell culture models and it is expected that these tools will be significantly improved in the future. It is also expected that use of closely related viruses for which there are animals models will also provide greater insight into KSHV biology.

The viral genome encodes at least 80 gene products, some with several splice variants. These gene products can be roughly divided into five main categories.

1. Genes involved in nucleotide metabolism, viral DNA replication and repair.
2. Genes involved in viral particle formation.
3. Genes involved in control of viral gene expression and genome maintenance.
4. Genes involved in alteration of the cellular milieu.
5. Genes involved in modulation of host responses.

For the purposes of this review, we will focus on the protein products of the genes in the last category, although several of these products also have functions that could place them elsewhere in the list. A number of gene products in this category have been identified as having homology with immunologically important cellular proteins and/or with other herpesvirus proteins known to have effects on the host's immunosurveillance mechanisms. To name only a few, they include: the viral complement control protein homolog (vCCPH or vCBP), first found in vaccinia and later studied in gamma-murine herpesvirus 68 (gamma-MHV68) and herpesvirus saimiri (HVS) (18-21); the vBcl-2 gene, which encodes a homolog of the cellular Bcl-2 gene (22), important in apoptosis; a homolog of the interferon regulatory factor called vIRF (23, 24); and two genes K3 and K5, which individually are found in other herpesviruses, but have no homology with known cellular proteins important in the immune response (25).

Like other members of the gamma–herpesviruses, KSHV establishes a life-long, persistent infection in the host organism. Crucial to the ability of the virus to establish and maintain this persistence is an evasion of host immune responses that would otherwise clear virus from the system. Through conjoined evolution with their host, the herpesviruses have developed a wide variety of mechanisms for either outwitting or adopting the host's immune responses. If one examines these immune evasion mechanisms from the viewpoint of an infectious disease clinician, then their spectrum is daunting. For KSHV, these mechanisms include: alteration of the host chemokine network through mimics of both the cellular chemokines and chemokine receptors (reviewed in 113); interference with a number of soluble innate immune effectors (13, 23); evasion of MHC class I-mediated and coordinated immune responses through down regulation of major immunomodulatory proteins (26-29); as well as blockage of innate apoptotic effects (22, 30-33).

3. EVASION OF INNATE HOST IMMUNITY

The host's first line of defense against any viral infection is the innate immune response. Although by definition this response is not as "educated" as the later antibody and cytotoxic T-lymphocyte (CTL) responses, it has a highly regulated set of checks and balances, fully as complicated as the adaptive response. The complement system is a case in point. Of the over 30 proteins involved in the complement cascade, nearly half are involved with regulation (reviewed in 34-36). In the recent years, the complexity of the positive and negative regulation of natural killer (NK)-mediated cell lysis is becoming more apparent with the identification of human homologs of the murine Ly-49 system (37). In addition, the numbers of cellular proteins involved in apoptosis and the intricacies of their network of cross talk are staggering (reviewed in 38-41). While this system of checks and balances is necessary, as can be seen in various immunodeficiency syndromes when this system breaks down, it also provides a number of targets for viral intervention and subversion.

3.1. The Complement System

The action of complement begins through one of three pathways, the classical, alternative or mannose-binding-lectin-associated serine protease (MASP) pathways (for review see (42). Each pathway has a slightly different trigger to initiate the complement cascade: natural IgM or elicited-IgG binding to foreign proteins for the classical pathway; the intrinsic degradation and deposition of the C3 complement protein for the alternative pathway; attachment of mannose-binding lectin in complex with the MASP-1 and MASP-2 serine proteases to mannose groups on the surface of bacterial cells for the MASP pathway. Although a slightly different set of initiating proteins are used in each case, each eventually leads to the activation of a series of downstream complement proteins which act to form the membrane attack complex (MAC). This MAC then lyses cells or pathogens on which it has been deposited. Alternatively, earlier components of the complement cascade, such as C3b, deposit on cells and microorganisms to acts as signals for targeting by neutrophils and monocytes.

Regulation of the complement cascade that is the result of any of these pathways is accomplished through two sets of proteins. The first, cascade-promoting set of proteins, are also members of the alternative pathway. Once C3b is deposited on a surface, whether through classical, alternative or MASP pathways, Factor B, Factor D and properdin all act to stabilize and amplify additional C3b deposition. In turn, this results in a logarithmic expansion in the number of MACs. Importantly, C3b deposition can also feed into the B-cell response, as will be discussed briefly in Section 4.2. The second set of regulatory proteins, the cascade-inhibiting set, is a group of soluble and cell-surface associated proteins. The most important of these is Factor H, a cofactor for the serine esterase Factor I.

If Factor H binds to C3b prior to the binding of Factor B then it not only prevents amplification of the C3b deposition, but it also aids Factor I in cleaving a small fragment off of C3b, causing its inactivation. Factor I is aided in its degradation of C3b, as well as C4b found in the classical pathway convertase, through the actions of CD46 (43). This molecule, also called membrane co-factor, is a negative regulator of complement. It is found on all nucleated human cells and probably plays a role in all three of the complement activation pathways (44). Human herpesvirus type 6 (HHV6) as well as the Edmonston strain of measles virus have hijacked CD46 with its ubiquitous expression as one of their cellular receptors (45, 46). One other complement inhibitor worth mention in this article is CD21 or complement receptor 2 (CR2). This molecule is found on mature B lymphocytes, epithelial cells, thymocytes and follicular dendritic cells (47-49). Binding of either inactivated C3b or C3d by this molecule increases the T-dependant B cell response by several orders of magnitude (50-53). In addition, the gamma-herpesvirus Epstein-Barr virus (EBV) utilizes this molecule as a cellular receptor through interactions with its gp350/220 surface glycoprotein (54). The spectrum of this article unfortunately prevents mentioning the numerous other checkpoints preventing the complement cascade from destroying self-tissue.

KSHV has taken advantage of this control system in the form of a homolog of the cellular complement control protein CD46 (13). The KSHV complement binding protein (vCBP) is a 1650 nucleotide long reading frame encoded by ORF 4 and like CD46, it has 4 short consensus repeat (SCR) or sushi domains. The first of these four domains is slightly shorter than the consensus ~60 amino acid sushi domain, while the other three have complete consensus homology. Through alternative splicing vCBP is encoded as both a membrane bound 550 amino acid form, with the putative trans-membrane region spanning residues 517-545, and a shorter secreted form lacking this membrane-spanning region (14). It has 34% identity with the CBP of Macaca mulatta rhadinovirus (MMRHV2) strains 26-95 and 17577 and 35% identity, mostly in the SCR, with human CD46. There are 11 putative sites for N-linked glycosylation with the eleventh having less probability of being glycosylated given the phenylalanine at the second position of the N-X-S/T consensus sequence. Following the SCRs are several regions rich in serine and threonine, giving rise to the possibility of O-linked glycosylation as is seen with human CD46 (44).

To date, little work has been published on this molecule. Using examples from other viral complement homologs and the function of CD46 however, some function can be hypothesized. It would be reasonable, given the ability of CD46 to aid Factor I in the lysis of C3b and C4b, that expression of vCBP most likely blocks complement responses being made by the host against the infected cell. This would provide some measure of protection during periods of viral reactivation. Further, the secreted form of the protein could protect circulating virus from attack by directing inactivation of C3 complexes deposited on circulating virus. An overall decrease in the amount of viral antigen in complex with complement would also help to block CD21-mediated stimulation of B lymphocytes. Given the paucity of work, how important is this gene to viral evasion of the host's immune responses? Some guess can be made by looking at the importance of molecules with similar roles in other herpesviruses.

Herpes simplex virus (HSV) type 1 encodes glycoprotein gC which acts as a cell-surface receptor for C3 and its activation products can also block the binding of both C5 and properdin to C3 (55-58). Lubinski *et al.* created viruses containing mutants of this gC lacking one or both of these functions (59). Upon injection of these viruses into C3 knockout C57BL/6 mice, it was found that there was little difference in the virulence of the mutants as compared with wild-type virus. However, when C3 levels were reconstituted through injection of purified human C3, the double mutant was 50-fold less virulent. Using sera depleted for various complement fractions, Friedman *et al.* have shown that the neutralization of gC null simplex mutants occurred in a C5-dependant manner (60). However, complement members downstream of C5 were not required.

Herpesvirus saimiri (HVS) encodes two proteins with complement-regulatory properties. The CCPH gene has homology to the cellular C3 convertase inhibitors. It contains four SCR domains and two alternatively spliced forms, one membrane-bound and one soluble (61). Fodor *et al.* have shown that CCPH is capable of blocking C3 convertase activity and cell lysis (61). In addition, expression of CCPH reduced surface deposition of C3d. HVS Orf15 or HVS vCD59 has 48% sequence identity with the human terminal complement inhibitor CD59 (also called membrane inhibitor of reactive lysis (MIRL), protectin, HRF20 or H19) (20, 62, 63). The protein is expressed during the viral lytic cycle and is attached to cells via a glycosyl-phosphatidylinositol anchor. While HVS CD59 is not capable of blocking C3b deposition, it was shown to protect BALB/3T3 cells from lysis by both human and rat serum (19). This activity seems plausible given the fact that human CD59 blocks complement lysis at a late point in the complement cascade interacting with C5b/C8 and C5b/C9 components of the MAC preventing the insertion of the membrane-inserted C9 homopolymer. Interestingly, human immunodeficiency virus type 1 virion particles carry an extensive amount of cellular CD59, which is capable of blocking the complement cascade (64).

3.2. Other Soluble Factors

3.2.1. The Interferon Response

The interferons (IFN) comprise a large family of proteins which play a role in metabolism, cell proliferation, hormone stimulation and importantly, anti-viral immunity. First discovered in 1957 by Isaacs and Lindenmann, it was observed that cultures of virally-infected cells produced a protein that rendered the cells resistant to infection by a number of other viruses (65). IFNs act by binding to cellular receptors and inducing signaling cascades through the membrane-associated Janus protein kinases (JAKs) (reviewed in 66-68). In turn, these kinases phosphorylate the signal transducers and activators of transcription

(STATs). Activated STAT1 and STAT2 heterodimers migrate to the nucleus where they activate the interferon response factors (IRFs). These IRFs are capable of binding to IFN-stimulated response elements (ISRE) in the promoter elements of various effector proteins, eliciting their production. The effector proteins include 2'5'-oligoadenylate synthetase, RNase L, several protein kinases and the tumor necrosis factor (TNF) receptor (69-72). Importantly, the IFN cascade has a number of checkpoints and regulatory proteins that affect the outcomes of receptor binding by interferon. This, like in the complement cascade, allows for intervention and blockade of the interferon response by a number of viruses.

The type I IFN response (IFN-alpha and IFN-beta) is crucial to the control of HSV type I replication. IFN-alpha has been shown to directly suppress viral replication and helps to induce specific anti-viral immunity (73-75). Leib *et al.* have shown that the attenuated phenotype displayed by a number of HSV null mutants is a result of the IFN-alpha/beta system (76). Viruses lacking genes encoding infected cell protein (ICP) 0, thymidine kinase, ribonucleotide reductase, virion host shutoff and ICP34.5, all with decreased ability to grow in cells, had up to a 1000-fold increase in viral titers in the eyes of experimentally infected IFN-alpha/beta receptor and IFN-alpha/beta/gamma receptor knock-out mice as opposed to wild-type mice (76). Although this study found little or no difference in viral titer in IFN-gamma receptor knock-out mice, more recently Vollstedt *et al.* have reported that the antibody-dependent, IL-12-induced IFN-gamma response is capable of controlling low MOI infections of HSV (77).

It should be noted that although most viral immune evasion efforts are directed at blocking the host immune attack, several viruses have subjugated the host's responses to their own ends. EBV takes advantage of the IFN response cascade to regulate its own latency program. IRF-7 acts as a negative regulator of the BamHI Q promoter (Qp) (78, 79). This promoter directs expression of EBV nuclear antigen (EBNA)–1 during Type I latency, as is seen in healthy carriers of EBV. In contrast, during Type III latency, the EBNA-I protein, along with a number of other viral proteins, is expressed from the BamHI C and/or BamHI W promoters resulting in full oncogenic transformation of the infected cells. This regulation is crucial for the virus since EBNA-1 is capable of blocking MHC class I-dependant responses being made against the virus (80). Only when immune surveillance is decreased can EBV risk switching to Type III latency with a larger number of viral proteins being expressed. To effect this subjugation of the host interferon response, the virus employs another of its latency program associated proteins, latent membrane protein (LMP)-1. This protein is able to induce the expression of IRF-7, thus selecting for altered EBV promoter usage (81). What advantage might this confer upon the virus? While Type I latency is sensitive to the effects of IFN-alpha, Type III latency is not. Expression of IFN-alpha potentially triggers switching of cells with Type I latency to a resistant Type III latency when the host IFN responses present a threat. This is all further complicated by the fact that the EBNA-2 gene product is capable of inducing IFN-beta expression while interfering with cellular type I IFN responses possibly by binding to p300/CBP complexes (82, 83).

KSHV orf K9, the first viral interferon regulatory factor, exhibits homology with the cellular IRFs and inhibits both type I and II interferon signal transduction (23, 24, 84). K9 is expressed as a 449 amino acid product with only 13% identity with human IRFs (85). It posses a tryptophan-rich potential DNA binding sequence in the amino terminus which might interact with ISRE promoter elements. Orf K9 is an early lytic gene that is expressed in response to TPA in PEL cell lines (86).

Li *et al.* have shown that vIRF directly interacts with p300 and displaces the p300/CBP-associated factor (PCAF)(87). This results in a decrease in the histone acetyltransferase activity of p300, hypoacetylation of histones H3 and H4 and a condensed chromosomal chromatin structure. These changes in the degree of histone acetylation result in decreased transcriptional activity from the early inflammatory gene (IFNA) promoter. Seo *et al.* (88) have demonstrated that vIRF can bind and block the activity of the transcriptional coactivator CREB-binding protein (CBP) *in vivo* and *in vitro*. Moreover, expression of vIRF can transform NIH3T3 cells through interactions with p300 as well as the IRF-1 and IRF-3 proteins (23). The orf K9 gene product can also interfere with IRF-mediated transcriptional activation of the ISG gene promoter. Lin *et al.* (89) has extended these results demonstrating blockage of IRF-3 but not IRF-7-mediated transactivation by vIRF. By binding IRF-3, K9 inhibits formation of IRF-3/CBP/p300 complexes, resulting in a blockage of the immediate early interferon genes.

Nakamura *et al.* (90) and Seo *et al.* (91) have shown that vIRF directly associates with the tumor suppressor protein p53, resulting in a dramatic inhibition of p53-mediated transcriptional activation and apoptosis. Signaling mediated activation of p53 is often achieved through post-translational modifications, including phosphorylation and acetylation (92). Activated p53 can then bind, in a sequence-specific manner, to the promoter elements of a number of genes and increase their transcription. Both Bax and p21 are induced by p53 resulting in apoptosis and G_1-phase cell cycle arrest, respectively (93, 94). The binding of p53 by vIRF blocks phosphorylation and activation of p53, inhibiting its ability to activate Bax and p21 transcription (90, 91).

As with the other immune evasion strategies employed by KSHV, the lack of a pathogenesis system forces us to answer the question of potential relevance of this mechanism to evasion of host immunity by looking at other viral systems employing similar mechanisms. The importance of the IFN response in controlling viral infection is underscored by the large number of different viruses that encode genes blocking some aspect of this immune control mechanism. The paramyxoviruses have been shown to evade IFN responses through a variety of different mechanisms, including the directed degradation of STAT1 and STAT2 (95-97). Morrison *et al.* (98) have

demonstrated that in addition to pirating the cellular IFN response to modulate its own expression patterns, EBV also blocks the anti-viral IFN response through down regulation of the IFN-gamma receptor by BZLF1, an immediate early gene product. By down regulating the IFN-gamma receptor, EBV blocks the ability of the cell to transmit an IFN-gamma signal and mount an anti-viral response. HSV encodes at least three separate modulators of the IFN response. US11 and ICP34.5 likely target a similar IFN response pathway, the double-stranded RNA (dsRNA)-dependant protein kinase PKR pathway (99). After IFN stimulates its transcription, PKR binds to dsRNA and is activated to phosphorylate eIF-2a, preventing translational initiation. US11 binds dsRNA, preventing the activation of PKR, while ICP34.5 redirects protein phosphatase 1-alpha to dephosphorylate eIF-2a, releasing the PKR-mediated block to translation (100). Yet another protein, ICP0, has been recently identified by Mossman *et al.* (101) as playing a role in blocking the IFN response by a mechanism distinct from ICP34.5, that was not fully elucidated. The mechanisms by which human cytomegalovirus (HCMV) influences the host IFN responses are more mysterious. Various HCMV proteins have been demonstrated to induce cellular IFN-response genes (102-106). However, Chin and Cresswell (107) have demonstrated that while HCMV induces the expression of viperin, an IFN-inducible gene, the localization of the protein is different from when it is induced by IFN. Interestingly the authors of this study point out that vesicular stomatitis virus infection of mouse spleen cells induces the expression and relocalization of the murine homolog of viperin, raising the question of whether this phenomena is a more general anti-IFN viral strategy. From these examples it is clear that vIRF is likely aiding KSHV in host immune evasion and persistence.

3.2.2. The Chemokine Network

The chemokines are a large family of proteins that interact with cellular 7-transmembrane, G-protein-coupled receptors (GPCRs) to recruit leukocytes to sites of inflammation in addition to having putative roles in lymphocyte development and angiogenesis (reviewed in 108 and 109). Orfs K6 and K4 of KSHV express proteins of 95 a.a. and 94 a.a. in length and share approximately 43% and 52% amino acid identity, respectively, with the cellular CC chemokine monocyte inflammatory protein-1alpha (MIP-1alpha) (13, 85). These two gene products share almost 60% amino acid identity, suggesting that they arose through a gene duplication event. A third gene ORF K4.1, which expresses an 114 a.a. protein, has sequence homology not only with MIP-1 beta, but also with several other members of the cellular CC chemokine family (13, 14, 85, 110). These three KSHV genes are commonly referred to, respectively, as vMIP-I, vMIP-II and vMIP-III.

In competition assays, vMIP-II has been shown to be able to bind and block signaling events from 11 of the 17 known chemokine receptors including CC chemokine receptor 1 (CCR1), CCR2, CCR5 and CCR8 as well as the CXC chemokine receptor CXCR4 (111-115),(116). However, this binding is clearly distinct from that of the cellular ligand since no Ca^{++} flux occurs after binding. As is suggested by this finding, vMIP-II is capable of blocking the chemoattractive effects of both CC and CXC chemokines: monocyte chemoattractant protein 1; MIP-1beta; RANTES (regulated on activation, normal T cell expressed and secreted); fractalkine (113). One exception to this antagonistic role is the binding of vMIP-II to CCR3, which results in Ca^{++} flux and chemotaxis of eosinophils (111).

The vMIP-I chemokine homolog is not nearly so promiscuous as vMIP-II, of all the receptors tested, binding has only been demonstrated for the CCR8 chemokine receptor (112, 116). Unlike vMIP-II with its primarily antagonistic role, it acts as an agonist of this receptor and causes Ca^{++} flux upon binding. The CCR8 receptor is found on monocytes and T lymphocytes of the Th_2 lineage (117, 118). Haque *et al.* have presented evidence that the CCR8 receptor is additionally present on endothelial cells and in skin lesions from KS patients, with diffuse staining of the associated spindle cells with an anti-CCR8 antibody (119). Combined with the finding of Boshoff *et al.* (111) that both vMIP-I and vMIP-II were highly angiogenic in the chorioallantoic assay, it is thus reasonable to envision a role for vMIP-I in lesion genesis, both as an endothelial cell attractant and as a neo-angiogenic factor.

vMIP-III is the least well characterized of these three viral chemokines. Stine *et al.* (114) have published that vMIP-III is capable of weakly competing with several chemokine/receptor pairs. In addition, vMIP-III strongly induces chemotaxis of cells expressing CCR4 and weakly induces chemotaxis of CCR5 expressing cells. Examination of KS lesion tissue by Western blot revealed vMIP-III expression. Finally, they also report that vMIP-III is angiogenic in the chorioallantoic assay (114).

The $CD4^+$ T cells are the major regulatory cells of the immune system. Through secretion of various lymphokines, they are able to influence the host's immune system toward either a cellular or humoral defense. The Th_1 T lymphocytes mainly secrete IL-2, IFN-gamma and lymphotoxin while the Th_2 T lymphocytes mainly secrete IL-4, IL-5, IL-6 and IL-10. Lymphokine secretion by the Th_2 cells aids in the maturation of antibody-secreting plasma cells whereas Th_1 lymphokines help to induce monocytes and macrophages. Taken together, the vMIPs are capable of inducing chemotaxis from at least three different chemokine receptors: vMIP-I on CCR8; vMIP-II on CCR3; vMIP-III on CCR4. Each of these chemokine receptors is expressed on T lymphocytes of the Th_2 lineage (120). Indeed, immunohistochemical examination of KS lesions shows a predominance of CCR3+ Th_2 lineage T lymphocytes (121). The lymphokine profile of KS lesions is slightly more puzzling with the presence of both Th_2 IL-6 and Th_1 IFN-gamma cytokines (122, 123). However, causing the host's immune system to shift away from a Th_1 response has obvious advantages and precedence in other viral systems. EBV harbors an IL-10 homolog that functions to suppress the Th_1 lymphokine IFN-gamma (124), while measles can inhibit IL-12, a chemokine critical to the generation of a Th_1 response (125). In addition to homologs of the cellular chemokine receptors, HHV-8 also expresses a homolog of the cellular IL-6 chemokine that

promotes the Th_2 response (85, 110, 126). Presumably, shifting the immune response toward a Th_2 profile aids in viral escape from the host immune responses, although greater experimental evidence is needed in proof of this hypothesis. There are some suggestions that this altered chemokine profile could aid in the development of the characteristic vascular KS lesions (127).

DNA sequence analysis of the KSHV genome predicts that the K14 open reading frame, designated viral OX2 (vOX2), exhibits a significant level of homology with cellular OX2. Cellular OX2 belongs to a group of leukocyte glycoprotein with a broad expression profile. Both stimulatory and tolerogenic roles for OX2 in antigen presentation have been proposed (128, 129). It has been shown to provide a costimulatory signal for activated T cells, leading to an increase of IL-4 and TGF-beta production, but not IL-2 production (129). In contrast, unlike conventional costimulatory molecules such as CD40, CD80 and CD86, OX2 has been shown to deliver negative signals to macrophages and monocytes upon antigen recognition, resulting in decreased graft rejection in animals (128, 130). Disruption of the OX2 gene in mice leads to expansion of the macrophage and granulocyte populations in mesenteric lymph nodes, supporting the role of OX2 in attenuation of signaling in myeloid lineage cells (131). Recently, an OX2 receptor (OX2R) has been identified as a novel protein having a restricted expression in cells of the myeloid lineage (132) and the OX2-OX2R interaction delivers a restrictive signal to macrophages and thus, limits their activation.

The KSHV vOX2 gene is expressed as a 271 a.a. protein with an approximate apparent weight of 55 kDa. There are five putative N-glycosylation sites in the extracellular region of the protein and experiments with N-glycosidase digestion suggest that most if not all of the sites are utilized (133). The vOX2 glycoprotein is expressed on the surface of transiently transfected B cell lines as well as KSHV-infected PEL cell lines and its expression is increased by TPA induction. Treatment of macrophage/monocytes and dendritic cells with a soluble, glycosylated vOX2-GST fusion protein resulted in the production of both IL-1 beta, TNF-alpha, IL-6 and MCP-1. Production of these chemokines was enhanced by costimulation with IFN-gamma. Purified monocytes/macrophage and dendritic cells also produced IL-1 beta and TNF-alpha after incubation with vOX2-GST. Thus, like cellular OX2, vOX2 targets myeloid lineage cells, but unlike cellular OX2, vOX2 provides a positive signal to these cells to induce proinflamatory cytokine production (133). While the exact role of these cytokines and the targets for their action is still unclear, it is apparent that there is the potential for this virally-directed cytokine array to misguide or disrupt the host immune responses for viral dissemination and/or to facilitate cytokine–mediated angiogenic proliferation.

As observed for modulation of the host antiviral IFN responses, modulation of the host cytokine responses is an often utilized viral immune evasion strategy. Further exploration of the role that cytokines play in the pathogenicity of KSHV will require new model systems. These models will have to address not only the role that KSHV alteration of the cytokine profile plays in immune evasion, but also the role it may play in the development of the characteristic KS lesions and in viral dissemination. Translation of these effects into homologous viral systems will likely prove more difficult than for other KSHV immune evasion strategies.

3.3. Apoptosis

Apoptosis or programmed cell death is a mechanism by which the immune system has enlisted every cell of the body to participate in its own death. Whether triggered through signaling by a cell surface receptor such as Fas or through signaling from intracellular molecules, apoptosis proceeds through a cascade of kinases, caspases, phophatases and DNAases, resulting in the breakdown of mitochondrial potential, DNA fidelity and cell membrane integrity. Apoptosis is used to “prune” defective cells, those that are virally-infected, and those cells that have reached the end of their programmed life-span. Circumvention of the apoptosis response by viruses can potentially lead to uncontrolled proliferation and an immortalized phenotype, allowing for completion of the viral lifecycle.

A variety of viruses, including all of the known gamma herpesviruses, have hijacked cellular proteins which when expressed in the wrong context can contribute to an oncogenic event. In the case of KSHV, one such acquired gene is a homolog of the cellular bcl-2 gene. The cellular bcl-2 proto-oncogene was first discovered as a translocated gene in ~80% of patients with follicular B-cell lymphoma (134). It is the prototypical member of an extended family of structurally related proteins containing four conserved domains (BH1-BH4) and a carboxy-terminal hydrophobic transmembrane region. Cellular Bcl-2 protein is capable of both homodimerizing and heterodimerizing with other Bcl-2 family members including Bax (135). A number of models have been put forth to explain the protective effects of cellular Bcl-2. It may act as an ion channels to prohibit the destabilization of mitochondrial membranes or modulate the activation of caspases and inhibit cytochrome c export from the mitochondria (135).

Orf16, the KSHV encoded Bcl-2 homolog, is expressed as a 175 a.a. protein and only possesses 15-20% homology with the cellular Bcl-2 gene (13, 14). This homology is largely restricted to the BH1 and BH2 heterodimerization and death-repressor domains (30). vBcl-2 is most likely expressed as a lytic protein during the viral lifecycle (30, 136). However, little work has been published on the specific functioning of vBcl-2. In an early report, Sarid *et al.* demonstrated that vBcl-2 was able to inhibit Bax toxicity in yeast, partially block the apoptotic effects of Bax overexpression in GM701 fibroblasts and “homodimerize” with cellular Bcl-2 (22). In contrast, Cheng *et al.* were also able to demonstrate anti-apoptotic effects of vBcl-2, but were not able to demonstrate dimerization with human Bcl-2, Bcl-x_L, Bax, or Bak (30). Further work to better define specific vBcl-2 binding partners and important regions has not been published. In

addition to a putative role in inhibiting the innate apoptotic response, Ojala *et al.* have posited a possible role for vBcl-2 in blocking apoptosis induced by v-cyclin, a KSHV protein (137). vCyclin is a D-type cyclin that associates with the cellular cyclin dependent kinase 6 (CDK6) (138). Overexpression of both vCyclin and CDK6 results in apoptosis upon cell entry into S-phase, however, Ojala *et al.* demonstrated that vBcl-2 could block this apoptosis (139). The vCyclin/CDK6 complex phosphorylates cellular Bcl-2, inactivating it and blocking its anti-apoptotic activity. The vBcl-2 homolog is not susceptible to this phosphorylation and thus, its activity is not down regulated by the vCyclin/CDK6 complex. This ability to modulate apoptosis by expression of vBcl-2 and vCyclin may be a viral strategy to increase viral transmission, allowing a controlled form of apoptosis to occur only when it will release large numbers of viral particles. Finally, the HVS vBcl-2 homolog has also been shown to inhibit apoptosis mediated by Fas, dexamethasone, irradiation and radical oxygen species (140).

Many forms of apoptotic cell death require the activation of the ICE-like family of cysteine proteases (caspases). The most direct pathway appears to be triggered by some members of the tumor-necrosis-factor receptor (TNF-R) superfamily (CD95, TNFR-1 and TRAMP) . These receptors relay death signals through a cytoplasmic sequence motif called a death domain (DD), which interacts with the DD of the adaptor molecules FADD and/or TRADD recruiting them to the membrane. FADD then associates with the ICE-like protease FLICE (caspase-8, MCH5, MACH) through death-effector domains (DEDs) present at the carboxyl terminus of FADD and the amino terminus of FLICE, leading to the assembly of a receptor-associated death –inducing signaling complex (DISC). DISC-associated FLICE subsequently initiates proteolytic activation of other ICE family members, which in turn leads to apoptosis (31).

Orf K13 of KSHV, also termed vFLIP, is a homolog of the cellular FLICE inhibitory protein. It is expressed as a multicistronic transcript with orf 72 (vCyclin) and 73 (LANA) through differential splicing events (141). This gene locus is expressed either as a tricistronic RNA transcript encoding all three proteins or a bicistronic message coding for just the vFLIP and vCyclin proteins (141). When levels of these messages were examined in the PEL cell line BCP-1, it was found that the tricistronic message was expressed in unstimulated cells and the level of the bicistronic message increased after butyrate stimulation (142). *In vivo*, the bicistronic transcript is expressed abundantly in cells from KS skin lesions with the levels of vFLIP expression increased in late stage lesions as compared with early stage lesions, while the tricistronic transcripts are found mostly in cells of the lymph node of KSHV-positive patients (143). Low *et al.* have recently reported that there is an IRES element in the KSHV FLIP protein that allows for expression from the bicistronic message (144). However, Grundhoff and Ganem have shown evidence for alternative splicing creating a monocistronic message for vFLIP (145).

KSHV vFLIP is expressed as a 189 amino acid protein. It has homology with proteins expressed by equine herpesvirus-2, bovine herpesvirus-4 and HVS (31, 146, 147). All of these proteins, like cellular FLICE, contain two death-effector domains. One of these may bind to the death-effector domains of FADD (MORT-1), TRADD, RIP, RAIDD or FLICE and interfere with FADD-FLICE interaction, thereby inhibiting the recruitment and activation of FLICE by Fas (148, 149). Djerbi *et al.* have shown that expression of vFLIP does not alter the levels of cell surface Fas receptor expression, but dramatically blocks anti-Fas receptor antibody-mediated apoptosis (150). As was shown previously by Thome *et al.* (31), Djerbi *et al.* demonstrated a lack of caspase-8 activity as well as decreased caspase-9 and –3 activity after Fas receptor stimulation in cells expressing vFLIP (150). Tumors appeared more rapidly and grew to a greater size upon injection of A20 cells expressing vFLIP into both syngeneic and semiallogeneic mouse strains as compared to injection of parental A20 cells. Belanger *et al.* has confirmed the inhibition of caspase-8 activation by vFLIP (151). In the presence of vFLIP, less procaspase-8 was converted to the active form after Fas-stimulation, presumably blocking the Fas-mediated apoptosis cascade. The importance of this blockage to caspase-8 activation by the vFLIPs is brought into question by the data of Garvey *et al.* (152). Mutants of the Molluscum contagiosum virus (MCV) vFLIP protein, MC159, that were unable to block apoptosis, were still able to bind caspase-8 as well as FADD. However, the authors of the study did not look for inhibition of caspase activity directly. Thus, the mechanism of KSHV vFLIP still requires further study.

Information about the *in vivo* importance of the vFLIP gene comes from the work of Glycofrydes *et al.* (153). Deletion of Orf71, a vFLIP homolog of HVS, results in a virus still able to transform cells, replicate and cause pathogenesis in animals. Additionally, the vFLIP-negative virus replicated with wild-type kinetics in owl monkey kidney (OMK) cells. Further, the deletion virus was able to transform PBMC from humans, Callithrix jacchus and Saguinus oedipus. S. oedipus experimentally infected with the vFLIP deletion virus developed lymphoma in the same time frame as those infected with the wild-type virus. Continuously growing, spontaneous T cell lines were established from a number of different organs of the infected animals. Oddly, as compared with cell lines established from wild-type infected animals, these cells were not more sensitive to Fas-mediated apoptosis. This suggests that other HVS genes may play a role in blocking apoptosis or that vFLIP has more importance to the prevention of apoptosis during other phases of viral replication.

3.4. Natural Killer Cell (NK) Responses

Natural killer cells (NK cells) play an important role both in innate and adaptive immunity. They prowl the body looking for evidence that a cell has been altered, either through infection by pathogen, assault by mutagen or simple mistake in replication. If the NK cell finds such evidence, it has the ability to both kill the altered cell directly through the action of perforin and granzyme, but

also to produce cytokines that enhance the adaptive cellular responses against the cell.

Although the task that they perform is simple, NK cell biology and regulation is quite complex. Based on CD56 cell surface expression, they can be divided into two groups, the CD56 low or dim and the CD56 high or bright populations (reviewed in 154). The CD56 dim population comprises approximately 90% of the circulating NK cells. This group generally expresses high levels of CD16 (Fc gamma receptor III) and is more cytotoxic in a resting state than the minority CD56 bright population. In contrast, the CD56 bright population generally has a low level of CD16 expression but the ability to produce immense amounts of cytokines following stimulation. The CD56 dim population provides much of the antibody-dependant cellular cytotoxicity (ADCC) and direct cell lysis activity ascribed to NK cells while the CD56 bright population helps to stimulate and coordinate the adaptive immune responses through cytokine elicitation.

Each of these NK cell populations is controlled by activating and inhibitory cell surface receptors (reviewed in 155-160). These receptors can be split into four classes. First are the killer cell immunoglobulin-like receptors (KIR). These receptors are capable of binding to several MHC class I types including HLA-A, -B and –C. Second are the C-type lectin receptor family which are expressed on the cell surface as a heterodimer between CD94 and one of a number of NKG2 molecules. These receptors bind to HLA-G as well as the non-classical MHC haplotype, HLA-E in conjunction with leader sequences from the classical MHC haplotypes. The final two classes, the natural cytotoxicity receptors (NCR) and Ig-like transcripts (ILT) or alternatively named leukocyte immunoglobulin-like receptor (LIR) family are newer members of the ever-growing NK cell receptor family. The NCR transmit signals across the NK cell membrane by associating with a variety of immunoreceptor tyrosine-based activation motif (ITAM)-containing molecules. These have been shown to include both CD3-zeta and Fc-epsilonRI-gamma. Several ILT members have been shown to bind to HLA molecules, but ligands for the NKR family and several other ILT family members are still unknown.

In addition to these four main classes, a number of orphan receptors have also been identified including 2B4, important in the pathology of X-linked lymphoproliferative disease (XLP) (161). While the NCR predominantly stimulate NK cell activity, each of the other classes of receptors encompass both stimulatory and inhibitory members contributing to the complexities of NK cell regulation. To further complicate matters, the CD56 bright and dim populations of NK cells express different levels of these receptors. Generally, the CD56 bright population has low levels of KIR, low levels of ILT, high levels of CD94/NKG2. The CD56 dim population has much higher levels of KIR, but low levels of CD94/NKG2, with intermediate levels of ILT. The differing levels of these receptors probably play a important role in the apparent phenotypes of these two populations.

Important to NK surveillance are various adhesion molecules expressed on the cell surface (154, 162). These include beta integrins, able to bind to intracellular adhesion molecule 1 (ICAM-1), and CD2, able to bind LFA3. These molecules stabilize the interaction between the NK cell and the potential target allowing various inhibitory and stimulatory receptors to find their ligands on the opposite cell. Antibodies that block the binding of the NK adhesion molecules to the target cell have been shown to block NK cell lysis (163, 164).

Induction of NK cell killing activity is controlled through the action of both positive and negative regulatory molecules. The most prominent of these negative regulatory molecules is MHC class I. If MHC class I has been down regulated from the surface of a cell through any of a variety of mechanisms, then it will not be available to interact with the KIR or CD94/NKG2 partner on the NK cell. This means that no inhibitory signal will be transmitted to the NK cell to prevent it from killing. If the NK cell also receives some other positive signal, such as those transmitted by the NCR when they interact with their ligand, the NK cell will be activated to either kill or release cytokines. Evidence from Burshtyn *et al.* would suggest that when the KIR binds to MHC class I on the target cell a signal is passed to the NK cell, which lowers its ability to adhere to the target cell (165). This signal and response is fairly rapid, on the order of several minutes. This again points to the importance of adhesion molecules for NK cell cytotoxicity.

KSHV expresses two viral proteins, which in concert, act to block both the cytotoxic T cell response and targeting by NK cells. K3 and K5, the 11^{th} and 14^{th} open reading frames from the left end of the genome respectively, encode proteins with approximately 40% identity (14, 25). This high identity, a number of shared motifs and similar general structure has lead to the hypothesis that they arose through a gene duplication event. K3 shows homology with an immediate early gene product of bovine herpesvirus-4, IE1 and the K3 gene of MHV-68, while K5 shows homology with the HVS orf12 protein (25, 166). They are early lytic cycle proteins and show increased expression in PEL cells treated with TPA (166, 167). Both contain two transmembrane regions and a PHD/LAP family zinc-finger domain at their amino termini (14). These types of zinc finger domains are found in a number of E3 ubiquitin ligases (168). Downstream of the transmembrane regions they contain a variety of modules identified as being important in protein-protein interactions as well as protein trafficking (169). The importance of these motifs will be discussed further in the next section, Section 4.2, on the mechanisms of KSHV evasion of cytotoxic T cell responses.

Even though the K3 and K5 protein share many common features, they are phenotypically distinct when expressed *in vitro*. Expression of the K3 protein results in strong down regulation of MHC class I of multiple allotypes from the cell surface through increased endocytosis and targeted degradation (26, 28). In contrast, K5 is able to only weakly mediate the down regulation of MHC class I HLA-A and –B (26, 28). As described above, with increased down regulation of MHC class I, a major

inhibitory KIR ligand, one would expect that cells expressing both K3 and K5 would be subject to increased NK cell lysis. However, in experiments looking at the susceptibility of cells expressing K3, K5 or both proteins to killing by NK cell lines, it was found that cells expressing K5 alone or K5 in conjunction with K3 were much more resistant to NK cell killing than those expressing K3 alone (29). In addition, K5 was able to protect BJAB from lysis by YTS cells, an NK cell line that lacks any KIR and therefore is not inhibited from killing even cells expressing normal MHC class I levels. Further characterization of cells expressing K5 revealed that, in addition to lowered levels of MHC class I, they had dramatically lowered amounts of both B7-2 and ICAM(27, 29). As outlined above, ICAM plays an important role in the initial adherence between NK cells and potential targets. By down regulating ICAM from the cell surface, K5 reduces the ability of the NK cell to maintain contact with the target cell and diminishes the likelihood of a positive signal being transmitted to the NK cell. Hence, K5 blocks NK cell mediated lysis, not by causing a negative signal to be transmitted, but by preventing a positive one.

Due to our inability to test mutants of KSHV for pathogenicity *in vivo*, the importance of this interference with NK responses is still unclear. From other viral systems, it is known that the NK response is critical to control viral spread.

4. EVASION OF ADAPTIVE HOST IMMUNITY

The adaptive immune system consists of two arms, the cellular, cytotoxic CD8-positive T cell arm and the humoral, antibody-producing B cell arm. Both typically require antigen presentation in conjunction with MHC and a costimulatory signal for full activation. Positive and negative control of these adaptive immune responses is as complicated as those that govern the various innate responses. Elicitation of an antibody response requires not only that the B cell itself recognizes the correct antigen, but also that the correct stimulation from an activated CD4-positive T helper cell be present to cause it to differentiate into a plasma cell. Elicitation of a cytotoxic T cell (CTL) response is similar, requiring specific recognition by the T cell of MHC class I presented peptide plus costimulation or T helper produced IL-2. Hence, there exist numerous possibilities for deregulation by viral proteins.

4.1. Evasion of CTL Responses

Herpesviruses, as a group, have devised a number of different strategies to deal with the MHC class I-restricted cytotoxic T lymphocyte (CTL) responses made against viral proteins. These include a variety of different mechanisms for either inducing MHC class I endocytosis, preventing its transport to the cell surface or inducing its degradation (reviewed in 170 and 171). As detailed in section 3.4, KSHV encodes at least two gene products involved in the endocytosis of MHC class I and prevention of antiviral CTL responses. Upon expression of the K3 gene in BJAB cells, a B cell line, class I molecules are endocytosed from the cell surface into internal organelles which are TGN-46 and AP-1 gamma-adaptin subunit positive (169). This staining pattern suggests relocalization of the MHC class I to the trans-Golgi network (TGN), however, confirmatory electron microscopy is still needed. Subsequently, the class I molecules are redirected to a lysosomal compartment (169). Two groups, Ishido *et al.* and the group of Coscoy and Ganem have shown that MHC class I molecules are degraded in cells expressing K3 (26, 28). Coscoy and Ganem further demonstrated that this breakdown is prevented by inhibitors of the lysosomal protease system such as NH_4Cl and chloroquine (26). Surprisingly, the increased rate of MHC class I endocytosis and its redirection to the lysosome by K3 are genetically separable events.

The C-terminal cytoplasmic region of K3 contains a number of motifs that have been demonstrated to mediate trafficking and interaction functions in other proteins. These include a Y-X-X-phi endocytosis motif, a potential SH3-binding domain, two regions of negatively charged residues and four amino acids conserved in both K3 and K5, NTRV (14). In addition, as detailed above, both K3 and K5 contain a zinc finger domain at their amino termini. Each of these domains was mutagenized and it was found that the PHD/LAP zinc finger domain, Y-X-X-phi motif and NTRV residues were critical for the ability of K3 to mediate an increased rate of class I endocytosis (169). Mutation of the negatively charged residues had little effect on the ability of K3 to increase the rate of MHC class I endocytosis. Paradoxically however, overall cell surface levels of class I molecules showed little decrease as compared with wild-type cells. Examination by confocal microscopy demonstrated that class I molecules were being transported to the TGN, but were not redirected to the lysosomal compartment. When cell lysates were examined by Western blot for the presence of MHC class I molecules, it was found that BJAB cells expressing K3 with the negatively charged residues mutated expressed as much class I as the parental BJAB cells. This is in stark contrast to the wild-type K3 expressing BJAB cells, which have almost undetectable levels of MHC class I protein (169).

Coscoy *et al.* have also shown that the PHD/LAP zinc finger domain is critical for MHC class I down regulation by K3 and K5 (172). Further, they have demonstrated that the down regulation of B7-2 and ICAM by K5 requires an intact PHD/LAP motif. Mutation of this domain abrogates the ability of K5 to down regulate these various molecules. In addition, they found that each of the targeted proteins is ubiquitinylated in the presence of K5. The PHD/LAP domain of K5 was expressed as a GST fusion protein and used in an *in vitro* ubiqitinylation assay. This fusion protein was capable of cooperating with an E2 ubiquitin ligase to mediate self-ubiquitinylation. However, the mechanism of protein endocytosis and the importance of ubiquitinylation to this process still need to be addressed. It is possible, given the work from our lab showing that endocytosis and degradation are separable, that ubiquitinylation is critical to proteolysis of the various proteins and occurs subsequent to endocytosis rather than being its cause.

The murine gamma-herpesvirus-68 K3 homolog, mK3, also contains a PHD/LAP zinc finger motif (173).

Like KSHV K3, expression of mK3 results in the removal of MHC class I from the surface (173, 174). Unlike KSHV however, mK3 interacts with class I molecules in the ER, possibly targeting them for proteosomal destruction through the addition of ubiquitin. Boname *et al.* were able to show the direct binding of mK3 to MHC class I, something that has not been demonstrated for KSHV (174). They also demonstrated the ubiquitinylation of class I in the presence of mK3, however the amount of MHC class I molecules modified by ubiquitin was small and detection required ^{35}S-labeling and extremely long exposure times. Again, further experiments showing the necessity of ubiquitinylation to MHC class I down regulation and degradation are required.

The internalization and degradation of MHC class I molecules from the surface of K3 expressing cells would be expected to have a dramatic influence on surveillance by CTL. An absence of class I molecules from the surface would mean that the cell has no way to present stimulatory peptides to activate CTL killing and signal that the cell itself is infected. Indeed, Stevenson *et al.* have published that cells expressing K3 are decreased in their ability to present peptides to T cell hybridomas in a MHC class I-restricted manner indicating both potential evasion of CTL lysis and activation (173). The *in vivo* importance of CTL evasion through down regulation of MHC class I can be inferred from the wide spectrum of viruses encoding genes that interfere with MHC class I antigen presentation. HCMV encodes four proteins, US3, US2, US11 and US6 which either retain class I in the ER (US3) (175), redirect it to the cytoplasm for degradation (US2 and US11) (176) or interfere with MHC class I peptide loading (US6) (177). Murine CMV (MCMV) also encodes a number of proteins capable of inhibiting MHC class I presentation of peptides. The m152 gene product acts much like HCMV US3 and traps class I molecules in the ER (178). Expression of m152 in a recombinant vaccinia virus conferred protection from lysis by vaccinia-specific CTL (179). Further, viruses lacking the m152 gene were more susceptible to CTL lysis during early replication than was the wild-type virus and mortality rates and viral burden with the deleted virus were significantly lower (180). The m6 gene product of MCMV causes degradation of MHC class I (181), while the role of the m4 gene product is more complex. While m4 can bind to MHC class I in the ER, this complex is still transported to the cell surface (182). Kavanagh *et al.* propose that there is altered assembly or recognition of the class I molecules on the surface of m4 expressing cells that leads to altered CTL lysis (182). HSV encodes ICP-47, which acts much like HCMV US6 and prevents loading of MHC class I with peptide (183). Goldsmith *et al.* have demonstrated that ICP-47 null viruses have reduced neurovirulence in wild-type mice or nude mice reconstituted with $CD8^+$ T cells as compared with wild-type mice acutely depleted of $CD8^+$ cells, demonstrating the importance of CTL evasion in simplex neurovirulence (184). As one last example, EBV EBNA-1 also inhibits peptide processing and presentation by MHC class I (185). The protein achieves this through encoding a repetitive sequence rich in glycine and alanine residues. This repeat sequence is able to block the ubiquiting/proteosome pathway from degrading both EBNA-1 and other proteins within the same cell. This inhibition prevents loading of MHC class I molecules with peptides for presentation and thus, limits CTL lysis of EBV-infected cells. Even with the supporting data from other viral models, the overall importance of this mechanism to KSHV immune evasion still needs clarification. This includes measurements of the degree of MHC class I down regulation under physiological conditions and experiments to better demonstrate how strongly small changes in class I cell surface levels affect immune surveillance.

4.2. Evasion of B cell responses

The development of a humoral immune response is governed in ways that are very similar to those that govern the cellular immune responses. Both responses require recognition of a specific peptide ligand and the delivery of co-stimulatory signal. In the case of B lymphocytes, peptide recognition is through the B cell receptor (BCR) and the co-stimulatory signal is delivered through the interactions of the B cell with activated cells of the CD4-positive T helper subset or through complement binding by the CD21/CD35 complement receptor molecules. This "alternative" pathway of activating B cells seems to be critical for the development of humoral antibody responses against a number of pathogens. Mice deficient for C3 or C4, two important complement proteins, have decreased allospecific IgG responses. To date, no KSHV gene that specifically targets host B cell responses has been identified. However, by less direct mechanisms, vCBP, mentioned earlier as being important at blocking the innate complement response, and K3 and K5, which play a role in altering NK and CTL responses, could potentially effect B cell responses.

Elimination of complement deposition on KSHV infected cells by vCBP means that even if antigen was presented on the surface of cells in conjunction with MHC class II, there would be no costimulation directly presented by the infected cell to trigger a B cell response. Additionally, down regulation of the MHC class I by K3 and K5 means that no T helper response can be generated to provide a conventional CD40/CD40L costimulatory signal. Coscoy and Ganem have demonstrated that BJAB cells expressing K5 are reduced in their ability to stimulate Jurkat T cells (27). While this system is quite artificial, it suggests that K5 is capable of limiting B cell antigen presentation that in turn would limit the T helper responses against KSHV.

While K3 and K5 likely have a role in the prevention of antibody production against KSHV by targeting the T helper subset, no specific viral genes have been shown to directly interfere with antibody-mediated neutralization of KSHV. For example, HSV encodes two glycoproteins, gL and gE that together bind to the Fc portion of antibodies, limiting the antibody-mediated neutralization of virus. It is likely that further examination of the glycoproteins of KSHV might yield proteins with similar function. Additionally, the mechanics of viral entry into the target cell probably interferes, at least partially, with the anti-viral antibody response. While specific

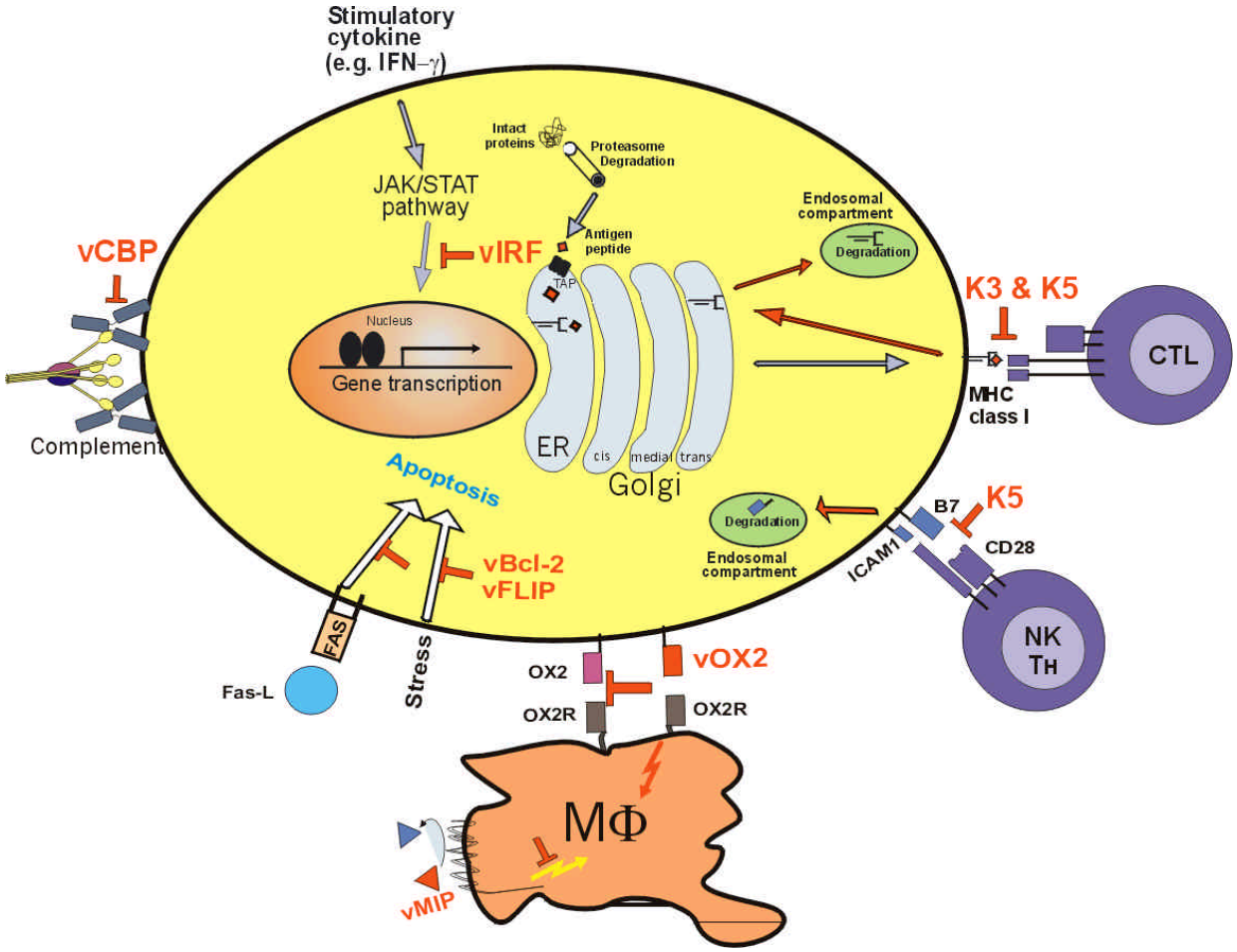

Figure 1. KSHV Immune Evasion Strategies. The KSHV gene products discussed in the text are shown in red letters next to the immune pathway that they block, indicated by a red T. Red arrows indicate either virally-induced signals, as from vOX2, or redirection of cellular gene products, in the case of both K3 and K5.

receptors have been identified as being important for viral entry, no clear evidence has been presented for any herpesvirus that the identified receptor is indispensable. Instead, it is more likely that viral entry occurs as a multi-step process and possibly can occur through several different mechanisms. If the host were able to block one mechanism, the virus would still be able to enter into target cells by another. The KSHV orf K8.1 gene product has been shown to bind to cell surface heparin sulfates, an activity that is also seen for HVS orf51 and HSV. This ability to bind to a wide variety of cell surface molecules allows for increased interaction of the viral particle with a large number of different cell types. Once in close contact with the cell the virus can wait for interaction with specific receptors that allow for viral entry. This strategy of interacting with two groups of receptors, one that mediates cell/virus juxtaposition and one that mediates entry, could allow the virus to occlude the more important entry ligand from humoral surveillance while the virus is in circulation and only expose it post cell binding just prior to cell entry. This strategy is employed by human immunodeficiency that utilizes two receptors, one of a variety of chemokine receptors to mediate juxtapositioning of the viral and cellular lipid membranes and CD4 to mediate viral fusion and entry. As yet, there is no evidence to support this mechanism for KSHV, but it is expected that this virus will have an equally complex and elegant strategy for evading the soluble humoral response.

5. DISCUSSION

In this review, we have outlined a number of potential strategies employed by KSHV to replicate in the presence of the ongoing host immune response. These strategies are schematized in Figure 1 and summarized in Table 1. This ability to evade the host's defenses is crucial to the ability of the virus to persist and spread. By studying each of these genes on a molecular level, researchers have gained insight into both KSHV biology and also the inner workings of the immune system. While the breadth of knowledge concerning the *in vitro* workings of KSHV genes continues to grow, information concerning the *in vivo* functioning lags. Greater use of closely related animal model systems such as MMRHV2, HVS and MHV68 along with improved culture systems should provide new understandings of the functional significance of these molecular mechanisms of immune evasion.

Table 1. KSHV immune evasion strategies

Innate Immunity	KSHV Gene
• Complement Cascade	vCBP
• Interferons (IFNs)	vIRF
• Cytokines	vMIP-1, vMIP-2, vMIP-3, vOX2
• Natural Killer Cell Lysis	K5
• Macrophage	vMIP-1, vMIP-2, vMIP-3, vOX2
• Apoptosis	vBcl-2, vFLIP
Adaptive Immunity	
• Cytotoxic T Lymphocyte Lysis	K3, K5
• Antibody Mediated Immunity	vCBP, Diverse receptor reservoir

6. ACKNOWLEDGEMENTS

The authors would like to thank Joong-Kook Choi for helpful discussions and review of the manuscript. This work was partly supported by U.S. Public Health Service grants CA82057, CA91819 and RR00168 and ACS grant RPG001102. Robert E. Means is supported through a fellowship from the Cancer Research Institute and Jae U. Jung is a Leukemia and Lymphoma Scholar.

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Key Words: KSHV (HHV-8), Immune evasion, K3, K5, vOX2, vIRF, Review

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