[Frontiers in Bioscience 12, 265-281, January 1, 2007]

Human Herpesvirus 8-Encoded Proteins with Potential Roles in Virus-Associated Neoplasia

John Nicholas

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231

TABLE OF CONTENTS

1. ABSTRACT

Human herpesvirus 8 (HHV-8) is a gamma-2 herpsvirus, related genetically to simian herpesvirus saimiri (HVS), the prototype virus of this subgroup of the gammaherpesvirus subfamily. HHV-8 DNA is present in all forms of Kaposis's sarcoma (KS) and primary effusion lymphoma (PEL), and in most forms of multicentric Castleman's disease (MCD), especially in HIV infected individuals. Of relevance to attempts to explain the molecular basis of HHV-8 associated neoplasia, are the unique genes specified by this virus, in particular angiogenic cytokines viral interleukin-6 (vIL-6) and viral CC-class chemokines (vCCL-1, vCCL-2, vCCL-3), mitogenic signaling membrane proteins variable ITAM-containing protein (VIP) and latency associated membrane protein (LAMP), pro-survival latently-expressed viral interferon regulatory factor (vIRF3), and the kaposin family of proteins that promote cell growth and cytokine production. Also of relevance are the angiogenic and cytokine-inducing viral G protein-coupled receptor (vGPCR), pro-proliferative and pro-survival latency proteins viral FLICE inhibitory protein (vFLIP) and latency-associated nuclear antigen (LANA), and G1-S phase cell-cycle promoter viral cyclin (v-cyclin), proteins specified also by other gamma-2 herpesviruses. The enormous progress on the characterization of the properties and biological activities of these proteins over the last ten years has provided insight into the potential mechanisms of HHV-8-induced neoplasia. Present data suggest that there operates a combination of cell transformation mediated by latently expressed proteins that promote cell proliferation and survival coupled with paracrine signaling functions mediated by either the viral cytokines or viral receptor-induced secreted cellular proteins. This review discusses the properties of the viral proteins believed to contribute to viral neoplasia via these mechanisms.

2. INTRODUCTION

Human herpesvirus 8 (HHV-8) is a gamma-2 subfamily herpesvirus that is associated with Kaposi's sarcoma (KS) and two rare forms of B cell malignancy, primary effusion lymphoma (PEL) and mutlicentric Castleman's disease (MCD). Before the discovery of HHV-8 in 1994, interleukin-6 (IL-6) had been implicated in KS and MCD as a pro-proliferative factor. The discovery of a functional homologue of IL-6 (viral IL-6, vIL-6) was therefore highly significant in attempts to explain the basis for HHV-8 induced disease. As KS is an angioproliferative disease in which various angiogenic, EC growth factors and pro-inflammatory cytokines are believed to be involved, the subsequent recognition of angiogenic activities of vIL-6, each of three HHV-8-encoded viral chemokines (vCCL-1, 2, 3) and the viral G protein-coupled receptor (vGPCR), and cytokine-inducing activities of the latter, expanded the candidate HHV-8 proteins mediating pathogenesis and the potential mechanisms of HHV-8-induced KS. Subsequently, evidence emerged that vascular endothelial growth factor (VEGF), a key angiogenic protein, was also important for PEL growth and dissemination in experimentally inoculated mice. Yet, each of the HHV-8 cytokines and vGPCR are expressed during productive (lytic) replication, and so any involvement would presumably be mediated in a paracrine fashion, from cells supporting lytic replication to latently infected and uninfected cells in the locality.

HHV-8 proteins expressed during latency comprise the ORF73-71 locus-encoded latency-associated antigen (LANA, ORF73), viral cyclin (v-cyclin, ORF72) and viral FLICE inhibitory protein (vFLIP, ORF71), a group of proteins called kaposins transcribed from an adjacent but distinct transcription unit, K12, and one of four viral interferon regulatory factors (vIRFs), namely vIRF3. As v-cyclin promotes G1-S transition and cell cycle progression, vFLIP mediates pro-survival signaling, LANA effects pro-proliferative and pro-survival functions, and kaposins induce cytokine expression and cell growth, each of these latency genes may contribute to HHV-8 neoplasia. vIRF3 blocks pro-apoptotic anti-viral interferon signaling and could also contribute. A model in which these latency functions effect cell proliferation, survival and transformation and in which paracrine activities mediated directly or indirectly by the lytically-expressed v-cytokines and vGPCR, perhaps at early stages of disease development, provides the most satisfactory mechanistic explanation of HHV-8-induced neoplasia, particularly KS.

In addition to the viral cytokines, vGPCR, and the four latency genes mentioned, there are two constitutive signaling membrane proteins encoded by genes and the left and right ends of the genome that may be involved in HHV-8 associated neoplasia. These genes are K1 and K15, specifying proteins VIP (variable ITAM-containing protein) and LAMP (latency associated membrane protein). While viral transcripts from K1 and K15 are most abundant during HHV-8 lytic replication, there is evidence that at least one form of LAMP is expressed during latency, and functional properties of VIP suggest that it may be of relevance to viral-induced cellular transformation if it is expressed during latency. VIP signals via Src-family kinases and mediates cell transformation in both *in vitro* and *in vivo* experimental models, and LAMP may specify anti-apoptotic functions.

This paper will review the properties of the v-cytokines, vGPCR, latency proteins and VIP and LAMP signaling receptors and discuss their possible contributions to HHV-8 induced neoplasia.

3. LATENCY PROTEINS

3.1. Latency-Associated Nuclear Antigen (LANA)

LANA is encoded by open reading frame (ORF) 73 and is part of a tricistronic transcription unit that includes ORFs 72 (v-cyclin) and ORF71 (vFLIP) (1-3). Two, alternatively spliced latency transcripts have been identified; one encodes all three ORFs [73, 72, 71 (5' to 3')] while the other splices out ORF73. The promoter that drives expression of this latency transcription unit lies a short distance upstream of ORF73 and contains elements both 3' and 5' of the transcription initiation site that are required for full activity (4, 5).

LANA is a pleiotropic protein, with functions that include latent origin binding and replication (6), chromosome tethering to enable viral genome segregation during cell division (7), pro-survival effects via inhibitory interactions with p53 (8), pro-proliferative functions via binding and inactivation of Rb and GSK3 to mediate release of E2F and activation/stabilization of β-catenin (9-11), respectively, and direct activation or repression of viral and cellular gene expression via interactions with transcription factors or promoter-recruitment of co-repressor complexes (5, 12-17) (Figure 1).

The Rb and p53 inactivating function are analogous to the those specified by viral oncoproteins of well-characterized tumor-inducing viruses, such as SV40 and adenovirus, and it would be reasonable to speculate that these activities of LANA could contribute to all forms of HHV-8 associated neoplasia. Thus, while the normal functions of these LANA activities may be to allow the latently infected cell to proliferate and survive, thus maintaining or expanding the latent viral reservoir, these activities could also lead to neoplasia by allowing the development and propagation of potentially transforming cytogentic changes. Also of potential relevance to HHV-8 pathogenesis is that one of the cellular genes induced by LANA is human telomerase reverse transcriptase (hTERT); transcription of hTERT appears to be mediated via interactions of LANA with the transcription factor Sp1 (17). hTERT activity is found at very low levels in normal cells but is activated in many cancers and allows survival of these cells. Clearly, LANA's activation of hTERT in latently infected cells could contribute to HHV-8 malignancies. Coupled with the predicted survival functions mediated by LANA through p53 inactivation and induction of hTERT expression and pro-proliferative effects of Rb inactivation (to release E2F and induce S-phase genes), the activation and stabilization of β-catenin is likely to contribute to cell proliferation and transformation and to HHV-8 disease. β-catenin is found at elevated

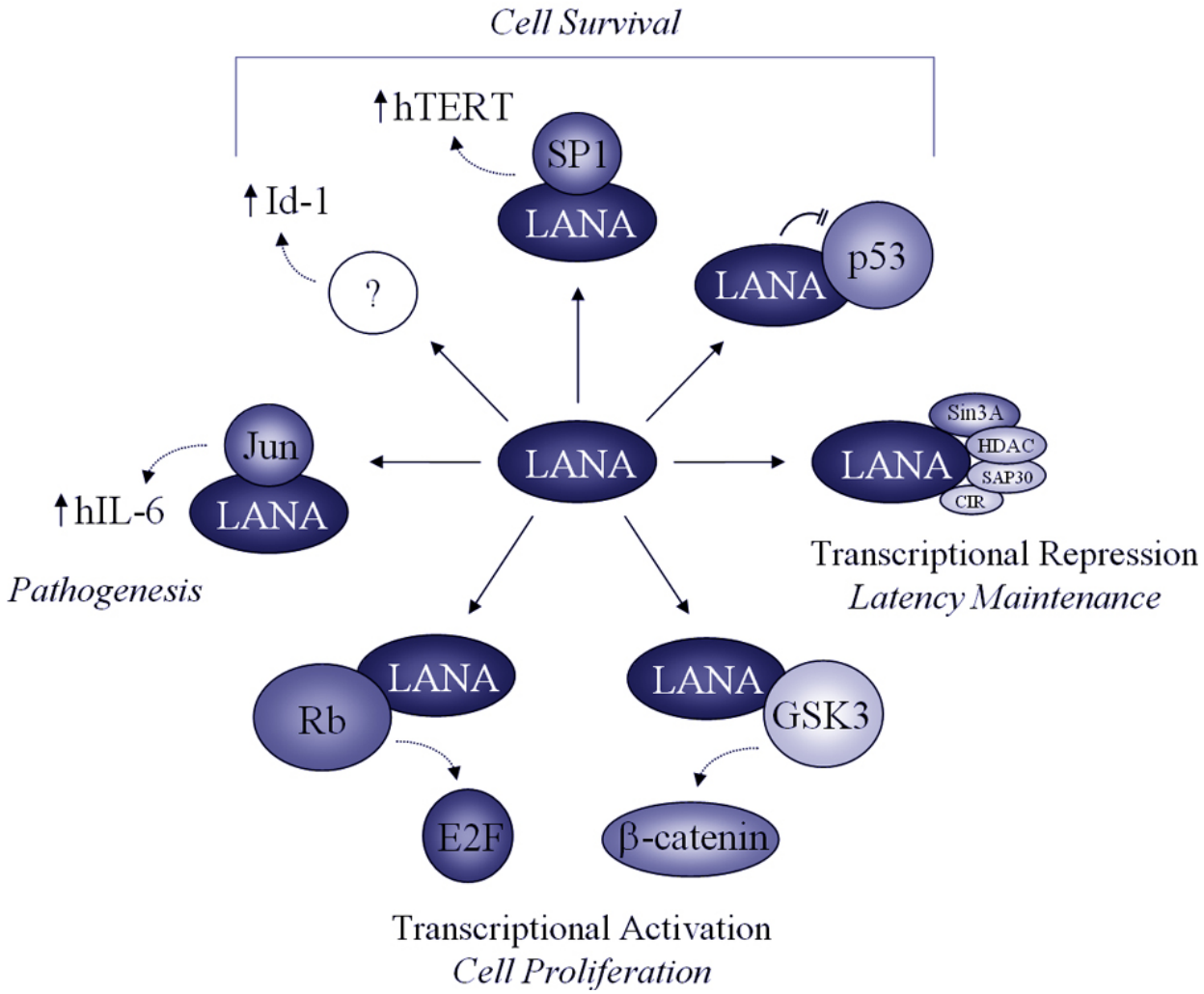

Figure 1. Overview of LANA functions. Pleiotropic activities of LANA include transcriptional activation, either directly at the promoter through interactions with transcription factors such as SP1 or Jun or via inactivation of glycogen synthase kinase 3(GSK3) or retinoblastoma protein (Rb) to stablize β-catenin and release E2F, respectively. β-catenin and E2F activation promote cell proliferation, which would be expected to expand the population of cells latently infected with HHV-8 and which could contribute to neoplasia. LANA can induce expression of hTERT (via SP1), Id-1 and hIL-6 (via Jun), and such gene regulation may also promote neoplasia via survival and proliferative effects as well as maintenance of the latently infected cell population. LANA also mediates transcriptional repression, one mechanism being through promoter recruitment of corepressor complexes. Repression of lytic genes would be important for latency maintenance. A further activity of LANA is the direct binding to and inhibition of the G1-S phase checkpoint protein and tumor suppressor, p53. Inhibition of p53 activity would be expected to promote cell survival and contribute to neoplasia.

levels in several cancers (18) and this can occur by various mechanisms. At least one mechanism by which LANA activates β-catenin is by nuclear sequestration of GSK3, a kinase that normally is cytoplasmic. One substrate of GSK3 is β-catenin, which when phosphorylated is targeted for proteasomal degradation. Therefore, by removing GSK3 from its site of action, LANA mediates activation of β-catenin. The biological relevance of this is emphasized by the presence of β-catenin at elevated levels in every primary effusion lymphoma (PEL) cell line examined, but not in EBV transformed B cells, for example, and that viral vector-transduced LANA can induce S-phase entry, the GSK3-binding region of LANA being required for this activity (9). Another group demonstrated independently that LANA-transduced human umbilical vein endothelial cells (HUVECs) had a higher rate of proliferation and prolonged life span compared to empty vector-transduced controls (19).

In addition to hTERT, other genes induced by LANA are human interleukin-6 (hIL-6), targeted via the transcription factor c-Jun that binds to the hIL-6 promoter (12), and the helix-loop-helix protein Id-1. Both proteins may play roles in HHV-8 pathogenesis. hIL-6 has been reported to stimulate KS cell growth, is found at elevated levels in the serum of MCD patients and to correlate with

disease severity (20, 21) and can support the clonal growth of PEL cells (22). In the case of Id-1, it has been demonstrated that expression of the protein in keratinocytes leads to their immortalization, accompanied by increased telomerase activity and Rb inactivation (23), and targeted expression of Id-1 in intestinal epithelial cells or thymocytes of transgenic mice leads to the development of intestinal adenomas and thymic lymphomas, respectively (24, 25). LANA expression in or HHV-8 infection of HUVECs has been shown to result in increased expression of Id-1, and Id-1 overexpression in primary ECs delays cell senescence (26, 27). KS tumor cells and KS-derived cells grown in culture express high levels of Id-1 (26).

3.2. Viral Cyclin

The viral cyclin (v-cyclin) gene, ORF72, is located immediately downstream of that for LANA, and, like LANA, is expressed during latency in PEL cell lines (1, 3). The HHV-8 D-type cyclin was shown to be functional, promoting cyclin dependent kinase 6 (cdk6) activation and G1-S cell cycle transition, but, unlike its cellular counterparts, being resistant to the inhibitory effects of the cdk inhibitors p16, p21 and p27 (28-30). In fact, HHV-8 v-cyclin can promote the cdk6-mediated phosphorylation and degradation of p27, and p27-phosphorylating cdk6-complexes have been identified in PEL cell lines and in KS, as have v-cyclin:cdk27 interactions (31-33). However, recent data indicate that in latently infected PEL cells, at least, v-cyclin:cdk6 mediates phosphorylation of serine-10 of p27, thereby enhancing its cytoplasmic localization rather than its degradation, promoted by threonine-187 phosphorylation (34). Interactions between v-cyclin and cdk6 have been demonstrated in *in vitro* replication assays to promote S-phase in addition G1-S transition (35). These results suggest a possible mechanism whereby HHV-8 might promote proliferation of latently infected cells and establish conditions that might lead to cell transformation and oncogenesis. Indeed, v-cyclin can promote cell growth in culture and induce tumors in transgenic mice when combined with loss of p53 functions (36), as might occur via LANA:p53 interactions in HHV-8 latently infected cells. It is perhaps worth noting, however, that the v-cyclin of herpesvirus saimiri (HVS) was found to be uninvolved in either productive replication or in development of lymphoma in experimentally infected animals (37).

Most other characterized gamma-2 herpesviruses encode a v-cyclin, that of HVS, the prototype gamma-2 herpesvirus, being the first to be discovered and shown to couple functionally to cdk6 (38, 39). The v-cyclin of murine gammaherpesvirus-68 (MHV-68) has been demonstrated to be required for lytic reactivation of virus from latently infected splenocytes of MHV-68 infected mice (40, 41). Thus, with respect to the functions of v-cyclins in normal virus biology, one role may be to provide the conditions necessary for lytic replication in normally non-permissive cells. It is noteworthy on this regard that HHV-8 v-cyclin promotes p27 threonine-187 phosphorylation, leading to p27 degradation, upon lytic reactivation in PEL cells (34). However, while it is likely that the gamma-2 herpesvirus v-cyclins share biological roles, the HHV-8 v-cyclin is currently the only v-cyclin demonstrated to be expressed during latency. Perhaps a role in maintaining the pool of latent virus by promoting proliferation of infected cells may be mediated by HHV-8 v-cyclin.

3.3. Viral FLIP

The viral FLICE [Fas-associated death domain IL-1β-converting enzyme (caspase8)] inhibitory protein, vFLIP, of HHV-8 is encoded by ORF71 (also known as K13, although it is in fact homologous to HVS ORF71). As shown initially for the vFLIP of HVS (42), HHV-8 vFLIP can block apoptosis induced by death receptor activation, and this activity also is mediated by vFLIPs from equine gammaherpesvirus-2 and the poxvirus molluscum contagiosum virus (43, 44). This activity of the viral vFLIPs therefore mimics the function of cellular FLIP proteins (cFLIPs, long and short form); all act by blocking the activating interaction of death receptor-bound adaptor protein FADD (Fas-associated death domain) with caspase 8 (45, 46). HHV-8 vFLIP appears to be expressed during lytic cycle replication as well as in latency, as specific vFLIP-encoding transcripts, in addition to a latently expressed transcript encoding LANA, vFLIP and v-cyclin, have been detected during lytic reactivation in PEL cells (47).

In addition the death receptor-inhibitory activity, HHV-8 vFLIP can interact with the regulatory component of the IκB kinase (IKK) complex to activate it and induce NFκB signaling, by the classical and alternative pathways (48-51). vFLIP-mediated activation of NFκB is able to protect cells against apoptosis induced by growth factor withdrawal (52) and is crucially important for survival of HHV-8 latently infected PEL cells (53). In the former case, protection is accompanied by increased expression of the pro-survival Bcl-2 family member Bcl-x_L. HHV-8 vFLIP has been demonstrated to transform Rat-1 fibroblasts, as determined by anchorage-independent growth in culture and formation of tumors in nude mice, and this activity is dependent on NFκB activation (54). However, another study using vFLIP-transduced A20 murine B lymphoma cells emphasized the importance of vFLIP-mediated anti-apoptotic functions for tumor growth, as tumor-promoting effects of vFLIP were seen only in immune competent mice, not in immune-deficient animals where vFLIP protection against T cell responses were not relevant (55). Combined, these data indicate that HHV-8 vFLIP is necessary for the normal growth and survival of HHV-8 transformed cells and that it may contribute to HHV-8 neoplasia by inhibiting both intrinsic and extrinsic apoptotic pathways via NFκB activation and FADD interactions, respectively. Presumably, these activities would normally serve to promote cell survival for the benefit of the virus, both during latency and also during productive replication where prolongation of cell viability in the face of pro-death signals induced by virus infection and replication would be expected to increase virus production.

Finally, it should be noted that the activation of NFκB signaling by vFLIP not only is important for cell

survival, but also can have other consequences. Furthermore, it is known that HHV-8 vFLIP can activate the JNK/AP1 pathway, which it does via its interaction with TNF receptor-associated factor 2 (TRAF2) (56, 57). One important effect of this dual activation of NFκB and AP1 is the induction of cellular IL-6 expression, and this has been demonstrated to occur in PEL cells (56). Interestingly, synergistic effects of vFLIP and LANA, previously shown to induce IL-6 expression (12), were found, suggesting that both of these latency genes contribute to IL-6 induction in latently infected cells. As outlined previously (LANA section) and discussed below (vIL-6 section), IL-6 is an angiogenic and mitogenic factor that is likely to play a significant role in KS, PEL and MCD.

3.4. Kaposins

The kaposins comprise a family of three proteins that are encoded by the K12 locus and expressed by via translation of alternative reading frames and utilization of non-AUG codons upstream of K12 in addition to the AUG codon that defines the start of the K12 ORF (58). The three translation products are referred to as Kaposins A, B and C. Kaposin A is encoded by ORF K12; kaposin B is translated from the most 5' CUG codon (in frame 2) in the major kaposin transcript and comprises 23 amino acid repetitive sequences derived from direct repeat (DR1 and DR2) elements but contains no K12-derived amino acids; kaposin C is translated from a downstream CUG codon (in frame 1) and comprises a fusion of DR1/DR2 and K12-encoded sequences. Transcripts ("T0.7") containing only K12 sequences have been identified in both KS and PEL cells, but when detected they were found to be of very low abundance (58). Translation of kaposin A from the abundant, larger transcript identified in BCBL-1 PEL cells would require internal initiation. It is now known that in addition to the DR2-DR1-K12 transcript initiating just 5' of DR2, there is another, spliced transcript that initiates some 5-kb upstream, 3' of ORF73 (59). Potential non-AUG initiation codons are present both in exon 1 (3' of ORF73) and exon 2 (starting 5' of DR2) that could be utilized to specify translation products differing at their N-termini from those encoded by the unspliced transcript. This spliced transcript, identified initially in primary PEL cells prior to culture, was found to be expressed also in multiple established PEL cell lines. Importantly, in the primary tissue, kaposin B was not detectable while kaposins A and C were expressed. This contrasts with the previously published data from BCBL-1 cells, in which kaposin B was the predominant protein (58).

With regard to the functions of the kaposins, the properties and biological activities of kaposins A and B have been investigated, and available data indicate that they could contribute to HHV-8 induced neoplasia. Kaposin A has been shown to mediate cell transformation in Rat-3 cell/nude mouse models (60). Insight into its mechanism of action was provided from independent studies showing that kaposin A could mediate signal transduction via membrane recruitment of the ARF GTPase-activating guanine nucleotide exchange factor (GEF) cytohesin-1 and that cytohesin-1 was necessary for kaposin A-induced cell transformation (Kliche *et al.*, 2001). Data relating to the function of kaposin B have been published recently (62). These elegant studies have revealed that the DR2-specified 23 amino acid repetitive sequences in the N-terminal half of the protein binds to a region (C-lobe) of the kinase MK2, a region targeted for phosphorylation and activation by p38 and bound by the C-terminal region of MK2 to effect auto-inactivation. Kaposin B binding to MK2 leads to activation of the kinase. As MK2 activity is known to stabilize mRNAs with AU-rich elements (AREs), including cytokine mRNAs, this suggests that kaposin B effects increased cytokine expression in HHV-8 infected cells, and indeed this activity of kaposin B has been demonstrated experimentally for mRNAs containing GM-CSF and IL-6 AREs (62). The biological significance of this activity of kaposin B is that it would be expected to lead to increased secretion of cytokines, such as IL-6, that are believed to play important roles in KS, PEL and MCD. In addition, the kaposin B-mediated increases in MKK6 kinase (encoded by an ARE-containing mRNA) and active forms of its target p38 could, along with increased cytokine production, be important for virus biology (although this has yet to be determined).

3.5. Viral Interefron Regulatory Factor 3 (vIRF3)

The HHV-8 genome contains coding sequences for four IRF homologues, namely vIRFs 1-4 specified by ORF K9 and ORFs K10, K10.5 and K11 spliced to upstream sequences (63-65). Of these, vIRF3 has been shown to be expressed during latency in PEL cell lines, whereas the other vIRFs appear to be expressed exclusively or predominantly as lytic genes (63, 64, 66-68). vIRF3 is expressed in the nucleus of PEL cell lines, and this has lead to its naming by some investigators as latency-associated nuclear antigen 2 (LANA2). It is important to note that while vIRF3 is a latent protein in PEL cell lines and has also been detected in lymphocytes in MCD tissue, it is undetectable in KS cells (68). It also appears to be absent in HHV-8 latently infected dermal microvascular endothelial cells (DMVECs) in culture (G. Hayward, pers. comm.). Therefore, while vIRF3 may be relevant to HHV-8 latent biology and disease, it seems that this is restricted to B cell populations and not of significance with respect to KS.

The fundamental role of vIRF3 in virus biology appears to be in blocking cellular IRF functions and IRF-stimulated pathways that lead to apoptosis. It has been reported that vIRF3 can inhibit that activities of IRF3 and IRF7 and, as a consequence, suppress the interferon-induction in response to virus infection (65). vIRF3 can also mediate protection against apoptosis by inhibition of p53 activity, which may involve direct interactions with the tumor suppressor (68), and can interfere with immune responses via inhibition of NFκB-activating IκB kinase β (IKKβ) (69). Therefore the overall biological effect of latently expressed vIRF3 parallels that of lytically expressed vIRFs 1 and 2 that inhibit activation or activities of cellular IRFs (70-74). While the latter activities would be predicted to enhance the efficiency of virus productive replication, by countering the cell's response to *de novo* infection and replication, the role of vIRF3 would be

predicted to promote survival of latently infected cells. Clearly, this activity could contribute to HHV-8 malignancies involving B cells (PEL, MCD), in which vIRF3 is expressed.

4. LYTIC PROTEINS

4.1. Viral Interleukin-6 (vIL-6)

The discovery of an IL-6 homologue in the HHV-8 genome was significant because IL-6 had been implicated in KS and MCD even before the discovery of HHV-8 (20, 21). That HHV-8 encoded its own version of this cytokine therefore suggested that vIL-6 may play a role in viral neoplasia. The amino acid sequences of vIL-6 protein is 25% identical to human IL-6 (hIL-6) and displays biological properties typical of this and other other cellular IL-6 proteins, such as support of IL-6-dependent murine B9 cell growth and induction of acute-phase genes in hepatocytes (75-77). Also in common with its cellular counterparts, vIL-6 mediates signaling through the gp130 signal transducer to activate Jak/STAT (primarily 1 and 3) and MAPK pathways (78-80). However, in contrast to endogenous IL-6 proteins, vIL-6 does not require the gp80 IL-6 receptor subunit (α-subunit) for formation of stable signaling complexes (78, 81). Notwithstanding, gp80 can be involved both physically and functionally in vIL-6 induced signaling complexes, stabilizing them and possibly modulating signal transduction, both quantitatively and qualitatively (80, 82; F. Hu & J. Nicholas, unpublished data).

In addressing the likely functions of vIL-6 in virus biology, it should be appreciated that while vIL-6 is expressed most abundantly during lytic replication, in both PEL and infected endothelial cell models, it is also expressed at low level in uninduced latently infected PEL cultures, in the absence of other lytic gene expression. It is conceivable, therefore, that vIL-6 plays a role during latency as well as during virus productive replication. Indeed, Chatterjee and colleagues (83) demonstrated that vIL-6 specifically was induced by treatment of PEL cells with IFNα, and effectively blocked the cell cycle arrest and apoptotic activities of IFNα. This suggests that at least one role of vIL-6 is to protect latently infected cells against anti-viral host defenses mediated by IFN, and could presumably perform a similar role during *de novo* infection or lytic reactivation. Other roles of vIL-6 during lytic replication remain speculative, but its mitogenic signaling and VEGF-inducing pro-angiogenic functions (77, 79, 84-86) suggest that it may be involved in establishing appropriate intracellular conditions for virus replication and extracellular conditions for dissemination of infected cells and virus from local sites of infection. Furthermore, as VEGF has been reported to enhance HHV-8 entry into cells via post-binding events (87), VEGF induced by vIL-6 (and other HHV-8 proteins, see below) may contribute to initial stages of HHV-8 infection via paracrine effects within an infected cell population.

With regard to the role of vIL-6 in HHV-8 associated disease, there is considerable evidence that it does indeed contribute to disease development. As already mentioned, VEGF is induced by vIL-6 and consequently angiogenesis is promoted by the viral cytokine (84). In murine models, cell lines stably expressing vIL-6, and secreting high levels of VEGF, are tumorigenic in nude mice and PEL cells introduced into nude mice develop lymphomatous effusions in a VEGF-dependent manner (84, 88). Also, vIL-6 expression in mice leads to increased hematopoiesis, plasmacytosis and organomegaly, features of MCD. Furthermore, vIL-6 is a mitogenic factor for PEL cells and therefore could contribute directly to PEL development (89). Similar pro-proliferative effects on gp130-expressing KS cells have been demonstrated and it has been reported that vIL-6 is able to induce endothelial expression of PTX3, an acute-phase protein, that would be expected to promote infiltration of inflammatory cells into sites of infection and contribute to the cytokine milieu supporting KS cell growth (86). VEGF induction by vIL-6 in endothelial/KS cells has not been investigated to date, but would be expected. Theoretically, then, there is support for the notion that vIL-6 can contribute to KS, PEL and MDC, but is there any evidence that vIL-6 is actually expressed in these tumors/effusions *in vivo*? Expression of vIL-6 has been probed for and found in KS, PEL and MCD primary tissue, although only some KS tissues (primarily advanced, nodular lesions) are positive for vIL-6, and vIL-6 has been detected in the sera of most PEL patients (90-97). In KS, PEL and MCD tissues, vIL-6 expression is found in only a minority of cells, presumably those undergoing abortive or productive lytic reactivation. That abortive rather than full lytic reactivation occurs is indicated by the fact that in PEL lines and KS tissue the proportion of cells expressing vIL-6 is higher than that staining positive for later lytic antigens (94, 95). Taken together, these findings suggest that vIL-6 expressed from a subset of HHV-8 infected cells during full or abortive reactivation could mediate mitogenic and angiogenic activities of relevance to HHV-8 associated malignancies (Figure 2).

4.2. Viral Chemokines (vCCLs)

There are three HHV-8 chemokines, named vCCL-1, vCCL-2 and vCCL-3, specified by ORFs K6, K4 and K4.1, respectively. The chemokines were previously called vMIP-1A/vMIP-I, vMIP-1B/vMIP-II and vBCK/vMIP-III (75, 77, 98, 99). All of the HHV-8 v-chemokines are expressed during productive replication and chemoattract Th2 cells, via their interactions either with the chemokine receptor CCR8 (vCCL-1, vCCL-2) or with CCR4 (vCCL-3), and therefore have been postulated to mediate immune evasion via polarization away from anti-viral Th1 responses (100-104). One of the chemokines, vCCL-2, is also able to interact as a neutral (non-signalling) ligand with a range of chemokine receptors, including CCR2, CCR5, CCR10, CXCR4, CX_3CR1, XCR1, and therefore to block agonist binding to these receptors, potentially mediating immune evasion via inhibition of immune cell infiltration into sites of lytic replication (105-108).

With regard to the potential roles of the v-chemokines as contributors to HHV-8 neoplasia, the most notable of their properties is their pro-angiogenic activities (103, 109). Thus, as for vIL-6, the induction of angiogenic

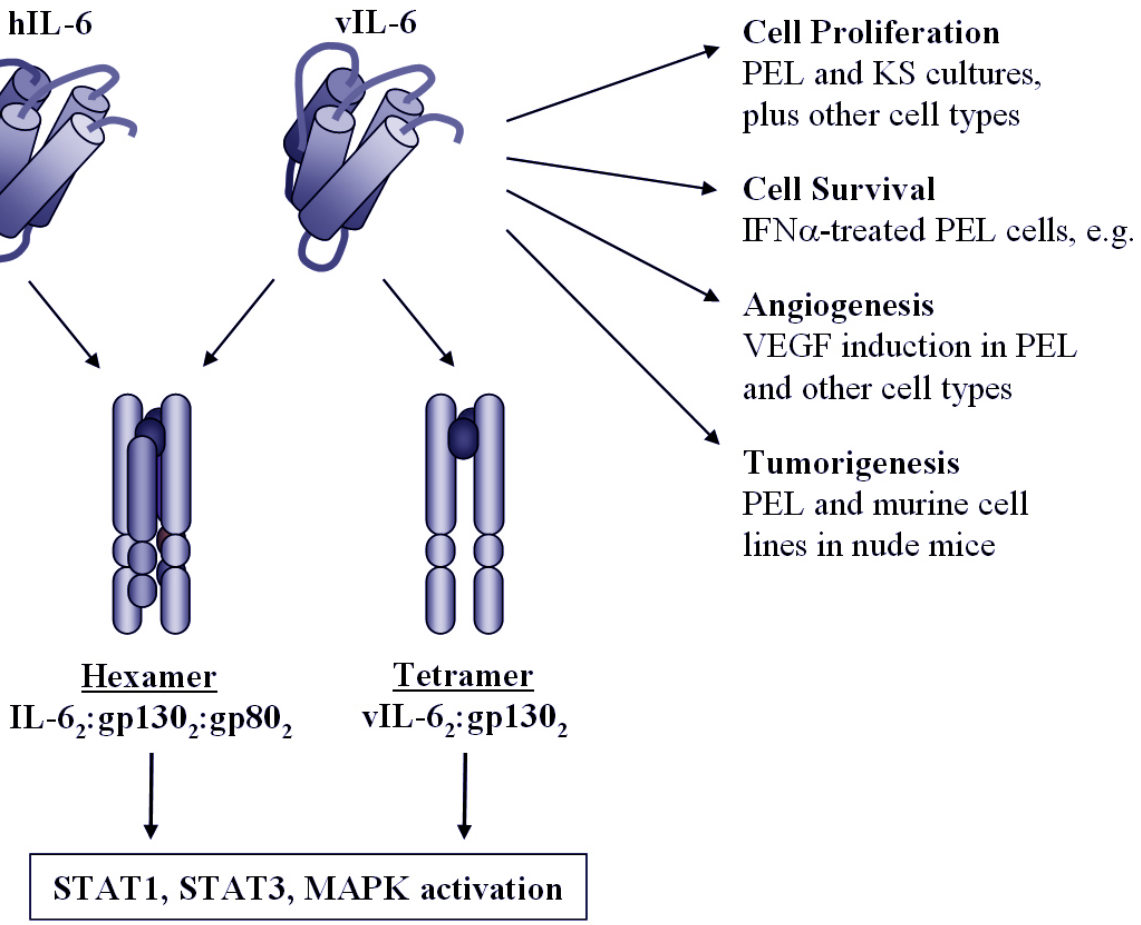

Figure 2. vIL-6 receptor complexes, signaling and activities. Like hIL-6, vIL-6 signals via dimerization and activation of the gp130 signal transducer. While vIL-6 can form hexameric complexes that include vIL-6, gp130 and the non-signaling α-subunit gp80 (IL-6R), analogous to the situation for hIL-6, gp80 is not required for vIL-6-induced gp130 dimerization and signaling. vIL-6 and hIL-6 both signal via STAT1, STAT3 and MAPK via Jak and SHP2 mediated mechanisms. Biological consequences of vIL-6 signal transduction include promotion of KS and PEL cell proliferation, PEL cell survival under conditions that would normally promote apoptosis, pro-angiogenic activity via induction of VEGF, for example, and pro-tumorigenic effects in murine model systems. These activities of vIL-6 suggest a role of the viral cytokine in HHV-8 associated pathogenesis.

factors would be expected to have a positive influence on endothelial/KS cell activation and proliferation and also to play a role in the progression and dissemination of PEL. It has been demonstrated that vCCL-1, at least, can induce VEGF expression in PEL cells, suggesting that autocrine signaling by the v-chemokine can promote the release of this and possibly other angiogenic factors (110). Pro-survival effects of vCCL-1 and vCCL-2, and also the CCR8 agonist I-309, have been noted in PEL cells (110) and vCCL-1 and I-309 can protect BW5147 murine lymphoma cells against dexamethasone-induced apoptotsis (111, 112). Pro-survival activities of the vCCLs would be expected to enhance virus production by countering the pro-apoptotic defenses of the host cell, but could also be of relevance to HHV-8-induced neoplasia.

4.3. Viral G Protein-Coupled Receptor (vGPCR)

Orthologues of the HHV-8 vGPCR are present in most other gamma-2 herpesviruses and are specified by ORFs 74. However, these vGPCRs are highly diverged with respect to their primary structures, and those that have been investigated functionally are distinguishable according to a number of criteria. For example, the HHV-8 and HVS vGPCRs are constitutively active whereas the MHV-68 vGPCR is ligand-dependent, the HHV-8, HVS and MHV-68 vGPCRs have distinct Gα protein-coupling profiles, and agonist stimulation of HHV-8 and HVS vGPCRs leads specifically to $G\alpha_q$-mediated signaling activation while $G\alpha_i$-mediated signaling is activated by agonist-bound MHV-68 vGPCR (113-115). Therefore, while the overall functions of these receptors in virus biology may be similar, presumably to establish intracellular conditions necessary for optimal virus replication and perhaps to induce the expression of secreted factors that have the same effect, their activities may be finely tuned to the cell types infected and virus genome contexts in which they found. Few studies have been reported on the functions of herpesvirus vGPCRs in lytic

replication. Two independent studies on MHV-68 vGPCR found that the receptor played a role in enhancing the efficiency of virus replication in culture and virus reactivation from latently infected B cells, although no effects were observed on lytic replication during primary infections of intranasally-innoculated mice (116, 117). In rat cytomegalovirus (RCMV), that encodes three vGPCRs, the R33-encoded receptor was found to be required for efficient replication *in vitro* and *in vivo* and for certain pathogenic effects in infected animals (118). Disruption of other vGPCRs in RCMV and murine CMV revealed phenotypes only *in vivo* , the vGPCR-altered viruses showing reduced growth and pathogenicity (119-121).

Whatever the precise role of HHV-8 vGPCR in virus biology, there is evidence that the receptor contributes to KS and possibly also to PEL and MCD via angiogenic and cytokine-inducing activities. The strongest evidence for a pathogenic role of vGPCR comes from the finding that transgenic or vector-transduced mice expressing the receptor, in endothelial cells or other cell types, develop endothelial lesions with remarkable resemblance to KS tumors (122-124). vGPCR is expressed in only a minority of cells within the lesions, but elevated levels of VEGF in these lesions have been noted. Therefore, the KS phenotype appears to be supported by vGPCR-induced paracrine signaling, presumably via VEGF and other cytokines induced in the vGPCR-expressing cells. A recent study has noted the induction of VEGF-family placental growth factor (PlGF), endothelial mitogen platelet derived growth factor B (PDGF-B), responsive receptors VEGFR-1 and PDGFRβ, and VEGF receptors 2 and 3 in the murine model, and determined through the use of a DOX-inducible system that continuous expression of vGPCR is required for maintenance of vGPCR-induced KS lesions (125). It is noteworthy that transgenic mice expressing and engineered version of HHV-8 vGPCR that is unable to bind chemokines, but is unaltered with respect to constitutive activity, fails to induce high rates of KS-like lesions in transgenic mice, implicating agonist-activated $G\alpha_q$/MAPK signaling and MAPK-effected VEGF induction as key to vGPCR pathogenicity (115, 126, 127). However, HHV-8 vGPCR is known activate a range pro-inflammatory, growth and angiogenic factors, such as TNFα, IL-1β, IL-2, IL-4, IL-6, IL-8, and bFGF, principally via NFκB activation, and these cytokines are also potential contributors to KS, and also to PEL and MCD (128, 129).

Further experimental evidence suggests a role of vGPCR and VEGF signaling in endothelial cell immortalization and therefore as contributors to KS. Thus, it has been shown that stable expression of vGPCR in primary endothelial cells leads to the outgrowth of cells that can be propagated indefinitely in culture and that expression of VEGF and VEGF receptors are induced in these cells (130). VEGF signaling was shown to be important for the growth and survival of these vGPCR-transduced endothelial cells, suggesting the vGPCR-induced establishment of a VEGF autocrine loop. However, if this scenario were to operate *in vivo* one would have to assume the occurrence of some abortive lytic replication by HHV-8 in endothelial cells, thereby allowing the expression of vGPCR in the absence of cell death that would result from full productive replication.

5. TERMINAL MEMBRANE SIGNALING PROTEINS

5.1. Variable ITAM-containing Protein (VIP)

HHV-8 VIP is specified by ORF K1, at the extreme left end of the genome. Transforming gammaherpesviruses HVS and EBV have genes at analogous genomic positions that encode signaling membrane proteins STP (saimiri transformation-associated protein) and LMP-1 (latency membrane protein-1), and these function as transforming proteins. STP and LMP-1 are not detectably homologous and neither of these proteins is homologous to HHV-8 VIP, but all three proteins are constitutively active signal transducers (131-136). HVS STP is required for HVS-mediated T cell transformation *in vitro* and for tumorigenesis in infected primate models, STP can mediate transformation of Rat-1 cells, and transgenic mice expressing STP develop T cell lymphomas or epithelial tumors (depending on the STP subtype) (137-139). LMP-1 is necessary for EBV-mediated immortalization of lymphocytes, can immortalize or fully-transform primary cells and cell lines in culture, and gives rise to B cell lymphomas in transgenic mice (140-144). Like STP and LMP-1, HHV-8 VIP can transform cells in culture, and K1 can also induce plasmablastic lymphomas and sarcomatoid tumors in transgenic mice expressing K1/VIP in multiple tissues (145, 146). Importantly, K1/VIP can substitute for STP in *in vitro* and *in vivo* transformation assays in the context of the HVS genome and virus infection, and when introduced into the murine gamma-2 herpesvirus MHV-68 K1/VIP was found to induce salivary gland adenocarcinomas in 25% of infected animals (145, 147). Thus, there is evidence to suggest that VIP may play a role in HHV-8-induced malignancies. However, "autocrine" transformation of the type demonstrated experimentally would require VIP to be expressed during latency, and this has not been demonstrated convincingly to date; indeed, evidence from PEL cells suggests that K1 is transcribed as an early lytic gene (64, 67, 148). The possibility remains, however, that VIP may be expressed during latency, either at very low levels that are not readily detectable or at higher levels in particular cell types, such as endothelial cells.

Apart from the possible role of VIP in HHV-8 malignancies via direct cellular transformation, the receptor may contribute to viral neoplasia, particularly KS, via the induction of angiogenic factors and inflammatory cytokines. VIP is known to activate SH2 domain-containing Src-family kinases, p85 subunit of PI3K, and PLCγ to initiate a range of downstream signaling cascades (133, 149-152). Of these, the PI3K/Akt pathway is of paramount importance in the regulation of cytokine expression, and VIP can induce cytokine expression via Akt-mediated NFκB activation (151). These cytokines include IL-6, IL-12 and GM-CSF. The angiogenic factors VEGF and matrix metalloproteinase 9 (MMP-9) are also induced by VIP, by a mechanism involving the SH2 binding motifs that comprise the C-tail ITAM

(immunoreceptor tyrosine-based activation motif), although the pathways required for induction of these proteins have not been determined (153). Pro-inflammatory and angiogenic factors are likely to contribute to KS, PEL and MCD by establishing the conditions for endothelial and B cell growth and promoting infiltration of inflammatory cells into sites of infection (90, 154).

What might the function of VIP be in virus biology? As already mentioned, VIP is an ITAM-containing signaling protein that activates a variety of pathways via Src-family tyrosine kinase, PI3K and PLCγ activation, but the biological consequences of this signaling are speculative at present. It is possible that dual mitogenic and survival signaling via these effector proteins allows efficient virus replication (149, 152). However, there is evidence that VIP may in fact block virus lytic replication, by acting as a lytic-latent switch and/or latency maintenance protein, although there are conflicting data in this regard (149, 155). The demonstrated downregulation of surface-expressed B cell receptor (BCR) complexes by VIP, analogous to EBV LMP-2 inhibition of B cell activation and latency maintenance, would be consistent with such a role (156). However, such a function of VIP clearly would require its expression during latency, and there is no firm evidence of this at present. A unique feature of VIP relative to other HHV-8 proteins is the fact that domains within its extracellular region are hypervariable, apparently the result of positive selection rather than drift (157, 158). However, the biological significance of this is unknown.

5.2. Latency Associated Membrane Protein (LAMP)

Encoded by K15, LAMP in its full-length form is predicted to comprise an integral membrane protein with twelve transmembrane domains, with cytoplasmic N- and C-termini (159, 160). The C-tail of LAMP contains SH2 and SH3 signaling motifs and sequences resembling CTAR-1 (C-terminal activation region-1) of LMP-1 that binds TNF receptor associated factors (TRAFs) (161, 162). The C-terminal region of LAMP has been demonstrated to bind TRAFs 1, 2 and 3, although apparently not with identical sequence requirements, and LAMP can activate NFκB signaling (160, 163). The SH2-binding motif (YEEVL), rather than the expected CTAR-like motif, is required for NFκB activation. The tyrosine residue of the YEEVL sequence is constitutively phosphorylated and along with the SH3-binding domain may be involved in TMP-mediated inhibition of B cell signalling, as CD8 antibody-induced oligomerization of a CD8-LAMP(C-tail) fusion protein was found to block IgM-induced intracellular calcium flux (159). Src-family protein tyrosine kinases Src, Lck, Hck, Yes and Fyn can associate with and phosphorylate the C-tail of LAMP, at least *in vitro* (163). In addition to NFκB activation, LAMP can mediate signal transduction via the Ras/Raf/MAPK pathway. Both NFκB and MAPK signaling are dependent on the YEEVL motif and TRAF2 and also appear to require other regions of the protein that are found in the full-length LAMP but not in "truncated" products of alternatively spliced mRNAs (163). In addition to potential mitogenic and survival signaling via Src-family kinases and NFκB activation, LAMP may also promote cell survival via interaction with the Bcl-2-related anti-apoptotic protein HAX-1 (164). While there is as yet no demonstration that this interaction promotes cell survival, LAMP and HAX-1 have been found to co-localize to mitochondria, consistent with the notion of their association *in vivo* and the possibility of such a function. The pro-mitogenic signal transducing and potential anti-apoptotic activities of LAMP could be relevant to HHV-8 neoplasia.

The expression of TMP is unclear. While a 23-kDa form of LAMP, a C-tail-containing product of a presumed proteolytic cleavage of the full-length 50-kDa protein, can be detected in latently infected PEL cells (BC-3), expression of K15 mRNA is essentially lytic, induced by TPA, with very low levels being detected in untreated PEL cells (BCBL-1, HBL-6) (64, 159, 164). Unusually, levels of the 23-kDa protein decline as K15 transcripts increase following TPA treatment (164). While it is difficult to explain these findings, the possibility does exist that the 23-kDa form of LAMP may be able to mediate signal transduction and possibly anti-apoptotic functions during latency. Although the predominant K15 transcript is the product of the splicing of eight exons, alternatively spliced forms of LAMP mRNA have been detected (163). The biological significance, if any, of the proteins encoded by these alternatively spliced mRNAs is unknown, but it has been established that they are only weakly active in signal transduction compared to full-length LAMP (163).

6. PERSPECTIVE

Research on the HHV-8 has been intense since the discovery of the virus in 1994, and much has been learned about the molecular biology of the virus and the possible mechanisms of virus-mediated pathogenesis. In contrast to the conventional paradigm of latency proteins being involved exclusively in herpesvirus neoplasia, via pro-proliferative and pro-survival effects on the cells in which they are expressed, the study of HHV-8, HHV-8 associated malignancies, and the functions of HHV-8 encoded cytokines and cytokine-inducing viral proteins, such as vGPCR, have indicated an alternative model in which latency and lytic functions combine to cause neoplasia (Figure 3). The main components of the model involve the mitogenic and survival functions of latently expressed LANA, v-cyclin, and vFLIP, coupled with the angiogenic and mitogenic functions specified by secreted vIL-6 and the v-chemokines as well as cellular cytokines and growth factors induced by these v-cytokines and by vGPCR. Other players may include the membrane proteins VIP and LAMP specified by the genome terminal ORFs K1 and K15, although the kinetics of expression and actual functions of these proteins in the life cycle of HHV-8 and in virus pathogenesis are far from clear. Latency proteins vIRF3 and the kaposins could also contribute to neoplasia via promotion of cell survival and pro-proliferative/cytokine inducing mechanisms, respectively.

It is unfortunate, although entirely understandable, that while most of the studies of viral gene expression kinetics and latency functions have been

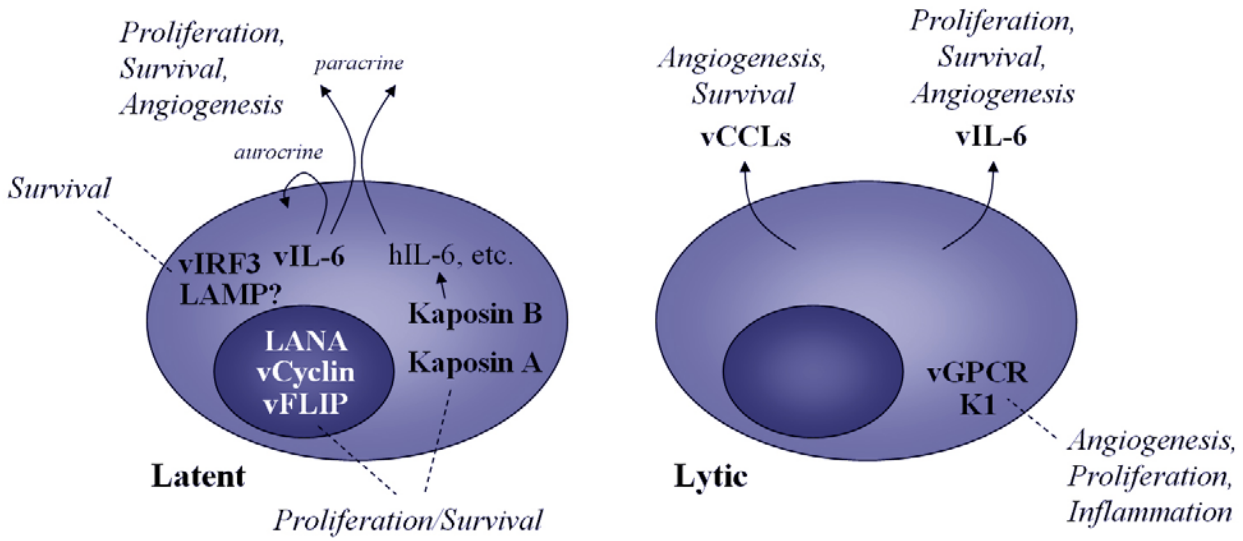

Figure 3. Hypothesized roles of HHV-8 latent and lytic proteins in virus-associated neoplasia. Both latent and lytic functions are likely to contribute to KS, PEL and MCD, lytic functions via paracrine promotion of cell proliferation and survival and also angiogenesis, and latent functions via pro-survival and mitogenic effects directly on latently infected cells. As vIL-6 has been demonstrated to be expressed in PEL and KS tissues independently of other lytic antigens and can be induced specifically by α-IFN in PEL cultures, vIL-6 may be expressed in latently infected cells and contribute to neoplasia in an autocrine fashion. LAMP (K15 protein) may also be expressed during latency, at least in PEL, and could promote cell survival via interactions with anti-apoptotic HAX-1. vIRF3, known to be expressed during latency in PEL cells, can promote cell survival via inhibitory interactions with cellular IRFs and p53. LANA, v-cyclin and vFLIP are expressed from the same latency transcription unit, and their pro-proliferative and survival functions are well established. Kaposins A and B have been shown to be expressed in PEL tissue and/or cell lines, and have either transforming (kaposin A) or cytokine mRNA stabilization (kaposin B) functions. An example of a cytokine that could be induced by kaposin B is hIL-6 (indicated). With regard to lytic viral genes implicated in neoplasia, the angiogenic viral cytokines vIL-6, vCCL-1, vCCL-2 and vCCL-3, and cellular cytokine-inducing vGPCR and VIP (K1 protein) receptors are key players. Pro-proliferative activity of vIL-6 and pro-survival functions of CCR8 agonists vCCL-1 and vCCL-2 could also contribute to HHV-8 neoplasia.

undertaken in PEL cells that are readily grown in culture and from which lytic replication and virus production can be induced, most of the models concerning virus-induced pathogenesis are based on KS, that is much more prevalent than the B cell malignancies PEL or MCD, and on endothelial cell biology. Thus, the central roles of inflammatory cytokines and angiogenic factors such as VEGF, bFGF, IL-6 and IL-8 in KS are fairly well established (154, 165), and so it is easy to imagine that vIL-6, the v-chemokines and vGPCR, that induce many of these factors, may contribute to KS. What is less clear is the precise interplay between these various viral and induced cellular factors, the expression levels required to mediate significant pathogenic effects, and the conditions under which these activities of these viral and cellular proteins result in KS. Endothelial culture models for HHV-8 latency, replication and virus- and viral protein-induced endothelial cell immortalization are available, although difficult to work with, and are beginning to be utilized more widely to try to address these complex issues. The work pioneered by Yang *et al.* (124) and similar subsequent studies by other groups (122, 123, 126) demonstrating the sufficiency of vGPCR functions and induced paracrine factors for the development of KS-like lesions in mice were seminal in that they provided firm supportive evidence for the paracrine model of HHV-8 pathogenesis. Related to this was a report from Enrique Mesri's laboratory demonstrating the ability of vGPCR to immortalize primary endothelial cells in culture by a mechanism involving induced VEGF/VEGFR-2 autocrine signaling (130). Similarly, HHV-8 infection of primary endothelial cells was found in independent studies to immortalize them, allowing long-term growth in culture, and to induce the expression of VEGF proteins and receptors (166, 167). There is evidence that paracrine angiogenic signaling is important not only in KS but also in PEL, as revealed by the elegant studies of Aoki and Tosato who demonstrated the requirement for PEL-secreted VEGF for PEL cell growth and dissemination in inoculated mice (88). As highlighted in this review, in addition to vGPCR there are several HHV-8 proteins, including vIL-6, the v-chemokines and VIP, that can induce the expression of VEGF species and that therefore have the potential to contribute to KS and PEL, and probably MCD also (168).

Where do we go from here? With regard to determining the role of HHV-8 in KS, what clearly is required is the development of culture systems that reflect accurately the *in vivo* situation, with respect to HHV-8 infection, persistence and reactivation, and also endothelial cell responses to HHV-8 infection. We also need a far better idea of the cellular gene expression profiles in HHV-

8 infected KS tumor and surrounding cells in order to determine the molecular mechanisms that account for KS disease. Unpublished work from Dr. Gary Hayward's laboratory (pers. comm.) indicates that there are major differences in the expression of important markers of resting vascular endothelial cells and those that are involved in neovascularization in HHV-8 infected ($LANA^+$) cells versus uninfected cells in KS tissues. These characteristic changes in cellular protein expression are reflected in human dermal microvascular endothelial cell (DMVEC) culture models, which also display the formation of spindloid cells and spindloid cell bundles upon infection by HHV-8 (169). Therefore, there is reason to believe that this culture system can provide an appropriate *in vitro* correlate of KS that can be used to study the properties and effects of HHV-8 genes in isolation and in the context of virus infection. With the development of an HHV-8 bacmid by Dr. S.-J. Gao (170), allowing us to undertake HHV-8 genetic manipulations, we now have the tools required to investigate the roles of viral genes in mediating changes in endothelial cell morphology and gene expression of relevance to KS pathogenesis, as well as to determine the roles of these genes in virus biology. We do not have an equivalent model of HHV-8 infection in B cells, as HHV-8 cannot immortalize or replicate in primary lymphocytes. However, if we could infect $HHV\text{-}8^-$ B cell lines to obtain latently infected cultures that can be induced chemically to turn on lytic replication and virus production, this would provide the basis for HHV-8 genetic and phenotypic analyses in this cell type and lead to a better understanding of HHV-8 biology and pathogenesis in B cells. It would allow direct comparison of infected versus uninfected B cells with regard to cellular gene expression profiles and growth characteristics, for example, and enable phenotypic analyses of viral mutants. Recent utilization of a B cell line derived from immortalized human BJAB cells to identify and replicate HHV-8 from patient samples and to derive latently infected cell lines provides hope that a generally useful B cell culture system can be obtained (171). Several other human B cell lines are available that could potentially be utilized. It appears as though the stage is set for further significant developments in the HHV-8 field.

7. ACKNOWLEDGEMENTS

I thank members of my laboratory, Daming Chen, Young Choi, Liping Feng, Fang Hu and Gordon Sandford, for their constant support, and also my colleagues Diane and Gary Hayward for sharing their insights. Support for work in my laboratory comes from NIH grants R01-CA76445 and R01-CA83519.

8. REFERENCES

1. D. Dittmer, M. Lagunoff, R. Renne, K. Staskus, A. Haase & D. Ganem: A cluster of latently expressed genes in Kaposis's sarcoma-associated herpesvirus. *J. Virol.* 72, 8309-8315 (1998)

2. R. Sarid, J.S. Wiezorek, P.S. Moore & Y. Chang: Characterization and cell cycle regulation of the major Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) latent genes and their promoter. *J. Virol.* 73, 1438-1446 (1999)

3. S.J. Talbot, R.A. Weiss, P. Kellam & C. Boshoff: Transcriptional analysis of human herpesvirus-8 open reading frames 71, 72, 73, K14, and 74 in a primary effusion lymphoma cell line. *Virology* 257, 84-94 (1999)

4. J. Jeong, J. Papin & D. Dittmer: Differential regulation of the overlapping Kaposi's sarcoma-associated herpesvirus vGCR (orf74) and LANA (orf73) promoters. *J. Virol.* 75, 1798-1807 (2001)

5. J.H. Jeong, J. Orvis, J.W. Kim, C.P. McMurtrey, R. Renne & D.P. Dittmer: Regulation and autoregulation of the promoter for the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. *J. Biol. Chem.* 279, 16822-16831 (2004)

6. M.E. Ballestas, P.A. Chatis & K.H. Kaye: Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. *Science* 284, 641-644 (1999)

7. M.A. Cotter & E.S. Robertson: The latency-associated nuclear antigen tethers the Kaposi's sarcoma-associated herpesvirus genome to host chromosomes in body cavity-based lymphoma cells. *Virology* 264, 254-264 (1999)

8. J. Friborg, W. Kong, M.O. Hottiger & G.J. Nabel: p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature* 402, 889-894 (1999)

9. M. Fujimuro, F.Y. Wu, C. ApRhys, H. Kajumbula, D.B. Young, G.S. Hayward & S.D. Hayward: A novel viral mechanism for dysregulation of β-catenin in Kaposi's sarcoma-associated herpesvirus latency. *Nat. Med.* 9, 300-306 (2003)

10. M. Fujimuro, J. Liu, J. Zhu, H. Yokosawa, & S.D. Hayward: Regulation of the interaction between glycogen synthase kinase 3 and the Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen. *J Virol.* 79, 10429-41 (2005)

11. S.A. Radkov, P. Kellam & C. Boshoff: The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. *Nat. Med.* 6, 1121-1127 (2000)

12. J. An, A.K. Lichtenstein, G. Brent & M.B. Rettig: The Kaposi's sarcoma-associated herpesvirus (KSHV) induces cellular interleukin 6 expression: role of the KSHV latency-associated nuclear antigen and the AP1 response element. *Blood* 99, 649-654 (2002)

13. A. Krithivas, D.B. Young, G. Liao, D. Greene & S.D. Hayward: Human herpesvirus 8 LANA interacts with proteins of the mSin3 corepressor complex and negatively regulates Epstein-Barr virus gene expression in dually infected PEL cells. *J. Virol.* 74, 9637-9645 (2000)

14. K. Lan, D.A. Kuppers, & E.S. Robertson: Kaposi's sarcoma-associated herpesvirus reactivation is regulated by interaction of latency-associated nuclear antigen with recombination signal sequence-binding protein Jκ, the major downstream effector of the Notch signaling pathway. *J Virol.* 79, 3468-78 (2005)

15. C. Lim, H. Sohn, Y. Gwack & J. Choe: Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) binds ATF4/CREB2 and inhibits its transcriptional activation activity. *J. Gen. Virol.* 81, 2645-2652 (2000)

16. C. Lim, Y. Gwack, S. Hwang, S. Kim & J. Choe: The transcriptional activity of cAMP response element-binding protein-binding protein is modulated by the latency associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus. *J. Biol. Chem.* 276, 31016-31022 (2001)
17. S.C. Verma, S. Borah & E.S. Robertson: Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus up-regulates transcription of human telomerase reverse transcriptase promoter through interaction with transcription factor Sp1. *J. Virol.* 78, 10348-10359 (2004)
18. R. Karim, G. Tse, T. Putti, R. Scolyer & S. Lee: The significance of the Wnt pathway in the pathology of human cancers. *Pathology* 36, 120-128 (2004)
19. T. Watanabe, M. Sugaya, A.M. Atkins, E.A. Aquilino, A. Yang, D.L. Borris, J. Brady & A. Blauvelt: Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen prolongs the life span of primary human umbilical vein endothelial cells. *J. Virol.* 77, 6188-6196 (2003)
20. S.A. Miles, A.R. Rezai, J.F. Salazar-Gonzalez, M. Vander Meyden, R.H. Stevens, D.M. Logan, R.T. Mitsuyasu, T. Taga, T. Hirano, T. Kishimoto & O. Martinez-Maza: AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc. Natl. Acad. Sci. USA* 87, 4068-4072 (1990)
21. K. Yoshizaki, T. Matsuda, N. Nishimoto, T. Kuritani, L. Taeho, K. Aozasa, T. Nakahata, H. Kawai, H. Tagoh, T. Komori, S. Kishimoto, T. Hirano & T. Kishimoto: Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. *Blood* 74, 1360-1367 (1989)
22. H. Asou, J.W. Said, R. Yang, R. Munker, D.J. Park, N. Kamada & H.P. Koeffler: Mechanisms of growth control of Kaposi's sarcoma-associated herpes virus-associated primary effusion lymphoma cells. *Blood* 91, 2475-2481 (1998)
23. R.M. Alani, J. Hasskarl, M. Grace, M.C. Hernandez, M.A. Isreal & K. Munger: Immortalization of primary human keratinocytes by the helix-loop-helix protein, Id-1. *Proc. Natl. Acad. Sci. USA* 96, 9637-9641 (1999)
24. D. Kim, X.C. Peng & S.-H. Sun: Massive apoptosis of thymocytes in T-cell-deficient *Id1* transgenic mice. *Mol. Cell. Biol.* 19, 8240-8253 (1999)
25. B.M. Wice & J.I. Gordon: Forced expression of Id-1 in the adult mouse small intestineal epithelium is associated with development of adenomas. *J. Biol. Chem.* 273, 25310-25319 (1998)
26. J. Tang, G.M. Gordon, M.G. Muller, M. Dahiya & K.E. Foreman: Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen induces expression of he helix-loop-helix protein Id-1 in human endothelial cells. *J. Virol.* 77, 5975-5984 (2003)
27. J. Tang, G.M. Gordon, B.J. Nickoloff & K.E. Foreman: The helix-loop-helix protein Id-1 delays onset of replicative scenscence in human endothelial cells. *Lab. Invest.* 82, 1073-1079 (2002)
28. Y. Chang, P.S. Moore, S.J. Talbot, C.H. Boshoff, T. Zarkowska, D. Godden-Kent, H. Paterson, R.A. Weiss & S. Mittnacht: Cyclin encoded by KS herpesvirus. *Nature* 382, 410 (1996)
29. M. Li, H. Lee, D.-W. Yoon, J.-C. Albrecht, B. Fleckenstein, F. Neipel & J.U. Jung: Kaposi's sarcoma herpesvirus encodes and functional cyclin. *J. Virol.* 71, 1984-1991 (1997)
30. C. Swanton, D.J. Mann, B. Fleckenstein, F. Neipel, G. Peters & N. Jones: Herpes viral cyclin/cdk6 complexes evade inhibition by CDK inhibitor proteins. *Nature* 390, 184-187 (1997)
31. M. Ellis, Y.P. Chew, L. Fallis, S. Freddersdorf, C. Boshoff, R.A. Weiss, X. Lu & S. Mittnacht: Degradation of $p27^{Kip}$ cdk inhibitor triggered by Kaposi's sarcoma virus cyclin-cdk6 complex. *EMBO J.* 18, 644-653 (1999)
32. A. Jarviluoma, S. Koopal, S. Rasanen, T.P. Makela & P.M. Ojala: KSHV viral cyclin binds to p27KIP1 in primary effusion lymphomas. *Blood* 104, 3349-3354 (2004)
33. D.J. Mann, E.S. Child, C. Swanton, H. Laman & N. Jones: Modulation of p27(Kip1) levels by the cyclin encoded by Kaposi's sarcoma-associated herpesvirus. *EMBO J.* 18, 654-663 (1999)
34. G. Sarek, A. Jarviluoma, & P. Ojala: KSHV viral cyclin inactivates p27KIP1 through Ser10 and Thr187 phosphorylation in proliferating primary effusion lymphomas. *Blood* 107, 725-732 (2006)
35. H. Laman, D. Coverley, T. Krude, R. Laskey & N. Jones: Viral cyclin-cyclin-dependent-kinase 6 complexes initiate nuclear DNA replication. *Mol. Cell. Biol.* 21, 624-635 (2001)
36. E.W. Verschuren, J. Klefstrom, G.I. Evan & N. Jones: The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss *in vitro* and *in vivo*. *Cancer Cell* 2, 229-241 (2002)
37. A. Ensser, D. Gycofrydes, H. Niphuis, E.M. Kuhn, B. Rosenwirth, J.L. Heeney, G. Niedobitek, I. Muller-Fleckenstein & B. Fleckenstein: Independence of herpesvirus-induced T cell lymphoma from viral cyclin D homologue. *J. Exp. Med.* 193, 637-642 (2001)
38. J. Nicholas, K.R. Cameron & R.W. Honess: Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. *Nature* 355, 362-365 (1992)
39. J.U. Jung, M. Stager & R.C. Desrosiers: Virus-encoded cyclin. *Mol. Cell. Biol.* 14, 7235-7244 (1994)
40. A.T. Hoge, S.B. Hendrickson & W.H. Burns: Murine gammherpesvirus 68 cyclin D homologue is required for efficient reactivation from latency. *J. Virol.* 74, 7016-7023 (2000)
41. L.F. van Dyk, H.W. Virgin & S.H. Speck: The murine gammaherpesvirus 68 v-cyclin is a critical regulator of reactivation from latency. *J. Viol.* 74, 7451-7461 (2000)
42. M. Thome, P. Schneider, K. Hofmann, H. Fickenscher, E. Meinl, F. Neipel, C. Mattmann, K. Burns, J.L. Bodmer, M. Schroter, C. Scaffidi, P.H, Krammer, M.E. Peter & J. Tschopp: Viral FLICE inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature* 386, 517-521 (1997)
43. J. Bertin, R.C. Armstrong, S. Ottilie, D.A. Martin, Y. Wang, S. Banks, G.H., Wang, T.G. Senkevich, E.S. Alnemri, B. Moss, M.J. Lenardo, K.J. Tomaselli & J.I. Cohen: Death effector domain-containing herpsvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 94, 1172-1176 (1997)
44. S. Hu, C. Vincenz, M. Buller & V.M. Dixit: A novel family of viral death effector domain-containing molecules that inhibit both CD-95- and tumor necrosis factor receptor-1-induced apoptosis. *J. Biol. Chem.* 272, 9621-9624 (1997)
45. A. Ashkenazi & V.M. Dixit: Death receptor signaling and modulation. *Science* 281, 1305-1308 (1998)

46. L.E. French & J. Tschopp: Inhibition of death receptor signaling by FLICE-inhibitory protein as a mechanism for immune escape of tumors. *J. Exp. Med.* 190, 891-894 (1999)
47. R. Sun, S.F. Lin, K. Staskus, L. Gradoville, E. Grogan, A. Haase & G. Miller: Kinetics of Kaposi's sarcoma-associated herpsvirus gene expression. *J. Virol.* 73, 2232-2242 (1999)
48. N. Field, W. Low, M. Daniels, S. Howell, L. Daviet, C. Boshoff & M. Collins: KSHV vFLIP binds to IKK-γ to activate IKK. *J. Cell. Sci.* 116, 3721-3728 (2003)
49. L. Liu, M.T. Eby, N. Rathore, S.K. Sinha, A. Kumar & P.M. Chaudhary: The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the IκB kinase complex. *J. Biol. Chem.* 277, 13745-13751 (2002)
50. H. Matta & P.M. Chaudhary: Activation of alternative NF-κB pathway by human herpes virus 8-encoded Fas-associated death domain-like IL-1β-converting enzyme inhibitory protein (vFLIP) *Proc. Natl. Acad. Sci. USA* 101, 9399-9404 (2004)
51. H. Matta, Q. Sun, G. Moses & P.M. Chaudhary: Molecular genetic analysis of human herpes virus 8-encoded viral FLICE inhibitory protein-induced NF-κB activation. *J. Biol. Chem.* 278, 52406-52411 (2003)
52. Q. Sun, H. Matta & P.M. Chaudhary: The human herpesvirus 8-encoded viral FLICE inhibitory protein protects against growth factor withdrawal-induced apoptosis via NF-κB activation. *Blood* 101, 1956-1961 (2002)
53. I. Guasparri, S.A. Keller & E. Cesarman: KSHV vFLIP is essential for the survival of infected lymphoma cells. *J. Exp. Med.* 199, 993-1003 (2004)
54. Q. Sun, S. Zachariah & P.M. Chaudhary: The human herpes virus 8-encoded viral FLICE-inhibitory protein induces cellular transformation via NF-κB activation. *J. Biol. Chem.* 278, 52437-52445 (2003)
55. M. Djerbi, V. Screpanti, A.I. Catrina, B. Bogen, P. Biberfeld & A. Grandien: The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors. *J. Exp. Med.* 190, 1025-1032 (1999)
56. J. An, Y. Sun, R. Sun & M.B. Rettig: Kaposi's sarcoma-associated herpesvirus encoded vFLIP induces cellular IL-6 expression: the role of the NF-κB and JNK/AP1 pathways. *Oncogene* 22, 3371-3385 (2003)
57. P.M. Chaudhary, A. Jasmin, M.T. Eby & L. Hood: Modulation of the NF-kappa B pathway by virally-encoded death effector domains-containing proteins. *Oncogene* 18, 5738-5746 (1999)
58. R. Sadler, L. Wu, B. Forghani, R. Renne, W. Zhong, B. Herndier & D. Ganem: A complex translational program generates multiple novel proteins from the latently expressed kaposin (K12) locus of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 73, 5722-5730 (1999)
59. H. Li, T. Komatsu, B.J. Dezube & K.M. Kaye: The Kaposi's sarcoma-associated herpesvirus K12 transcript from a primary effusion lymphoma contains complex repeat elements, is spliced, and initiates from a novel promoter. *J. Virol.* 76, 11880-11888 (2002)
60. S. Muralidhar, A.M. Pumfery, M. Hassani, M.R. Sadaie, M. Kishishita, J.N. Brady, J. Doniger, P. Medveczky & L.J. Rosenthal: Identification of kaposin (open reading frame K12) as a human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) transforming gene. *J. Virol.* 72, 4980-4988 (1998)
61. S. Kliche, W. Nagel, E. Kremmer, C. Atzler, A. Ege, T. Knorr, U. Koszinowski, W. Kolanus & J. Haas: Signaling by human herpesvirus 8 *kaposin A*, through direct membrane recruitment of *cytohesin-1*. *Mol. Cell* 7, 833-843 (2001)
62. C. McCormick & D. Ganem: The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. *Science* 307, 739-741 (2005)
63. C. Cunningham, S. Barnard, D.J. Blackbourn & A.J. Davison: Transcription mapping of human herpesvirus 8 genes encoding viral interferon regulatory factors. *J. Gen. Virol.* 84, 1471-1483 (2003)
64. G. Jenner, M.M. Alba, C. Boshoff & P. Kellam: Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. *J. Virol.* 75, 891-902 (2001)
65. B. Lubyova & P. Pitha: Characterization of a novel human herpesvirus 8-encoded protein, vIRF3, that shows homology to viral and cellular interferon regulatory factors. *J. Virol.* 74, 8194-8201 (2000)
66. F.D. Fakhari & D. Dittmer: Charting latency transcripts in Kaposi's sarcoma-associated herpsvirus by whole-genome real-time quantitative PCR. *J. Virol.* 76, 6213-6223 (2002)
67. M. Paulose-Murphy, N.-K. Ha, C. Xiang, Y. Chen, L. Gillim, R. Yarchoan, P. Meltzer, M. Bittner, J. Trent & S. Zeichner: Transcription program of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) *J. Virol.* 75, 4843-4853 (2001)
68. C. Rivas, A.-E. Thlick, C. Parravicini, P.S. Moore & Y. Chang: Kaposi's sarcoma-associated herpesvirus LANA2 is a B cell-specific latent viral protein that inhibits p53. *J. Virol.* 75, 429-438 (2001)
69. T. Seo, J. Park, C. Lim & J. Choe: Inhibition of nuclear factor κB by viral interferon regulatory factor 3 of Kaposi's sarcoma-associated herepsvirus. *Oncogene* 23, 6146-6155 (2004)
70. C.C. Flowers, S.P. Flowers & G.J. Nabel: Kaposi's sarcoma-associated herpsvirus viral interferon regulatory factor confers resistance to the antiproliferative effect of interferon-α. *Mol. Med.* 4, 402-412 (1998)
71. S.-J. Gao, C. Boshoff, S. Jayachandra, R.A. Weiss, Y. Chang & P.S. Moore: KSHV *ORF K9* (vIRF) is an oncogene which inhibits the interferon signaling pathway. *Oncogene* 15, 1979-1985 (1997)
72. S. Kirchhoff, T. Sebens, S. Baumann, A. Krueger, R. Zawatzky, M. Li-Weber, E. Meinl, F. Neipel, B. Fleckenstein & P.H. Krammer: Viral IFN-regulatory factors inhibit activation-induced cell death via two positive regulatory IFN-regulatory factor 1-dependent domains in the CD95 ligand promoter. *J. Immunol.* 168, 1226-1234 (2002)
73. M. Li, H. Lee, J. Guo, F. Neipel, B. Fleckenstein, K. Ozato & J.U. Jung: Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor. *J. Virol.* 72, 5433-5440 (1998)
74. J.C. Zimring, S. Goodbourn & M.K. Offermann: Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1-mediated transcription. *J. Virol.* 72, 701-707 (1998)

75. P.S. Moore, C. Boshoff, R.A. Weiss & Y. Chang: Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* 274, 1739-1744 (1996)
76. F. Neipel, J.-C. Albrecht, A. Ensser, Y.-Q. Huang, J.J. Li, A.E. Friedman-Kien & B. Fleckenstein: Human herpesvirus 8 encodes a homologue of interleukin-6. *J. Virol.* 71, 839-842 (1997)
77. J. Nicholas, V.R. Ruvolo, W.H. Burns, G. Sandford, X. Wan, D. Ciufo, S.B. Hendrickson, H.-G. Guo, G.S. Hayward & M.S. Reitz: Kaposi's sarcoma-associated human herpesvirus-8 encodes homologues of macrophage inflammatory protein-1 and interleukin-6. *Nat. Med.* 3, 287-292 (1997)
78. J. Molden, Y. Chang, Y. You, P.S. Moore & M.A. Goldsmith: A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit. *J. Biol. Chem.* 272, 19625-19631 (1997)
79. J. Osborne, P.S. Moore & Y. Chang: KSHV-encoded viral IL-6 activates multiple IL-6 signaling pathways. *Human Immunol.* 60, 921-927 (1999)
80. X. Wan, H. Wang & J. Nicholas: Human herpesvirus 8 interleukin-6 (vIL-6) signals through gp130 but has structural and receptor binding properties distinct from those of human IL-6. *J. Virol.* 73, 8268-8278 (1999)
81. D. Chow, X. He, A.L. Snow, S. Rose-John & K.C. Garcia: Structure of extracellular gp130 cytokine receptor complex. *Science* 291, 2150-2155 (2001)
82. M.J. Boulanger, D.C. Chow, E. Brevnova, M. Martick, G. Sandford, J. Nicholas & K.C. Garcia: Molecular mechanisms for viral mimicry of a human cytokine: activation of gp130 by HHV-8 interleukin-6. *J. Mol. Biol.* 335, 641-654 (2004)
83. M. Chatterjee, J. Osborne, G. Bestetti, Y. Chang & P.S. Moore: Viral interleukin-6-induced cell proliferation and immune evasion of interferon activity. *Science* 298, 1432-1435 (2002)
84. Y. Aoki, E.S. Jaffe, Y. Chang, K. Jones, J. Teruya-Feldstein, P.S. Moore & G. Tosato: Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. *Blood* 93, 4034-4043 (1999)
85. R. Burger, F. Neipel, B. Fleckenstein, R. Savino, G. Ciliberto, J.R. Kalden & M. Gramatzki: Human herpesvirus type 8 interleukin-6 homologue is functionally active on human myeloma cells. *Blood* 91, 1858-1863 (1998)
86. M. Klouche, N. Brockmeyer, C. Knabbe & S. Rose-John: Human herpesvirus 8-derived viral IL-6 induces PTX3 expression in Kaposi's sarcoma cells. *AIDS* 16, F9-18 (2002)
87. P.W. Ford, K.E. Hamden, A.G. Whitman, J.A. McCubrey & S.M. Akula: Vascular endothelial growth factor augments human herpesvirus-8 (HHV-8/KSHV) infection. *Cancer Biol. Ther.* 3, 876-881 (2004)
88. Y. Aoki & G. Tosato: Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas. *Blood* 94, 4247-4254 (1999)
89. K.D. Jones, Y. Aoki, Y. Chang, P.S. Moore, R. Yarchoan & G. Tosato: Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells. *Blood* 94, 2871-2879 (1999)
90. Y. Aoki, R. Yarchoan, J. Braun, A. Iwamoto & G. Tosato: Viral and cellular cytokines in AIDS-related malignant lymphomatous effusions. *Blood* 96, 1599-1601 (2000)
91. Y. Aoki, R. Yarchoan, K. Wyvill, S. Okamoto, R.F. Little & G. Tosato: Detection of viral interleukin-6 in Kaposi's sarcoma-associated herpesvirus-linked disorders. *Blood* 97, 2173-2176 (2001)
92. P. Brousset, E. Cesarman, F. Meggetto, L. Lamant & G. Delsol: Colocalization of the viral interleukin-6 with latent nuclear antigen-1 of human herpesvirus-8 in endothelial spindle cells of Kaposi's sarcoma and lymphoid cells of multicentric Castleman's disease. *Hum Pathol.* 32, 95-100 (2001)
93. J.S. Cannon, J. Nicholas, J.M. Orenstein, R.B. Mann, P.G. Murray, P.J. Browning, J.A. DiGiuseppe, E. Cesarman, G.S. Hayward & R.F. Ambinder: Heterogeneity of viral IL-6 expression in HHV-8-associated diseases. *J. Inf. Dis.* 180, 824-828 (1999)
94. C.-J. Chiou, L.J. Poole, P.S. Kim, D.M. Ciufo, J.S. Cannon, C.M. ap Rhys, D.J. Alcendor, J.-C. Zong, R.F. Ambinder & G.S. Hayward: Patterns of gene expression and a transactivation function exhibited by the vGCR (ORF74) chemokine receptor protein of Kaposi's sarcoma-associated herpesvirus. *J.Virol.* 76, 3421-3439 (2002)
95. C. Parravicini, B. Chandran, M. Corbellino, E. Berti, M. Paulli, P.S. Moore & Y. Chang: Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *Am. J. Pathol.* 156, 743-749 (2000)
96. K.A. Staskus, R. Sun, G. Miller, P. Racz, A. Jaslowski, C. Metroka, H. Brett-Smith & A.T. Haase: Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *J. Virol.* 73, 4181-4187 (1999)
97. J. Teruya-Feldstein, P. Zauber, J.E. Setsuda, E.L. Berman, L. Sorbara, M. Raffeld, G. Tosato & E.S. Jaffe: Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma. *Lab Invest.* 78, 1637-1642 (1998)
98. F. Neipel, J.-C. Albrecht & B. Fleckenstein: Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenicity? *J. Virol.* 71, 4187-4192 (1997)
99. J. Nicholas, V. Ruvolo, J. Zong, D. Ciufo, H.-G. Guo, M.S. Reitz & G.S. Hayward: A single 13-kilobase divergent locus in the Kaposi's sarcoma-associated hepesvirus (human herpesvirus 8) genome contains nine open reading frames that are homologous to or closely related to cellular proteins. *J. Virol.* 71, 1963-1974 (1997)
100. D.J. Dairaghi, R.A. Fan, B.E. McMaster, M.R. Hanley & T.J. Schall: HHV-8-encoded MIP-I selectively engages chemokine receptor CCR8: agonist and antagonist profiles of viral chemokines. *J. Biol. Chem.* 274, 21569-21574 (1999)
101. M.J. Endres, C.G. Garlisi, H. Xiao, L. Shan & J.A. Hedrick: The Kaposi's sarcoma-related herpesvirus

(KSHV)-encoded chemokine vMIP-I is a specific agonist for the CC chemokine receptor (CCR)8. *J. Exp. Med.* 189, 1993-1998 (1999)
102. S. Sozzani, W. Luini, G. Bianchi, P. Allavena, T.N. Wells, M. Napolitano, G. Bernardini, A. Vecchi, D. D'Ambrosio, D. Mazzeo, F. Sinigaglia, A. Santoni, E. Maggi, S. Romagnani & A. Mantovani: The viral chemokine macrophage inflammatory protein-II is a selective Th2 chemoattractant. *Blood*, 92, 4036-4039 (1998)
103. J.T. Stine, C. Wood, M. Hill, A. Epp, C.J. Raport, V.L. Schweickart, Y. Endo, T. Sasaki, G. Simmons, C. Boshoff, P. Clapham, Y. Chang, P. Moore, P.W. Gray & D. Chantry: KSHV-encoded CC chemokine vMIP-III is a CCR4 agonist, stimulates angiogenesis, and selectively chemoattracts TH2 cells. *Blood* 15, 1151-1157 (2000)
104. K.S. Weber, H.J. Grone, M. Rocken, C. Klier, S. Gu, R. Wank, A.E. Proudfoot, P.J. Nelson & C. Weber: Selective recruitment of Th2-type cells and evasion from a cytotoxic immune response mediated by viral macrophage inhibitory protein-II. *Eur. J. Immunol.* 31, 2458-66 (2001)
105. S. Chen, K.B. Bacon, L. Li, G.E. Garcia, Y. Xia, D. Lo, D.A. Thompson, M.A. Siani, T. Yamamoto, J.K. Harrison & L. Feng: *In vivo* inhibition of CC and CX_3C chemokine-induced leukocyte infiltration and attenuation of glomerulonephritis in Wistar-Kyoto (WKY) rats by vMIP-II. *J. Exp. Med.* 188, 193-198 (1998)
106. T.N. Kledal, M.M. Rosenkilde, F. Coulin, G. Simmons, A.H. Johnsen, S. Alouani, C.A. Power, H.R. Luttichau, J. Gerstoft, P.R. Clapham, I. Clark-Lewis, T.N. Wells & T.W. Schwartz: A broad-spectrum chemokine antagonist encoded by Kaposi's sarcoma-associated herpesvirus. *Science* 277, 1656-1659 (1997)
107. H.R. Luttichau, I. Clark-Lewis, J. Gerstoft & T.W. Schwartz. The herpesvirus 8-encoded chemokine vMIP-II, but not the poxvirus-encoded chemokine MC148, inhibits the CCR10 receptor. *Eur. J. Immunol.* 31, 1217-1220 (2001)
108. L. Shan, X. Qiao, E. Oldham, D. Catron, H. Kaminski, D. Lundell, A. Zlotnik, E. Gustafson & J.A. Hedrick: Identification of viral macrophage inflammatory protein (vMIP)-II as a ligand for GPR5/XCR1. *Biochem. Biophys. Res. Comm.* 268, 938-941 (2000)
109. C. Boshoff, Y. Endo, P.D. Collins, Y. Takeuchi, J.D. Reeves, V.L. Schweickart, M.A. Siani, T. Sasaki, T.J. Williams, P.W. Gray, P.S. Moore, Y. Chang & R.A. Weiss: Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* 278, 290-294 (1997)
110. C. Liu, Y. Okruzhnov, H. Li & J. Nicholas: Human herpesvirus 8 (HHV-8)-encoded cytokines induce expression of and autocrine signaling by vascular endothelial growth factor (VEGF) in HHV-8-infected primary-effusion lymphoma cell lines and mediate VEGF-independent antiapoptotic effects. *J. Virol.* 75, 10933-10940 (2001)
111. J. Louahed, S. Struyf, J.B. Demoulin, M. Parmentier, J. van Snick, J. van Damme & J.C Renauld: CCR8-dependent activation of the RAS/MAPK pathway mediates anti-apoptotic activity of I-309/ CCL1 and vMIP-I. *Eur. J. Immunol.* 33, 494-501 (2003)
112. J. van Snick, F. Houssiau, P. Proost, J. van Damme & J.C. Renauld: I-309/T cell activation gene-3 chemokine protects murine T cell lymphomas against dexamethasone-induced apoptosis. *J. Immunol.* 157, 2570-2576 (1996)
113. J.P. Couty, E. Geras-Raaka, B.B. Weksler & M.C. Gershengorn: Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor signals through multiple pathways in endothelial cells. *J. Biol. Chem.* 276, 33805-33811 (2001)
114. M.M. Rosenkilde, K.A. McLean, P.J. Holst & T.W. Schwartz: The CXC chemokine receptor encoded by herpesvirus saimiri, ECRF3, shows ligand-regulated signaling through G_i, G_q, and $G_{12/13}$ proteins but constitutive signaling only through G_i and $G_{12/13}$ proteins. *J. Biol. Chem.* 279, 32524-32533 (2004)
115. D. Verzijl, C.P. Fitzsimons, M. van Dijk, J.P. Stewart, H. Timmerman, M.J. Smit & R. Leurs: Differential activation of murine herpesvirus 68- and Kaposi's sarcoma-associated herpesvirus-encoded ORF74 G protein-coupled receptors by human and murine chemokines. *J. Virol.* 78, 3343-3351 (2004)
116. B.J. Lee, U.H. Koszinowski, S.R. Sarawar & H. Adler: A gammaherpesvirus G protein-coupled receptor homologue is required for increased viral replication in response to chemokines and efficient reactivation from latency. *J. Immunol.* 170, 243-251 (2003)
117. N.J. Moorman, H.W. Virgin & S.H. Speck: Disruption of the gene encoding the γHV68 v-GPCR leads to decreased efficiency of reactivation from latency. *Virology* 307, 179-190 (2003)
118. P.S. Beisser, G. Grauls, C.A. Bruggeman & C. Vink: Deletion of the R78 G protein-coupled receptor gene from rat cytomegalovirus results in an attenuated, syncytium-inducing mutant strain. *J. Virol.* 73, 7218-7230 (1999)
119. P.S. Beisser, C. Vink, J.G. van Dam, G. Grauls, S.J. Vanherle & C.A. Bruggeman: The R33 G protein-coupled receptor gene of rat cytomegalovirus plays an essential role in the pathogenesis of viral infection. *J. Virol.* 72, 2352-2363 (1998)
120. N.J. Davis-Poynter, D.M. Lynch, H. Vally, G.R. Shellam, W.D. Rawlinson, B.G. Barrell & H.E. Farrell: Identification and characterization of a G protein-coupled receptor homolog encoded by murine cytomegalovirus. *J. Virol.* 71, 1521-1529 (1997)
121. S.J. Kaptein, P.S. Beisser, Y.K. Gruijthuijsen, K.G. Savelkouls, K.W. van Cleef, E. Beuken, G.E. Grauls, C.A. Bruggeman, & C. Vink: The rat cytomegalovirus R78 G protein-coupled receptor gene is required for production of infectious virus in the spleen. *J Gen Virol.* 84, 2517-30 (2003)
122. H.-G. Guo, M. Sadowska, W. Reid, E. Tschachler, G. Hayward & M. Reitz: Kaposi's sarcoma-like tumors in a human herpesvirus 8 ORF74 transgenic mouse. *J. Virol.* 77, 2631-2639 (2003)
123. S. Montaner, A. Sodhi, A. Molinolo. T.H. Bugge, E.T. Sawai, Y. He, Y. Li, P.E. Ray & J.S. Gutkind: Endothelial infection with KSHV genes *in vivo* reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. *Cancer Cell* 3, 23-36 (2003)
124. T.-Y. Yang, S.-C. Chen, M.W. Leach, D. Manfra, B. Homey, M. Wiekowski, L. Sullivan, C.-H. Jenh, S.K. Narula, S.W. Chensue & S.A. Lira: Transgenic expression of the chemokine receptor encoded by human herpesvirus 8

induces an angioproliferative disease resembling Kaposi's sarcoma. *J. Exp. Med.* 191, 445-454 (2000)
125. K.K. Jensen, D.J. Manfra, M.G. Grisotto, A.P. Martin, G. Vassileva, K. Kelly, T.W. Schwartz, and S.A. Lira: The human herpes virus 8-encoded chemokine receptor is required for angioproliferation in a murine model of Kaposi's sarcoma. *J. Immunol.* 174, 3686-3694 (2005)
126. P.J. Holst, M.M. Rosenkilde, D. Manfra, S.-C. Chen, M.T. Wiekowski, B. Holst, F. Cifire, M. Lipp, T.W. Schwartz & S.A. Lira: Tumorigenesis induced by the HHV8-encoded chemokine receptor requires ligand modulation of high constitutive activity. *J. Clin. Invest.* 108, 1789-1796 (2001)
127. A. Sodhi, S. Montaner, V. Patel, M. Zohar, C. Bais, E.A. Mesri & J.S. Gutkind: The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting ion hypoxia-inducible factor 1α. *Cancer Res.* 60, 4873-4880 (2000)
128. S. Pati, M. Cavrois, H.-G. Guo, J.S. Foulke, J. Kim, R.A. Feldman & M. Reitz: Activation of NF-κB by the human herpesvirus 8 chemokine receptor ORF74: Evidence for a paracrine model of Kaposi's sarcoma pathogenesis. *J. Virol.* 75, 8660-8673 (2001)
129. M. Schwarz & P.M. Murphy: Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor constitutively activates NF-κB and induces proinflammatory cytokine and chemokine production via a C-terminal signaling determinant. *J. Immunol.* 167, 505-513 (2001)
130. C. Bais, A. Van Geelen, P. Eroles, A. Mutlu, C. Chiozzini, S. Dias, R.L. Silverstein, S. Rafii & E.A. Mesri: Kaposi's sarcoma associated herpesvirus G protein-coupled receptor immortalizes human endothelial cells by activation of the VEGF receptor-2/ KDR. *Cancer Cell* 3, 131-143 (2003)
131. J.U. Jung & R.C. Desrosiers: Association of the viral oncoprotein STP-C488 with cellular ras. *Mol. Cell. Biol.* 15, 6506-6512 (1995)
132. M. Lagunoff, R. Majeti, A. Weiss & D. Ganem: Deregulated signal transduction by the K1 gene product of Kaposi's sarcoma-associated herpesvirus. *Proc. Natl. Acad. Sci USA* 96, 5704-5709 (1999)
133. H. Lee, J. Guo, M. Li, J.-K. Choi, M. DeMaria, M. Rosenzweig & J.U. Jung: Identification of an immunoreceptor tyrosine-based activation motif of K1 transforming protein of Kaposi's sarcoma-associated herpesvirus. *Mol. Cell. Biol.* 18, 5219-5228 (1998)
134. H. Lee, J.-K. Choi, M. Li, K. Kaye, E. Kieff & J.U. Jung: Role of cellular tumor necrosis factor receptor-associated factors in NF-κB activation and lymphocyte transformation by herpesvirus saimiri STP. *J. Virol.* 73, 3913-3919 (1999)
135. H.-P. Li & Y.-S. Chang: Epstein-Barr virus latent membrane protein 1: structure and functions. *J. Biomed. Sci.* 10, 490-504 (2003)
136. R.K. Moorthy & D.A. Thorley-Lawson: Biochemical, genetic, and functional analyses of the phosphorylation sites on the Epstein-Barr virus-encoded oncogenic latent membrane protein LMP-1. *J. Virol.* 67, 2637-2645 (1993)
137. S.M. Duboise, J. Guo, S. Czajak, R.C. Desrosiers & J.U. Jung: STP and Tip are essential for herpesvirus saimiri oncogenicity. *J. Virol.* 72, 1308-1313 (1998)
138. J.U. Jung, J.J. Trimble, N.W. King, B. Biesinger, B.W. Fleckenstein & R.C. Desrosiers: Identification of transforming genes of subgroup A and C strains of herpesvirus saimiri. *Proc. Natl. Acad. Sci. USA* 88, 7051-7055 (1991)
139. S.C. Murthy, J.J. Trimble & R.C. Desrosiers: Deletion mutants of herpesvirus saimiri define an open reading frame necessary for transformation. *J. Virol.* 63, 3307-3314 (1989)
140. K.M. Kaye, K.M. Izumi & E. Kieff: Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc. Natl. Acad. Sci. USA* 90, 9150-9154 (1993)
141. V.R. Baichwal & B. Sugden: The multiple membrane-spanning segments of the BNLF-1 oncogene from Epstein-Barr virus are required for transformation. *Oncogene* 4, 67-74 (1988)
142. E. Kilger, A. Kieser, M. Baumann & W. Hammerschmidt: Epstein-Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J.* 17, 1700-1709 (1998)
143. D. Wang, D. Liebowitz & E. Kieff: An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* 43, 831-840 (1985)
144. D. Wang, D. Liebowitz, F. Wang, C. Gregory, A. Rickenson, R. Larson, T. Springer & E. Kieff: Epstein-Barr virus latent infection membrane protein alters the human B lymphocyte phenotype: deletion of the amino terminus abolishes activity. *J. Virol.* 62, 4173-4184 (1988)
145. H. Lee, R. Veazey, K. Williams, M. Li, J. Guo, F. Neipel, B. Fleckenstein, A. Lackner, R.C. Desrosiers & J.U. Jung: Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herepsvirus. *Nat. Med.* 4, 435-440 (1998)
146. O. Prakash, Z.-Y. Tang, X. Peng, R. Coleman, J. Gill, G. Farr & F. Samaniego: Tumorigenesis and aberrant signaling in transgenic mice expressing the human herpesvirus-8 K1 gene. *J. Natl. Cancer Inst.* 94, 926-935 (2002)
147. J. Douglas, B. Dutia, S. Rhind, J.P. Stewart & S.J. Talbot: Expression in a recombinant murid herpesvirus 4 reveals the *in vivo* transforming potential of the K1 open reading frame of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 78, 8878-8884 (2004)
148. M. Lagunoff & D. Ganem: The structure and coding organization of the genomic termini of Kaposi's sarcoma-associated herpesvirus (human herepsvirus 8) *Virology* 236, 147-154 (1997)
149. M. Lagunoff, D.M. Lukac & D. Ganem: Immunoreceptor tyrosine-based activation motif-dependent signaling by Kaposi's sarcoma-associated herpesvirus K1 protein: effects on lytic viral replication. *J. Virol.* 75, 5891-5898 (2001)
150. B.-S. Lee, S.-H. Lee, P. Feng, H. Chang, N.-H. Cho, & J.U. Jung: Characterization of the Kaposi's Sarcoma-Associated Herpesvirus K1 Signalosome. *J. Virol.* 79, 12173-12184 (2005)

151. F. Samaniego, S. Pati, J.E. Karp, O. Prakash & D. Bose: Human herpesvirus 8 K1-associated nuclear factor-kappa B-dependent promoter activity: role in Kaposi's sarcoma inflammation? *J. Natl. Cancer Inst. Monogr.* 28, 15-23 (2001)
152. C.C. Tomlinson & B. Damania: The K1 protein of Kapoi's sarcoma-associated herpesvirus activates the Akt signaling pathway. *J. Virol.* 78, 1918-1927 (2004)
153. L. Wang, N. Wakisaka, C.C. Tomlinson, S.M. DeWire, S. Krall, J.S. Pagano & B. Damania: The Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) K1 protein induces expression of angiogenic and invasion factors. *Cancer Res.* 64, 2774-2781 (2004)
154. B. Ensoli, M. Stürzl & P. Monini: Cytokine-mediated growth promotion of Kaposi's sarcoma and primary effusion lymphoma. *Semin. Cancer Biol.* 10, 367-381 (2000)
155. B.-S. Lee, M. Paulose-Murphy, Y.-H. Chung, M. Connlole, S. Zeichner & Jung JU: Suppression of tetradecanoyl phorbol acetate-induced lytic reactivation of Kaposi's sarcoma-associated herpesvirus by K1 signal transduction. *J. Virol.* 76, 12185-12199 (2002)
156. B.-S. Lee, X. Alvarez, S. Ishido, A.A. Lackner & J.U. Jung: Inhibition of intracellular transport of B cell antigen receptor complexes by Kaposi's sarcoma-associated herpesvirus K1. *J. Exp. Med.* 192, 11-21 (2000)
157. F.C. Kasolo, M. Monze, N. Obel, R.A. Anderson, C. French & Gompels UA: Sequence analyses of human herpesvirus-8 strains from both African human immunodeficiency virus-negative and -positive childhood endemic Kaposi's sarcoma show a close relationship with strains identified in febrile children and high variation in the K1 glycoprotein. *J. Gen. Virol.* 79, 3055-3065 (1998)
158. J.-C. Zong, D.M. Ciufo, D.J. Alcendor, X. Wan, J. Nicholas, P.J. Browning, P.L. Rady, S.K. Tyring, J.M. Orenstein, C.S. Rabkin, I.-J. Su, K.F. Powell, M. Croxson, K.E. Foreman, B.J. Nickoloff, S. Alkan & G.S. Hayward: High-level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus genome defines four major virus subtypes and multiple variants or clades in different human populations. *J. Virol.* 73, 4156-4170 (1999)
159. J.-K. Choi, B.-S. Lee, S.N. Shim, M. Li & J.U. Jung: Identification of the novel K15 gene at the rightmost end of the Kaposi's sarcoma-associated herpesvirus genome. *J. Virol.* 74, 436446 (2000)
160. M. Glenn, L. Rainbow, F. Aurade, A. Davison & T.F. Schulz: Identification of a spliced gene from Kaposi's sarcoma-associated herpesvirus encoding a protein with similarities to latent membrane protein 1 and 2A of Epstein-Barr virus. *J. Virol.* 73, 6953-6963 (1999)
161. S.R. Brodeur, G. Cheng, D. Baltimore & D.A. Thorley-Lawson: Localization of the major NF-□B-activating site and the sole TRAF3 binding site of LMP-1 defines two distinct signaling motifs. *J. Biol. Chem.* 272, 19777-19784 (1997)
162. G. Mosialos, M. Birkenbach, R. Yalamanchili, T. VanArsdale, C. Ware & E. Kieff: The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. *Cell* 80, 389-399 (1995)
163. M.M. Brinkmann, M. Glenn, L. Rainbow, A. Kieser, C. Henke-Gendo & T.F. Schulz: Activation of mitogen-activated protein kinase and NF-kB pathways by Kaposi's sarcoma-associated herpsvirus K15 membrane protein. *J. Virol.* 77, 9346-9358 (2003)
164. T.V. Sharp, H.-W. Wang, A. Koumi, D. Hollyman, Y. Endo, H. Ye, M.-Q. Du & C. Boshoff: K15 protein of Kaposi's sarcoma-associated herpesvirus is latently expressed and binds to HAX-1, a protein with antiapoptotic function. *J. Virol.* 76, 802-816 (2002)
165. F. Samaniego, P.D. Markham, R. Gendelman, Y. Watanabe, V. Kao, K. Kowalski, J.A. Sonnabend, A. Pintus, R.C. Gallo & B. Ensoli: Vasular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma (KS) are induced by inflammatory cytokines and synergize to promote vascular permeability and KS lesion development. *Am. J. Pathol.* 152, 1433-1443 (1998)
166. O. Flore, S. Rafii, S. Ely, J.J. O'Leary, E.M. Hyjek & E. Cesarman: Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature* 394, 588-592 (1998)
167. R. Masood, E. Cesarman, D.L. Smith, P.S. Gill & O. Flore: Human herpesvirus-8-transformed endothelial cells have functionally activated vascular endothelial growth factor/vascular endothelial growth factor receptor. *Am. J. Pathol.* 160, 23-29 (1999)
168. J. Nishi, K. Arimura, A. Utsunomiya, S. Yonezawa, K. Kawakami, N. Maeno, O. Ijichi, N. Ikarimoto, M. Nakata, I. Kitajima, T. Fukushige, H. Takamatsu, K. Miyata & I. Maruyama: Expression of vascular endothelial growth factor in sera and lymph nodes of the plasma cell type of Castleman's disease. *Br. J. Haematol.* 104, 482-485 (1999)
169. D.M. Ciufo, J.S. Cannon, L.J. Poole, F.Y. Wu, P. Murray, R.F. Ambinder & G.S. Hayward: Spindle cell conversion by Kaposi's sarcoma-associated herpesvirus: formation of colonies and plaques with mixed lytic and latent gene expression in infected primary dermal microvascular endothelial cells cultures. *J. Virol.* 75, 5614-5626 (2001)
170. S.-J. Gao, J.-H. Deng & F.-C. Zhou: Productive lytic replication of a recombinant Kaposi's sarcoma-associated herpesvirus in efficient primary infection of primary human endothelial cells. *J. Virol.* 77, 9738-9749 (2003)
171. P. Gasperini, M. Barbierato, C. Martinelli, P. Rigotti, F. Marchini, G. Masserizzi, F. Leoncini, L. Chieco-Bianchi, T.F. Schulz, & M.L. Calabro: Use of a BJAB-derived cell line for isolation of human herpesvirus 8. *J Clin Microbiol.* 43, 2866-2875 (2005)

Key Words: Human herpesvirus 8, HHV-8, Kaposi's sarcoma, primary effusion lymphoma, multicentric Castleman's disease, viral interleukin-6, vIL-6, vGPCR, vCCL-1, vCCL-2, vCCL-3, vIRF, v-cyclin, vFLIP, kaposin, VIP, K1, LAMP, K15, Review

Send correspondence to: Dr John Nicholas, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231, Johns Hopkins Oncology Center, 1650 Orleans Street, Baltimore, MD 21231, Tel: 410-502-6801, Fax: 410-502-6802, E-mail: nichojo@jhmi.edu