

Kaposi's sarcoma (KS) is a distinctive neoplasm that differs in many respects from most solid tumors. This low grade malignancy contains many foci of inflammatory and endothelial cell origins. The dominant partially transformed cell type is the spindle cell, which produces a cornucopia of proinflammatory and angiogenic factors believed to play an important role in the recruitment of additional cells to the sarcoma.

More than four years have passed since Chang and colleagues discovered a novel viral genome in KS specimens (1), identifying it as a member of the gamma herpes family. The KS-associated herpesvirus (KSHV, also known as human herpesvirus 8 or HHV8) is believed to play a crucial role in the development of KS neoplasia. The discovery of KSHV led to the identification of a number of viral ORFs which corresponded to known cellular homologues, including chemokines and their receptors (2, 3). This is in keeping with research over the past fifteen years that has identified numerous viral ORFs with sequence homology to cellular genes. Many of these genes vary only slightly from their cellular counterparts, and are putative mediators of viral pathogenesis, including viral entry and immune dysregulation, as demonstrated most elegantly by the identification of chemokine receptors as coreceptors required for entry and infection of cells by HIV-1 (4).

At least 15 accessory genes of KSHV have been identified, some of which regulate cell cycle and extracellular signaling. For example, ORF 16, the viral *bcl-2* analogue, can block apoptosis; and ORF 72, the viral cyclin D, can play a role in cell cycle progression. Among the viral growth ligands is ORF K2, the IL-6 homologue, which can act in a paracrine or possibly autocrine manner to stimulate cell growth. ORFs K4 and K6 encode viral chemokine analogues (virokines).

Among the accessory genes identified to date, one has become a major focus of the investigations into the KS oncogenic/pathogenic process. ORF 74, named KSHV G protein-coupled receptor (KSHV-GPCR), encodes a chemokine receptor homologue. In late 1997, Cesarman and colleagues (5) showed that KSHV-GPCR encoded a constitutively active G protein-coupled receptor that stimulated the phosphoinositide-protein kinase C pathway, resulting in the proliferation of transfected NRK-49F cells. Almost simultaneously, Mesri and colleagues (6) found that KSHV-GPCR activated two other protein kinase pathways, JNK/SAPK and p38MAPK. Further, Mesri's group found that in addition to the oncogenic proliferative signal mediated by KSHV-GPCR, this viral-encoded receptor-like gene also supported angiogenic development in a manner similar to its cellular homologue, the IL-8 receptor  $\beta$  (which has subsequently been renamed CXCR2). These previous findings are critical to our current understanding of the role of KSHV in KS pathogenesis.

A subgroup of CXC ( $\alpha$ ) chemokines contain a tripeptide, glutamic acid-leucine-arginine (ELR), motif. This ELR motif,

found at positions 4–6 from the NH<sub>2</sub> terminus of human IL-8, NAP-2, ENA-78, GRO- $\alpha$ ,  $\beta$ ,  $\gamma$ , and GCP-2, has been shown to be essential but not sufficient for high affinity binding and activation of the human KSHV-GPCR cellular homologue, CXCR2 (7). Specifically, CXCR2 binds IL-8  $\sim$  GRO- $\alpha$   $\sim$  NAP-2  $>$  ENA-78 with affinities ranging from 10 to 2 nM. Furthermore, these CXCR2 ligands have been shown to have angiogenic activity.

In contrast, several CXC chemokines such as PF-4 (platelet factor-4), IP-10 (interferon-induced protein-10), MIG (monokine induced by IFN- $\gamma$ ), and I-TAC (interferon-inducible T cell  $\alpha$  chemoattractant) do not contain the ELR motif and are known to be angiostatic. IP-10, MIG, I-TAC, and CC chemokines MCP-4, Eotaxin, and SLC are known ligands for CXCR3, which is expressed by T cells.

Although KSHV-GPCR was initially thought to be a constitutively activated receptor, more recent studies demonstrate that different chemokines can modulate its activity level. In this issue, Clark-Lewis and associates further explore the regulation of KSHV-GPCR by chemokines and chemokine variants (8). They demonstrate that KSHV-GPCR binds IL-8  $\sim$  NAP-2  $>$  PF-4  $\sim$  I-309  $\sim$  GRO- $\alpha$   $>$  RANTES  $>>$  MIP-1 $\beta$  and MCP-1, with a  $K_d$  of 25 nM for IL-8. While KSHV-GPCR binds chemokines of both the CC and CXC subfamilies, unlike its cellular counterpart, not all chemokines induce KSHV-GPCR to signal. Using chemical synthesis, Clark-Lewis and associates have modified IL-8, PF-4, and IP-10 to show that KSHV-GPCR transfectants respond to ELR-containing variants by increased production of inositol phosphate (IP). Presumably, this is the proliferative signal transduced by KSHV-GPCR. In contrast, IP-10, a non-ELR-containing chemokine, suppressed IP levels. Thus, KSHV-GPCR binds to both ELR- and non-ELR-containing chemokines but with opposite signaling outcomes, suggesting that KSHV-GPCR combines the opposing angiogenic and angiostatic functions of human CXCR2 and CXCR3.

What advantage to the virus is modulation of angiogenesis by KSHV-GPCR? Because KS tumors are highly vascularized, the proangiogenic signals must prevail. Perhaps virokines and endogenous chemokines are differentially regulated, thereby providing a permissive environment for HHV8. Nevertheless, it is difficult to imagine what conditions would greatly favor KSHV-GPCR-regulated growth, given that the endogenous expression of IL-8 and of IP-10 are inducible by various viral infections and proinflammatory cytokines. However, only IL-8 has been shown to be induced by UVB, suggesting that this might be a contributing factor in the occurrence of KS on the extremities of patients.

Presumably, the KSHV-encoded virokines promote neovascularization and hence viral survival and growth of the KS tumor. However, these virokines have been shown to antagonize the endogenous chemokine receptors, but it has not been determined if they act as an agonist or an antagonist of KSHV-GPCR. In addition, the virokines are expressed late in the kinetics of virus replication, suggesting that their role is in maintenance and spread of the infection but not in its initiation. In contrast, vMIPII has been shown to inhibit HIV infection and may serve as a peptide model for anti-HIV therapeutics (9).

Although our knowledge is incomplete, it is clear that the full characterization the KSHV-encoded chemokine receptor and chemokines has great potential to explain the role chemokines and chemokine receptors play in KS and other viral infections.

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