

CHEMOKINE SUBVERSION BY HUMAN HERPESVIRUSES

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Abbreviations

DC, dendritic cells; EBV, Epstein-Barr virus; gB, glycoprotein B; GAG, glycosaminoglycans; gC, glycoprotein C; gH, glycoprotein H; gG glycoprotein G; gL, glycoprotein L; GPCRs, G protein-coupled receptors; HCMV, human cytomegalovirus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; IP, inositol phosphate; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma-associated virus; MHC-I, major histocompatibility complex I; ORF, open reading frame; PLC, phospholipase C; SgG2, soluble glycoprotein G2; SCID, severe combined immunodeficiency; vCKs, viral chemokines; vCKBPs, viral chemokine binding proteins; vCKRs, viral chemokine receptors; VEGF, vascular endothelial growth factor; VZV, varicella zoster virus.

Abstract

Viruses use diverse molecular mechanisms to exploit and evade the immune response.

Herpesviruses, in particular, encode functional chemokine and chemokine receptor homologs pirated from the host, as well as secreted chemokine binding proteins with unique structures.

Multiple functions have been described for herpesvirus chemokine components, including attraction of target cells, blockade of leukocyte migration, modulation of gene expression and cell entry by the virus. Here we review current concepts about how human herpesvirus chemokine, chemokine receptors and chemokine binding proteins may be used to shape a proviral state in the host.

Introduction

Herpesviruses are large DNA viruses divided into α , β , and γ subfamilies that cause acute, latent and chronic infections in mammalian hosts [1]. The high prevalence of herpesviruses in human populations worldwide may be related to their ability to establish life-long latency and to their exceptional capacity to modulate the host immune response. At the molecular level, herpesvirus survival strategies are mediated in part by viral genes pirated from the host. The host chemokine system, which, among other functions, coordinates leukocyte trafficking, is a particularly rich source of such genes. In particular, human herpesviruses (HHVs) encode both viral chemokines (vCKs) and viral chemokine receptors (vCKRs) [2–4], which are structurally homologous to human chemokines and human G protein-coupled chemokine receptors (GPCRs), respectively. They also encode viral chemokine binding proteins (vCKBPs), which lack homology to any other known protein, yet bind promiscuously to many chemokines and interfere with their cellular activities. These herpesvirus-encoded proteins may modulate host chemokine functions to ultimately subvert the antiviral immune response. In addition, novel functions unrelated to conventional

chemokine activities and leukocyte trafficking have been identified. For example, most HHVs deploy vCKs in the viral envelope as key components of cell entry.

vCKs, vCKRs, and vCKBPs are differentially represented among the HHV subfamilies. Only α and β HHVs are known to encode vCKBPs (Figure 1), whereas only the β and γ HHV subfamilies have been shown to encode vCKRs (Figure 2). On the other hand, possibly due to their implication in common cell entry mechanisms, all known HHVs encode at least one vCK (Figure 3). Here we review the current state of knowledge of the HHV chemokine system, whose main biological and biochemical properties are summarized in Table 1.

ALPHAHERPESVIRUSES

The *alphaherpesvirinae* subfamily includes the neurotropic herpesviruses herpes simplex virus 1 (HSV-1), HSV-2 and varicella-zoster virus (VZV). After initial infection of epithelial cells in the oropharynx or genital tract, these viruses establish latency in ganglia of the peripheral nervous system [5]. Upon reactivation, they typically cause painful but self-limiting infections, including cold sores (HSV-1), genital ulcers (HSV-2) and shingles (VZV) [5]. However, they can also cause more serious conditions, including stromal keratitis, acute encephalitis and post-herpetic neuralgia. Unlike in β and γ HHVs, which are mostly lymphotropic, no vCKRs have been identified in α HHVs. Instead, all 3 α HHVs express vCKBPs in their viral envelope: glycoprotein G (gG) in HSV-1 and HSV-2 and glycoprotein C (gC) in VZV. Interestingly, these are the only known vCKBPs that do not inhibit but instead potentiate the activity of their chemokine ligands (Table 1). The conserved envelope protein gL of this HHV subfamily has been shown to adopt the canonical chemokine fold.

vCKBPs encoded by alphaherpesviruses

HSV-1 and HSV-2 gG

The *US4* ORF of HSV-1 and HSV-2 encodes a viral envelope vCKBP termed gG. Interestingly, the sequence of gG is more divergent (less than 14% amino acid identity) than any other envelope glycoprotein common to HSV-1 and HSV-2. Furthermore, HSV-2 gG (gG2) but not HSV-1 gG (gG1) can be cleaved and released from the virion as a soluble vCKBP (SgG2) [6,7]. However, despite these structural and topological differences, gG1 and gG2 are functionally very similar. They both bind the same small set of human chemokines with CCL22, a gG2-specific ligand, as the only exception (Figure 1) [8]. Surprisingly, unlike gG of non-human alphaherpesviruses, gG1 expressed in the viral envelope or the plasma membrane of infected cells and SgG2, enhance the chemotactic activity of their ligands by a mechanism that has been proposed to mimic that of glycosaminoglycan (GAG)-chemokine interactions [8,9]. Consistent with this, gG itself is a GAG-binding protein whose interaction with chemokines does not block ligand binding to signaling receptors [10]. Therefore, it was suggested that gG attached to the cell surface via GAGs or expressed in the plasma membrane of the infected cell may bind chemokines to present them to their cognate cellular receptors [10]. In addition, gG has been reported to delay receptor internalization upon chemokine binding [10]. How HSV would benefit from chemokine potentiation by gG is currently unknown. Existing hypotheses include the recruitment to the site of infection of more cells susceptible of being infected and the induction of an activated proviral state in cells neighboring the infected cell.

VZV gC

An orthologue of HSV gG is missing in VZV. Instead, VZV expresses an envelope glycoprotein named gC that has been recently characterized as a vCKBP able to bind 27

different chemokines with nanomolar affinity (Figure 1) [11]. Like HSV gG, gC boosts the chemotactic activity of its ligands (Table 1); however, its role in VZV pathogenesis is not yet defined. It has been reported that a gC deletion mutant of VZV has reduced capacity to reach the dermis from the epidermis [12]. Since the activity of some GPCRs is known to loosen epithelial tight junctions [13], it was proposed that gC may facilitate viral dissemination across the epidermis [11].

vCKs encoded by alphaherpesviruses

gL

Herpesvirus cell entry is a complicated process that requires the action of multiple viral glycoproteins; however, gB and the heterodimer gH/gL play a conserved central role in all HHVs [14]. It is assumed that upon binding to specific cellular receptors, gH/gL dimers activate gB to initiate fusion with the target cell plasma membrane [15]. In certain HHVs, gH/gL can interact with additional viral proteins to form diverse complexes that may ultimately determine the cellular tropism of the virus. In fact, in β HHVs, gH/gL dimers need these supplementary interacting partners to be efficiently exposed in the viral envelope [16]. On the contrary, uncomplexed gH/gL dimers are known to mediate cell entry of α HHVs by their interaction with cellular integrins (Table 1) [17]. Importantly, it has been shown that gL of α HHVs adopts a classical chemokine fold. In the gH/gL dimer of HSV-2, gL folds as a C chemokine with a single disulfide bond [18], validating computational predictions of chemokine homology to α HHV gL [19]. This opens up the possibility that gH/gL dimers may use cellular GPCRs in addition to integrins to induce α HHV entry in particular cell types.

BETAHERPESVIRUSES

Human cytomegalovirus (HCMV) and the 3 roseolovirus subtypes HHV-6A, HHV-6B and HHV7 comprise the HHV *betaherpesvirinae* subfamily. Although typically asymptomatic in healthy adults, β HHVs constitute an important health concern in neonates and infants [20,21]. In addition, HCMV infections are a significant risk factor for graft rejection in transplants. A link between HHV-6 and multiple sclerosis and other neuroinflammatory conditions has also been proposed [22,23]. The 230 kb genome of HCMV is the largest for the herpesviruses and encodes more than 160 ORFs [24]. Four of these ORFs encode GPCR homologues US27, US28, UL33 and UL78. US28 is a promiscuous vCKR binding 10 human chemokines and one vCK, whereas the remaining 3 homologues are classified as orphan receptors, with no chemokine ligands identified to date (Figure 2). Similarly, vCXCL1 is the only one of the 4 different HCMV-encoded chemokines — vCXCL1, vCXCL2, UL128, and UL130 — whose cellular receptors have been identified (Figure 3). Furthermore, HCMV *UL21.5* ORF encodes the only herpesvirus vCKBP outside the α HHV subfamily (Figure 1). On the other hand, 2 vCKR named U12 and U51 are conserved in all roseoloviruses and are most homologous to CXCR4 (HHV6-U12, 19% identity), CCR3 (HHV6-U51, 14% identity), CCR7 (HHV7-U12, 20% identity), and CCR4 (HHV7- U51, 18% identity). As might be expected from these quite divergent sequences, U12 and U51 bind to distinct repertoires of chemokines (Figure 2). In contrast, ORF *U83* encodes a vCK unique to HHV-6 named vCCL4. Interestingly, HHV-6A vCCL4 (vCCL4a) and HHV-6B vCCL4 (vCCL4b) display remarkably different receptor specificities (Table 1 and Figure 3).

vCKBPs encoded by betaherpesviruses

HCMV UL21.5

ORF *UL21.5* of HCMV encodes a secreted vCKBP shown so far to bind only CCL5 (Figure 1) [25]. However, it is important to note that a comprehensive chemokine screening of this vCKBP has not yet been reported. The UL21.5 interaction with CCL5 is known to block binding of the chemokine to its cellular receptors [25], although inhibition of CCL5-mediated chemotaxis has not yet been assessed. Interestingly, UL21.5-encoding mRNA is packaged into virions [26], which might allow HCMV to interfere with cellular migration even before the initiation of viral transcription.

vCKs encoded by betaherpesviruses

HCMV vCXCL1

ORF *UL146* of HCMV encodes vCXCL1, a vCK that shares 31% amino acid identity to human CXCL8. Consistent with this, vCXCL1 is known to act as an agonist at the cellular CXCL8 receptors CXCR1 and CXCR2 [27], which are primarily expressed in neutrophils. Since neutrophils are known to contribute to the vascular dissemination of HCMV, it has been proposed that vCXCL1 may promote virus spreading by recruiting neutrophils to the site of infection [27,28]. It has also been reported that vCXCL1 signals through CX3CR1 to attract NK cells [29]. However, the affinity of the vCXCL1 interaction with CX3CR1 is significantly lower than that with CXCR2, which raises concerns about the relevance of the interaction *in vivo*.

Located in a hypervariable region of the HCMV genome termed U_L/b' , vCXCL1 is one of the most structurally diverse genes across HCMV strains (up to 60% amino acid variability). However, the *N*-terminal ELR motif that characterizes all ligands of CXCR1 and CXCR2 is unaltered in all vCXCL1 variants [30]. Although some functional differences have been found among these different vCXCL1 genotypes [31], the correlation between vCXCL1

variant and clinical outcome remains controversial. Nevertheless, it is important to note that lab strains lacking the U_L/b' genomic region are attenuated in vivo [32], which suggests that ORFs located in this area, including vCXCL1, may be important HCMV virulence factors.

HCMV vCXCL2

UL147, another ORF in the U_L/b' genomic region of HCMV, encodes a second viral CXC chemokine named vCXCL2 [33]. vCXCL2 lacks an N-terminal ELR motif and is most highly related to CXCL9 (27.5% amino acid identity). However, its putative cellular receptor and biological activities remain undefined.

HCMV UL128 and UL130

The cell entry protein gL of β HHVs lacks the chemokine homology observed for its counterparts in α HHVs. However, gH/gL dimers of HCMV can interact with UL128 and UL130, CC and C chemokine homologs encoded by the virus, respectively, as well as the accessory protein UL131A to form the pentameric complex gH/gL/UL128-UL130-UL131A [34]. This complex is required for HCMV infection in epithelial, endothelial and monocytic cells but dispensable in fibroblasts, where a different gH/gL/gO complex plays a major role [35–37]. Importantly, the ablation of any one of the 5 components of the complex suffices to disrupt HCMV infectivity in these cell types. For instance, HCMV lab strains carrying deletions of the U_L/b' genomic region, where UL131A is located, are unable to infect non-fibroblast cells [38]. Although the mechanism by which the pentamer promotes HCMV cell entry remains unknown, vaccine strategies targeting this envelope complex are effective in blocking HCMV entry in key cell types [39,40], including cytotrophoblasts [41], which are thought to be the main placental target for congenital HCMV infections.

A recent crystal structure of the pentamer confirmed the protein-protein interactions within the complex as well as a chemokine fold for UL128 and UL130 [42]. In addition, this

study claimed that the great flexibility of the complex could allow UL128 and UL130 to interact with cellular chemokine receptors to promote viral cell entry. However, a bona fide cellular receptor for the pentamer remains to be identified. Furthermore, whether these 2 proteins can act as soluble vCKs, independently of their complex with gH/gL and UL131A is not clear. Chemokine-like activities have not been ascribed to UL130 yet and those reported for UL128 appear to contradict one another. Initially, it was shown that recombinant UL128 impaired monocyte chemotaxis induced by CCL5 and CCL2 [43]. However, a later study showed that UL128 protein induced chemotaxis of human peripheral blood mononuclear cells [44]. Further investigation will be required to resolve these controversies and to clarify whether the cellular effects of complexed and free UL128 and UL130 can be mediated by classical chemokine receptors.

HHV-6 vCCL4

The conserved ORF *U83* of HHV-6A and HHV-6B encodes two related but functionally distinct chemokines, vCCL4a and vCCL4b, respectively. While vCCL4a is known to induce chemotaxis by signaling through CCR1, CCR4, CCR5, CCR6, and CCR8, vCCL4b is a specific CCR2 agonist (Figure 3) [45,46]. Both vCKs could presumably attract cells to the site of infection to facilitate viral spread and latency, but the broader specificity of vCCL4a could allow HHV-6A to attract a higher diversity of cells and expand the virus cell tropism. Interestingly, two splicing variants of these vCKs are expressed during the virus cell cycle. Full-length vCCL4 is expressed mostly at late stages associated with active virus replication, whereas truncated forms of the chemokine *N*-terminus are produced early after virus cell entry and during latency [45,47]. Importantly, truncated vCCL4a and vCCL4b maintain the receptor specificity of the full-length vCKs. However, while the splicing truncation of vCCL4b does not affect its agonist activity at CCR2, the short form of vCCL4a is known to antagonize those receptors activated by full-length vCCL4a [45,48]. This could potentially

provide HHV-6A with the interesting ability to fine-tune its chemokine modulatory effects to benefit specific phases of viral infection.

vCKRs encoded by betaherpesviruses

HCMV US27

HCMV US27 is a late-phase protein predominantly expressed in intracellular vesicles suggestive of a role in endocytosis [49]. Its primary sequence is most highly homologous to CX3CR1 (22% amino acid identity) and contains the familiar DRY box motif at the end of transmembrane helix III as well as a C-terminal domain rich in serine and threonine residues. This latter domain is thought to target the receptor to intracellular vesicles since a chimeric US27 construct containing the C-terminus of CXCR3 trafficked to the plasma membrane rather than to the Golgi [50].

To date, US27 remains orphan, with neither ligands nor specific signaling pathways identified yet. However, a recent study found it was able to enhance signaling mediated by endogenous CXCR4, resulting in augmented calcium mobilization and chemotaxis [51]. Expression of US27 in transfected cells appears to boost cell proliferation and to protect cells from the apoptotic stimuli [52,53]. Conversely, a HCMV variant devoid of US27 was equally infective as the wildtype virus but produced significantly fewer progeny [54]. Collectively, this suggests a role for US27 in enhancing HCMV infectivity by promoting viral particle productivity and host cell survival.

HCMV US28

US28 is most highly homologous to CX3CR1 (35% identity). US28 is expressed early after HCMV infection and was detected by immunohistochemical analysis of tumor sections taken from glioblastoma patients [55]. US28 is known to be constitutively active, coupling via $G\alpha_q$ and $G\alpha_i$ proteins to a variety of signaling pathways. Several of these pathways are linked

to cell proliferation and angiogenesis, notably phospholipase C (PLC) activation which is associated with the transcription factors NFAT, NF- κ B, CRE, and SRF [56]. Studies led by the lab of Martine Smit have shown constitutive signalling of US28 in a variety of cell backgrounds, leading to the upregulation of several molecules associated with cell proliferation, notably cyclin D, VEGF, COX-2 and β -catenin [57–60]. The ability of US28 to constitutively activate G proteins appears to rely on an intact DRY motif at the end of transmembrane helix III since US28 mutant R129A is unable to activate PLC or known transcription factors [55,57,58]. The role of G protein independent signaling (e.g. via β -arrestins) has not been fully elucidated for US28. However, since cells expressing the R129A mutant are still able to successfully form tumors in a nude mouse model, it would appear that these signaling pathways might be important *in vivo* [57].

The tumorigenic potential of the US28 constitutive activity has driven approaches to target this vCKR with small molecule antagonists. The prototypic US28 antagonist VUF2274 has been shown to act as an inverse agonist of US28, with an IC_{50} of 776 nM for the inhibition of PLC in human fibroblasts infected with the AD169 strain of HCMV [61]. Interestingly, VUF2274 was initially described as a specific antagonist of CCR1 which shares only 30% homology with US28 [62]. Similarly, compounds based on the structure of CXCR3 antagonists have been reported to also act as inverse agonists of US28 [63].

In addition to its constitutive signaling, US28 has also been shown to interact with several host chemokines, including the CC chemokines CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL13, CCL26 and CCL28, and, consistent with its CX3CR1 homology, CX3CL1 [64,65]. Since US28 has been shown to be expressed on the viral envelope, it has the potential to aid the entry of HCMV into CX3CL1⁺ target cells like endothelial cells. Supportive of this, US28-deleted viral isolates were shown to be defective in cell-to-cell spread in epithelial cells [66]. Also based in the US28 binding to CX3CL1, a novel strategy to inhibit HCMV infection *in vivo* has been designed using a CX3CL1 variant with enhanced

affinity and specificity for US28 fused to a cytotoxic domain of *Pseudomonas* Exotoxin A. This fusion molecule was shown to specifically target and kill HCMV infected cells expressing US28 in vitro and in a humanized SCID mouse model [67]. The recent derivation of a crystal structure of US28 in complex with CX3CL1 may serve to further hone the specificity of such a molecule [68].

In terms of a physiological consequence of chemokine binding, smooth muscle cells expressing US28 have been shown to migrate along gradients of CCL5, which is inhibited by CX3CL1 [69]. There appears to be an element of signaling bias, since in the same study, when US28 was expressed in macrophages, CX3CL1 was able to induce chemotaxis whereas CCL5 was devoid of activity. In the context of atherosclerosis, HCMV infection may be envisaged to perturb cell migration in the vessel wall. In addition, US28 has been shown to acts as a co-receptor for HIV-1 entry in vitro [70], although the relevance of this finding for HIV pathogenesis is unclear.

HCMV UL33

Expression of UL33 is reportedly restricted to the later stages of HCMV infection, suggesting that it may play a role in the dissemination of the virus [49]. UL33 can promiscuously couple to $G\alpha_q$, $G\alpha_s$, and $G\alpha_{i/o}$ proteins, constitutively activating inositol phosphate (IP) production and the transcription factor CREB (cyclic AMP responsive element binding) [71].

In contrast to its constitutive activity, Tschische and co-workers showed that in HEK 293T cells, UL33 and UL78 could form heterodimers with US28 inhibiting the constitutive NF- κ B activation mediated by US28. This modulation exhibited signaling bias since activation of the $G\alpha_q$ /PLC pathway was unaffected by the expression of either receptor [72]. In a similar vein, Tadagaki *et al.* showed using both HEK 293T and THP-1 expression systems, that UL33 and UL78 could form heterodimers with CCR5 or CXCR4 when co-

expressed [73]. Co-expression of the vCKRs with either human chemokine receptor had little effect upon chemokine binding at either receptor but did negatively modulate downstream functions including chemotaxis and endocytosis. Notably, the ability of HIV to use CCR5 and CXCR4 as a co-receptor for viral entry was impaired, leading to the suggestion that inducing the formation of such heteromers could be a novel therapeutic approach to inhibit HIV infection [73].

HCMV UL78

UL78 is expressed early after HCMV infection, but it appears to be dispensable for viral replication in fibroblasts [74]. However, UL78 expression has been shown to impact virus growth in both endothelial and epithelial cells [75]. To date, no activating ligands or signaling properties have been attributed to UL78, although as aforementioned, UL78 can negatively regulate host and viral chemokine receptor signaling [72,73].

HHV-6 and HHV-7 U12

U12 is a late gene expressed during lytic infection with HHV-6 [76]. Isegawa and colleagues reported that expression of HHV-6 U12 in K562 erythroleukemia cells resulted in responses to CCL2, CCL3, CCL4 and CCL5 in intracellular calcium flux assays, mediated by pertussis toxin-insensitive G proteins [76]. Although all chemokines bound to U12 with nanomolar affinity, calcium flux responses varied in potency and efficacy, with CCL3 and CCL4 requiring micromolar concentrations to elicit a response. In contrast, expression of HHV-7 U12 in K562 cells calcium flux responses to CCL19, CCL17, CCL21 and CCL22 [77]. Surprisingly, while U12 itself is unable to respond to CCL19 or CCL22, co-expression of U12 with the host receptors CCR4 or CCR7 results in the cells becoming responsive to both CCL19 and CCL22, suggestive of U12 forming U12:CCR4 and U12:CCR7 heterodimers with modified functionality [78].

HHV-6 and HHV-7 U51

Infection of cord blood mononuclear cells with HHV6-A or HHV6-B revealed U51 to be expressed early after viral infection [79]. Expression of HHV-6 U51 in COS-7 cells showed that the receptor responds to CCL2, CCL5 and CCL11 in calcium mobilization assays [80]. Assays in which IP accumulation was measured in unstimulated cells revealed that U51 could also signal constitutively, which was enhanced by the addition of CCL5 [80]. Heterologous binding assays and receptor endocytosis assays carried out by the same group on HHV-6 U51 expressing cells revealed CX3CL1 and XCL1 to be additional U51 ligands [80]. In addition, it was shown that CCL2, CCL5, CCL11, CCL7, CCL13 and Kaposi's sarcoma associated virus (KSHV)-encoded vCCL2 could all compete the binding of radiolabeled CCL5 to U51 [81]. On the other hand, HHV-7 U51 was shown to display the same chemokine binding profile of HHV-7 U12, inducing calcium flux responses upon interaction with CCL19, CCL17, CCL21 and CCL22 [82]. Interestingly, unlike with HHV-7 U12, none of these chemokine interactions with HHV-7 U51 resulted in active cell chemotaxis [82]. This, plus the different expression kinetics of U12 and U51, suggests that despite binding the same set of chemokines, these 2 vCKRs may play distinct roles in HHV-7 pathogenesis.

GAMMAHERPESVIRUSES

Two human pathogens, EBV and KSHV are included in the *gammaherpesvirinae* family. Both of these γ HHVs are oncogenic; however, while EBV is able to induce malignant cell transformation in cancers such as Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's lymphoma [83,84], the KSHV-induced eponymous tumor, Kaposi's sarcoma (KS), is mostly polyclonal and angiogenic and inflammatory in nature [85]. Importantly, although KSHV is typically associated with AIDS patients, it has also been reported in HIV-free sub-Saharan and Mediterranean populations and as a secondary

complication after allogeneic transplantation [86]. KSHV also causes primary effusion lymphoma as well as multicentric Castleman's Disease, which like KS is also a non-cancerous proliferative disease. Three distinct vCKs — vCCL1, vCCL2 and vCCL3 — and one vCKR, ORF74, fully equip KSHV to hijack the host chemokine system at multiple levels (Table 1). EBV is a relatively small herpesvirus, having only 84 ORFs within its 184 kb genome, and encodes only one ORF encoding a putative GPCR, the orphan receptor BILF1.

vCKs encoded by gammaherpesviruses

KSHV vCCL1

ORF *K6* of KSHV encodes a vCK, vCCL1 (formerly named vMIP-I) that is most highly related to CCL18 (50% amino acid identity). Initially, it was shown that vCCL1 was able to induce calcium flux in CCR5-expressing cells and to block HIV infection in cells transfected with the HIV-coreceptors CCR5, CCR3 and CXCR6 [87–89]. However, consistent with its high CCL18 homology, 3 independent studies have now established vCCL1 as a specific agonist at the CCL18 receptor CCR8 [90–92]. Besides interfering with the anti-viral response and immune cell migration to the site of infection, vCCL1 could benefit KSHV at other levels. For instance, vCCL1 is known to attract CCR8⁺ vascular endothelial cells [93], which could promote angiogenesis and vascular dissemination of the virus. In addition, like the human CCR8 ligand CCL1, vCCL1 binding to CCR8 induces anti-apoptotic signaling [94]. This could prevent host cell apoptosis and ensure both productive KSHV replication and healthy neighboring cells for virus dissemination.

KSHV vCCL2

ORF *K4* of KSHV encodes vCCL2 (formerly named vMIP-II), the best characterized vCK. vCCL2 is undoubtedly the most highly promiscuous of all known chemokines, capable of binding to chemokine receptors from all four structural subgroups. In particular, vCCL2

can interact with CCR1, CCR2, CCR3, CCR5, CCR8, CCR10, CXCR4, CX3CR1 and XCR1 [64,92,95]. Unlike most vCKs described previously, the interaction of vCCL2 with its cellular receptors does not result in calcium flux or chemotaxis signals but instead blocks the binding of cognate human chemokine ligands. Therefore, vCCL2 is considered a broad spectrum chemokine receptor antagonist that might serve KSHV by seriously impairing the recruitment of anti-viral immune cells to the site of infection. Of note, vCCL2 has also been reported to be an agonist at CCR3 and CCR8 [96], which could potentially benefit KSHV by attracting CCR3⁺ or CCR8⁺ Th2 cells. Furthermore, vCCL2 is known to be a ligand for the atypical chemokine receptor ACKR3 (previously named CXCR7) [97], which might affect the availability of vCCL2 during KSHV infection, and for the vCKRs HCMV US28, HHV-6 U51 and KSHV ORF74 (Figure 3) [64,81,98]. In particular, it has been shown that vCCL2 is able to downregulate the constitutive signaling activity of ORF74 [99]. This has been proposed to foster immune evasion during specific phases of KSHV infection.

Besides its biological role in KSHV infection, a broad-spectrum inhibitor like vCCL2 may have potential applications as a pharmaceutical. For instance, peptides derived from vCCL2 have been designed to block CXCR4- and CCR5-mediated HIV cell entry [100,101]. In addition, recombinant vCCL2 has been proved to have a significant therapeutic effect in multiple mouse models of inflammation [102,103]. Furthermore, its anti-inflammatory and angiogenic activities might be used to improve graft survival after organ transplantation [104–106].

KSHV vCCL3

vCCL3 (also termed vMIP-III) encoded by ORF *K4.1* of KSHV, is most highly homologous to human XCL1. Consistent with this, vCCL3 is a potent agonist at the human XCL1 receptor XCR1 [92], which is mainly expressed by a subpopulation of dendritic cells that might be latently infected by KSHV. In addition, vCCL3 induces chemotaxis of Th2

cells by signaling through CCR4 [107]. This could presumably help KSHV to establish a pro-viral immunological environment at the site of infection. However, it is important to note that while micromolar doses (0.1-10 μ M) of vCCL3 were required to induce CCR4 activation, nanomolar concentrations (0.1-10 nM) sufficed to activate XCR1, suggesting its greater relevance for XCR1.

gL

As for α HHVs, gL of γ HHVs was predicted to be related to chemokines [19]. This was confirmed by the crystal structure of the gH/gL heterodimer of EBV [108]. Interestingly, while α HHV gL is more highly homologous to C chemokines, γ HHV gL adopts a classical CC chemokine fold. gH/gL dimers in the γ HHV viral envelope mediate cell entry by their interaction with integrins and ephrin type A receptor 2 in EBV and KSHV, respectively [109,110]. Whether a classical chemokine receptor could act as an entry coreceptor for gH/gL is currently unknown. It is also important to note that while gH is a transmembrane protein, gL possesses an *N*-terminal signal peptide and is known to be secreted when transfected in the absence of gH [111]. Therefore, the idea of gL acting as a soluble vCK in α and γ HHV infections must be considered.

vCKRs encoded by gammaherpesviruses

KSHV ORF74

ORF74, the only GPCR within the KSHV 160 kb genome, is undoubtedly the best studied HHV vCKR. ORF74 has 27% homology with CXCR2 and has been shown to be expressed in KS lesions [112]. A pivotal role for ORF74 in KS was supported by a transgenic mouse expressing ORF74 under the direction of the human CD2 promoter. This mouse developed characteristic erythematous maculae that developed predominantly into skin tumors, surrounded by spindle cells and infiltrating leukocytes, similar to the lesions

observed in human KS [113].

ORF74 is constitutively active, coupling to a variety of G proteins in transfected cells including $G\alpha_q$, $G\alpha_i$ and $G\alpha_{12/13}$ [114–116]. In turn, this activates a variety of intracellular signaling pathways which likely accounts for the oncogenic nature of ORF74, including activation of ERK1/2, p38, JNK, RhoA and Rac1 [114,115,117,118]. The molecular basis for the constitutive activation of ORF74 is not fully elucidated, although an abundance of mutagenesis studies points to a role for mutations in what are normally highly conserved residues within GPCRs, such as the DRY motif in helix III and conserved aspartate and glutamate residues in helices VI and VII. For a discussion of the finer points of these studies, the reader is pointed to an excellent review by de Munnik and colleagues [119]. As might be anticipated with promiscuous G protein coupling, signaling bias is in evidence. For example, ORF74 expression in COS-7 but not in HEK 293T cells resulted in the constitutive activation of the ERK signaling pathway [115,117].

In keeping with its homology to CXCR2, ORF74 binds the majority of CXCR2 ligands including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8 (Figure 2) [98]. ORF74 has also been shown to bind CXCL4, CXCL10, and CXCL12, which are agonists for CXCR3 and CXCR4 [98,120]. Not shackled by class restriction, ORF74 also binds the CC chemokines CCL1 and CCL5 [121]. Chemokine binding to ORF74 is associated with modulation of its constitutive activity. CXCR2 ligands tend to display a broad range of agonist activities at ORF74, acting as both full and partial agonists in assays of IP accumulation and β -arrestin recruitment [98,122]. Conversely, non-CXCR2 ligands such as CXCL10 and CXCL12 function as full inverse agonists, inhibiting the constitutive activity of ORF74 [98,120,123]. In addition to modulation by host chemokines, as mentioned previously, vCCL2 has been shown to act as a partial inverse agonist for ORF74 [99].

EBV BILF1

BILF1 possesses only 15% identity to the chemokine receptor CXCR4 but has been shown to form heterodimers with CXCR4, negatively regulating its function by scavenging $G\alpha_i$ proteins [124]. Expressed early in EBV cellular infection, BILF1 is a constitutively active orphan receptor, coupling to $G\alpha_i$ proteins and resulting in NF- κ B activation in a COS-7 cell transfectant model [125,126]. As in the case of HCMV US28, there appears to be tissue bias with respect to the BILF1 constitutive activity, since when expressed in Burkitt's lymphoma or lymphoblastoid B cells, NF- κ B-independent CRE activation was observed [125]. BILF1 activity is dependent on the integrity of an EKT motif, analogous to the DRY motif commonly found in GPCRs, since mutation of this motif resulted in reduced $G\alpha_i$ coupling [127]. Surprisingly, this signaling-deficient BILF1 mutant still induces tumor growth in a xenograft mouse model, which suggests that G protein-independent signaling pathways may be implicated [127].

BILF1 also forms an association with MHC-I molecules, enhancing the internalization of MHC-I from the cell surface, and targeting MHC-I for lysosomal degradation, which is thought to play a role in the ability of EBV infected cells to evade CD8⁺ T cell responses [128,129]. This immune-evasive function requires the C-terminus of BILF1 [130].

Concluding Remarks

About 3 decades ago, the first vCKRs were identified in HCMV. This discovery established that DNA viruses could encode proteins to target and hijack specific components of the host immune response. With the subsequent identification of many additional vCKs, vCKBPs, and vCKRs, it soon became evident that the host chemokine system is a preferred target for herpesvirus survival strategies. However, we are just beginning to understand how these viral chemokine components might coordinate with other immune evasion mechanisms to facilitate viral spread and persistence. The specificity of HHVs for human hosts impedes progress in defining biologically relevant functions for HHV-encoded chemokine systems in

the context of a natural infection. In addition, not all HHV-encoded vCKs, vCKBPs and vCKRs described in this review possess a clear orthologue in non-human herpesviruses, and those that do may display opposite activities (e.g., α HHV vs non-human alphaherpesvirus gG). The development of new or humanized non-human herpesvirus models as well as recombinant non-human herpesviruses expressing HHV-encoded vCKs, vCKRs and vCKBPs will help to elucidate how the chemokine system modulates HHV pathogenesis and to identify new approaches for treating and preventing HHV infections.

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Disclosure Statement

The authors declare that they have no conflicts of interests.

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Figure Legends

Figure 1. vCKBPs encoded by HHVs. Graphic representation of the vCKBPs identified in alphaherpesvirus (HSV1, HSV2 and VZV) and betaherpesvirus (HCMV). Chemokines bound by each vCKBP are indicated. Since it has been shown that HCMV UL21.5 mRNA is packaged into virions, the expression of UL21.5 protein from this encapsidated mRNA as well as from the viral DNA genome is represented. vCKBPs are depicted in the color of the corresponding HHV. sgG2, soluble gG2.

Figure 2. vCKRs encoded by HHVs. Graphic representation of the vCKRs identified in betaherpesvirus (HCMV, HHV6 and HHV7) and gammaherpesvirus (EBV and KSHV). Chemokines bound by each vCKR are indicated. Unknown ligands for orphan vCKRs are shown in gray. Lightning bolt symbol indicates vCKRs with reported constitutive activity.

Figure 3. vCKs encoded by HHVs. Graphic representation of the vCKs identified in alphaherpesvirus (HSV1, HSV2 and VZV), betaherpesvirus (HCMV, HHV6A and HHV6B) and gammaherpesvirus (EBV and KSHV). vCKs are shown in the same color as the corresponding virus. Other non-chemokine viral proteins (gH and HCMV gL and UL131A) are shown in gray. Cellular receptors for each vCK are indicated. Agonistic and antagonistic activities are represented with green and red arrows, respectively. Unknown receptors for vCKs are shown in red. s-vCCL4a/b, spliced variants of vCCL4a/b.

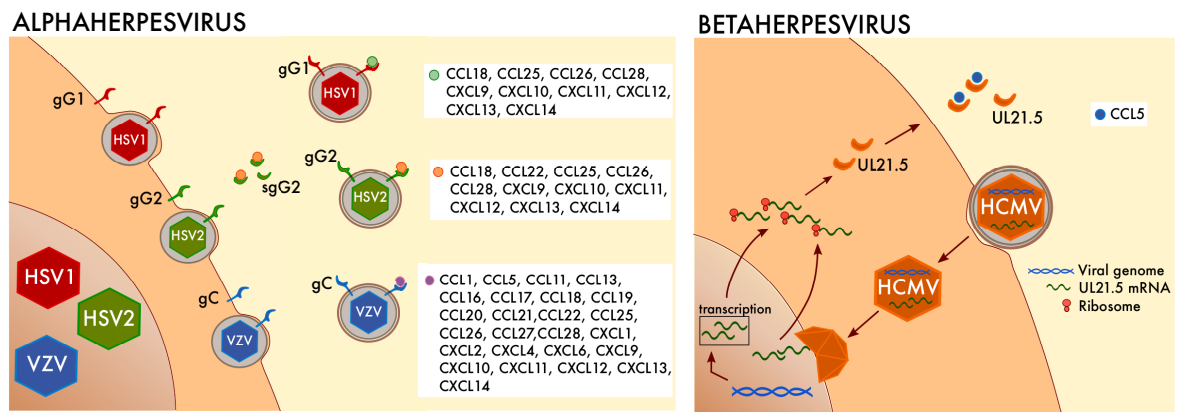


Figure 1

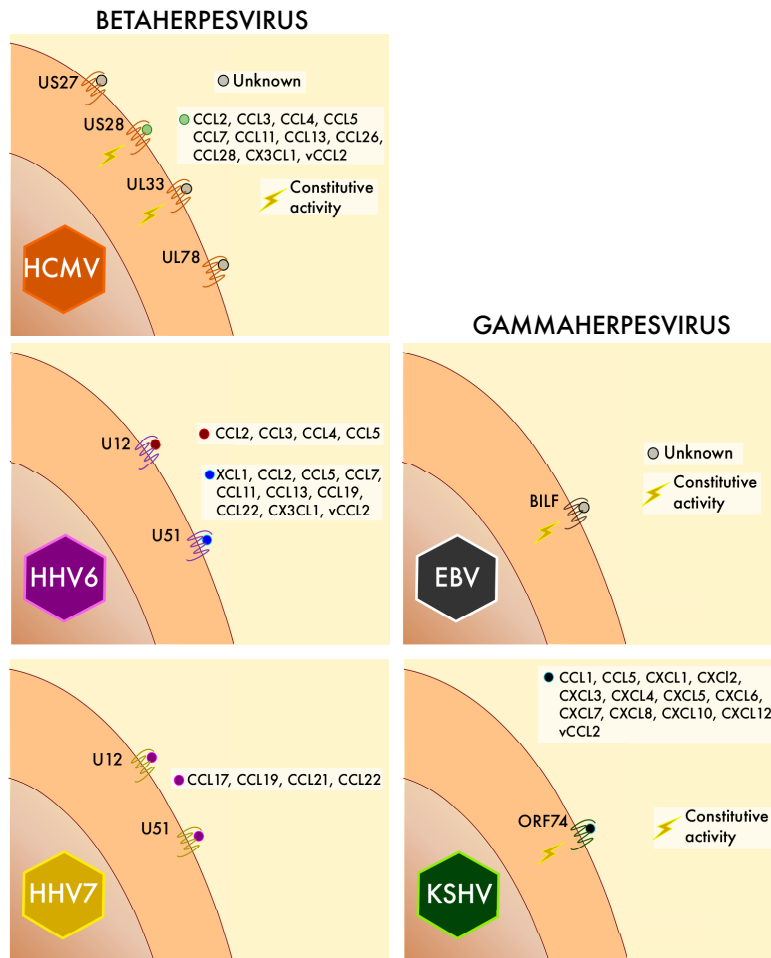


Figure 2

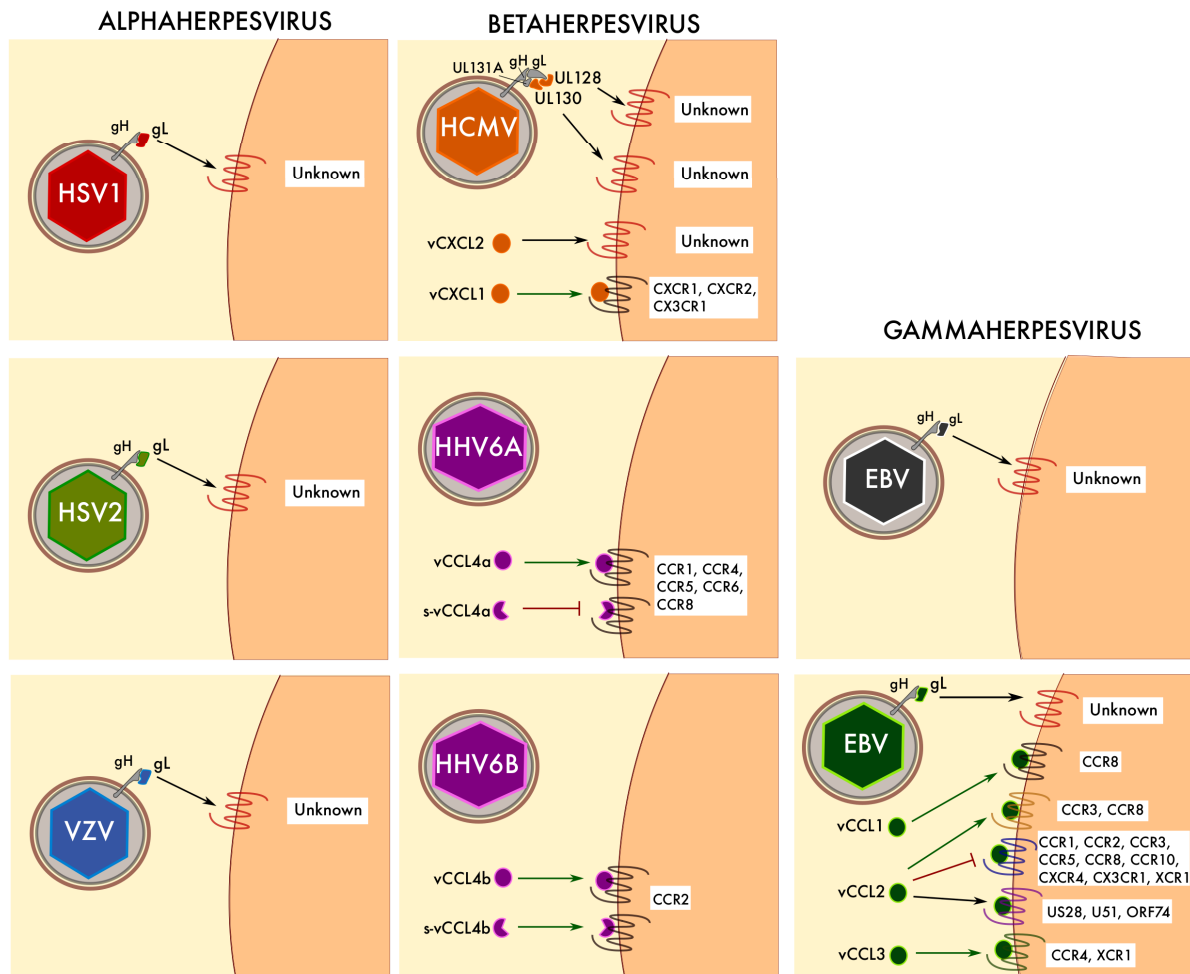


Figure 3