Commentary

Viral G Protein–coupled Receptor and Kaposi's Sarcoma: A Model of Paracrine Neoplasia?

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The most recently identified human herpesvirus, Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV-8), has been found to be a necessary, although perhaps not sufficient, etiologic agent for all forms of KS (1, 2). This virus is also invariably present in a rare subset of malignant lymphomas, primary effusion lymphomas (PELs), and in a significant percentage of patients with multicentric Castleman's disease, an angiolymphoproliferative disorder (3, 4). Both KS and PEL occur more frequently, but not exclusively, in HIV-infected individuals, and all cases of HIV-related multicentric Castleman's disease are infected with KSHV. These findings suggest an important role of immunosuppression and HIV infection in KSHV-mediated pathogenesis. Although it remains controversial whether KS is a malignant neoplasm, KS lesions probably evolve from a reactive, inflammatory/angioproliferative process into true clonal cancers. Thus, KS is generally considered a malignancy, especially because of its frequent multifocal and aggressive behavior. Given the definitive association of KSHV with two different human malignancies, it is not difficult to consider KSHV to be a human oncogenic virus.

KSHV is a gammaherpesvirus that is homologous to EBV and herpesvirus saimiri (HVS), human and simian viruses, respectively, that are able to transform lymphoid cells in culture and cause malignant lymphomas in some circumstances. Genomic sequencing has revealed that KSHV contains several genes with likely oncogenesis-related functions that subvert pathways involved in cellular activation, proliferation, differentiation, and survival. Many of these genes are viral homologues of cellular genes, including those encoding viral cyclin D, IFN regulatory factors (IRFs), viral IL-6 (vIL-6), BCL-2, FLICE-inhibitory protein (FLIP), three chemokines (viral macrophage inflammatory protein [vMIP]-I, -II, and -III) and last, but not least, a G protein–coupled receptor (KSHV GPCR). Three of these genes have been found to be transforming in mouse fibroblast assays (vIRF, KSHV GPCR, and vIL-6), and two are homologous to cellular oncogenes (vBCL-2 and v-cyclin D). Two additional viral genes having no direct cellular counterpart, those encoded by open reading frame K1 (containing an immunoreceptor tyrosine-based activation motif [ITAM]) and K12, have also been found to be transforming in certain experimental assays. Therefore, KSHV qualifies as the virus with the most putative oncogenes identified to date. Thus, the following dilemma: why are KSHV-associated neoplasms so rare in the general population in spite of a seroprevalence of KSHV infections of at least 5% in Western countries? Why is KSHV only poorly transforming after infecting cells in culture? This may be partially explained by the concept that KSHV-mediated pathogenesis will be determined by the specific pattern of viral gene expression and the specific cellular background in which these genes are expressed, both of which are highly dependent on host factors (5, 6). Most of the genes found or suspected to be transforming are lytic genes, which are only transcribed in a subset of cells in KS lesions and PEL. However, the pathogenic potential of a single gene product thus sparsely expressed is elegantly demonstrated in the article by Yang et al. in this issue (7).

The report by Yang et al. provides the first transgenic mouse model for KS using a single KSHV gene, the chemokine receptor KSHV GPCR. In this study, it was found that expression of KSHV GPCR in hematopoietic cells of transgenic mice leads to angioproliferative lesions that exhibit most of the characteristics of KS. This viral receptor is encoded by open reading frame 74, and has closest homology to human IL-8 receptors type A (CXC chemokine receptor 1 [CXCR1]) and B (CXCR2) (8). KSHV GPCR is a constitutively signaling receptor that can bind chemokines from the CXC and the CC families, but does not require ligand for its activation (9). However, specific chemokines can further enhance and others inhibit this constitutive activity (10–12). KSHV GPCR has been found to have several unique properties among viral chemokine receptors, besides its ligand-independent activity. KSHV GPCR has been demonstrated to be transforming when transfected into NIH-3T3 cells (13). In addition, and perhaps more important to the pathogenesis of KS than a direct oncogenic role, it was shown that signaling by KSHV GPCR leads to the upregulation of expression of vascular endothelial growth factor (VEGF), thereby inducing angiogenesis via a paracrine mechanism(s) (13).

In addition to the chemokine receptor KSHV GPCR,

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J. Exp. Med. © The Rockefeller University Press • 0022-1007/2000/02/417/05 $5.00
Volume 191, Number 3, February 7, 2000 417–421
http://www.jem.org

KSHV encodes three chemokine ligands with interesting functional characteristics. vMIP-I and -II can promote angiogenesis (14). vMIP-II has been shown to inhibit HIV infection in vitro (15). Both vMIP-I and -II can bind the human CC chemokine receptor 8 (CCR8). vMIP-I acts as an agonist (16, 17) of CCR8. vMIP-II has been suggested to behave as an antagonist (16) or as an agonist and Th2 lymphocyte chemoattractant (18) by two different groups of investigators. As CCR8 is preferentially expressed in Th2 lymphocytes, expression of these viral chemokines may have immunomodulatory effects, perhaps mediating some of the inflammatory aspects of KS. vMIP-II can also inhibit signaling of KSHV GPCR, perhaps providing this virus with an additional control or feedback mechanism (11). Other examples of chemokines and chemokine receptors encoded by herpesviruses include two CXC chemokines encoded by CMV genes UL146 and UL147. vCXC-1 (UL146) has chemotactic activity for neutrophils similar to that of human IL-8. This suggests that vCXC-1 may be involved in dissemination during acute infection and may account for the presence of neutrophils in CMV disease (19). CMV also encodes three proteins with homology to CC chemokine receptors (US27, US28, and UL33), which are thought to be able to sequester cellular chemokines (20). HHV-6 also encodes chemokine (U83 [21]) and chemokine receptor (U12 and U51) homologues (22). Interestingly, EBV lacks viral chemokine receptor or chemokine homologues, but it can induce the cellular expression of both (23–25). Similarly, herpes simplex virus can activate a variety of cellular chemokines involved in its pathogenesis (26). Many animal herpesviruses have also pirated chemokine and chemokine receptor genes. Therefore, it is common for herpesviruses to use this family of receptor–ligand signaling pathways for their propagation and subversion of immune responses. In addition, a novel mechanism for subversion of chemokine responses by a herpesvirus has recently been identified, and is reported in this issue by Parry et al. (27). It involves the production of a secreted protein by the murine gammaherpesvirus 68, which can bind and sequester a variety of chemokines in spite of having no sequence similarity to chemokine receptors.

It has been suggested that stimulation of angiogenesis via paracrine mechanisms is important in KSHV-mediated pathogenesis (13, 28). This idea is supported by observations made after in vitro KSHV infection of primary human endothelial cell cultures (29). Infection of these cultures by KSHV results in dramatic phenotypical changes. These include a change from a cobblestone to a spindle cell morphology, greatly prolonged life span verging on immortalization, expression of telomerase, and colony formation in soft agar. In addition, infection by KSHV results in the sustained upregulation of VEGF receptor 2 (VEGFR-2, KDR, flk-1), the principal mitogenic receptor for VEGF. The surprising finding was that KSHV could only be found in a small proportion (5–10%) of the cells in these cultures. Supernatants from infected cultures exhibit some of the effects of actual infection such as upregulation of VEGFR-2 expression, suggesting involvement of paracrine mechanisms leading to the continuous and unregulated proliferation of endothelial cells in culture.

Although KSHV GPCR has been demonstrated to induce cellular proliferation and transformation when overexpressed in murine cells, and KSHV has been found to be transforming in endothelial cell cultures, the paper by Yang et al. (7) provides the first evidence that a KSHV gene can induce angioproliferative lesions in animals. In addition, the pathology obtained in the KSHV GPCR transgenic mice is remarkably similar to KS, with angiogenesis and $CD34^+$ spindle cell proliferation. Interestingly, the KS-like angioproliferative lesions were obtained when the transgene was placed under the control of a CD2 promoter, and expressed by T and NK cells. These KSHV GPCR–expressing cells were found to infiltrate the lesions, and have a similar distribution as cells expressing VEGF. Therefore, the authors suggested that KSHV GPCR is not directly transforming, but rather can activate the expression of cellular factors, in particular VEGF, that in turn recruit and stimulate the proliferation of endothelial and spindle cells, thereby inducing the formation of KS-like lesions (Fig. 1). While the selection of a CD2 promoter was somewhat arbitrary, the resulting expression of KSHV GPCR in only a few cells in the lesions in the transgenic mice may be similar to KSHV GPCR expression within KS lesions in humans. In lymphoma cell lines, KSHV GPCR exhibits an early lytic pattern of expression (30, 31). In KS lesions, there is a small subset of cells expressing lytic genes that appear to be productively infected with KSHV (32, 33), and according to one recent report (24), KSHV GPCR is expressed by these scattered cells. Therefore, since in this novel mouse model KSHV GPCR expression in a few infiltrating cells is able to recapitulate the induction of angioproliferative lesions resembling KS, it can be argued that in KS lesions the expression of this viral receptor by a subset of infected cells could have a major contribution to KSHV-induced angiogenesis. This finding suggests that KSHV GPCR is a critical viral gene involved in the pathogenesis of KS, and points to the dramatic effect of paracrine stimulation of angiogenesis mediated by VEGF secretion in KS pathogenesis.

Yang et al. (7) suggest that while KSHV GPCR is involved in induction of proliferative lesions in the transgenic animals, it functions primarily by "paracrine" mechanisms. Although the CD2-driven KSHV GPCR was expressed by T and NK cells in lymphoid organs, no lymphoproliferative diseases were identified. This implies that while KSHV GPCR can induce transformation of immortalized mouse fibroblasts, it may not cause mouse T cell proliferation and transformation, arguing either for cell type specificity or for the requirement of additional genetic events for transformation. However, an explanation based solely on a mechanism mediated by VEGF secretion by KSHV GPCR–expressing T cells does not explain two important features of this model: Why are angioproliferative lesions caused by excess VEGF production not observed in lymphoid organs? And why do these T cells infiltrate certain sites in which they are able to survive long enough to induce an angioproliferative lesion? These facts suggest that the KSHV

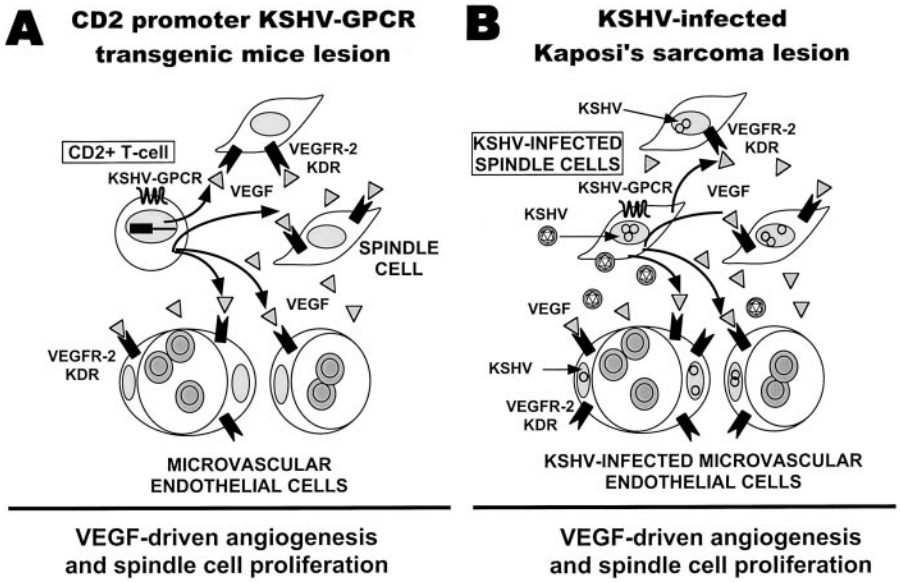

Figure 1. Potential mechanisms of KSHV GPCR–mediated paracrine neoplasia in the CD2 promoter KSHV GPCR transgenic mice and KSHV-infected KS lesions. (A) $CD2^+$ T or NK cells in the lesions express KSHV GPCR and overexpress VEGF (triangles), inducing angiogenesis and spindle cell proliferation in a paracrine fashion. (B) In KS lesions, most of the spindle cells and microvascular endothelial cells are latently infected with KSHV (episomes are shown as small circles in the cell nucleus). A few KSHV-infected spindle cells, in which KSHV undergoes lytic replication, express KSHV GPCR. These cells, according to the data from the CD2 promoter KSHV GPCR transgenic mice, upregulate VEGF secretion and have the potential for paracrine induction of spindle cell proliferation and angiogenesis.

GPCR–expressing T cells in the lesions are functionally different from their counterparts in lymphoid organs and peripheral circulation. This difference may be conferred by recruitment and activation at sites of inflammation, which may also explain the preferential development of KS-like lesions in skin and mucosal sites in the transgenic mice. However, alternative but more speculative models can be proposed. As KSHV GPCR is transforming, at least in the NIH-3T3 model of oncogenesis, it may as well cause stochastic genetic alterations in cells in which it is expressed. In the transgenic T cell population, it could lead to a transformed phenotype with particular homing, survival, and VEGF-secreting characteristics. These transformed T cells could infiltrate tissues where they might give rise to tumors in a paracrine fashion, resembling Hodgkin's disease, in which a few transformed Reed-Sternberg cells can support a dramatic proliferation of reactive lymphoid cells. Another alternative explanation for the findings by Yang et al. is the possible transient nature of the expression of the transgene in endothelial or KS spindle cell precursors, during which time it might have induced cellular genetic changes sufficient for subsequent tumor development. This possibility is supported by the finding that hematopoietic and endothelial cells have a common precursor (34, 35). There is no information available regarding CD2 expression by murine hemangioblasts, and it may therefore be hypothesized that as these cells differentiate, CD2 and, in the transgenic animals, KSHV GPCR, are no longer expressed. This is a mechanism well documented for human T lymphotropic virus 1 (HTLV-1), where the major transforming protein, Tax, is only expressed in early stages of tumor formation and is not required in established T cell malignancies. Such "hit and run" explanations have also been proposed for CMV and EBV (36, 37). Therefore, a more direct, although transitory role for KSHV GPCR in transformation cannot be definitely excluded. In KS lesions in humans, KSHV can be identified in practically all tumor cells, as assessed by latent gene expression (38, 39), suggesting a requirement for the virus for tumor maintenance in vivo, in contrast to the murine model. However, KS cell lines "lose" the viral genome after several passages in vitro, and three fully transformed and tumorigenic cell lines have been obtained from KS lesions that lack KSHV (40, 41). These findings are consistent with the idea that KSHV-independent mechanisms can support the growth of KS cells in culture, probably after KSHV-induced cellular genetic alterations.

The ability of a single KSHV gene, KSHV GPCR, to recapitulate KS-like pathogenesis when expressed by a small subset of tumor infiltrating lymphocytes strongly points to a role for this, and perhaps other lytically expressed KSHV genes, in the pathogenesis of KS. The findings reported by Yang et al. provide the first KSHV gene transgenic mouse model of KS and a fascinating model of paracrine neoplasia and angioproliferative disorders.

Submitted: 17 December 1999
Accepted: 11 January 2000

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