Under the Microscope

AIDS - Associated Kaposi's Sarcoma: A Double Jeopardy

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Until the human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) epidemic, Kaposi's sarcoma (KS) was an uncommon tumor categorized into 3 epidemiological types:

1. Classical, as originally described by Moritz Kaposi in 1872, occurs in elderly men of eastern European and Mediterranean ancestry;
2. African-endemic, found in sub-Saharan Africa, affects children and young adults;
3. Immunosuppresion-associated, seen predominantly in organ-transplant recipients treated with immunosuppresive drugs.

In the 1980s KS emerged as the leading neoplasm associated with HIV infection and AIDS (AIDS-KS), particularly in homosexual and bisexual HIV-1 infected individuals (1,2). Despite the different epidemiologies, all forms of KS often look similar histologically, composed predominantly of proliferating spindle-shaped cells which form slit-like vascular spaces, and a prominent infiltration of macrophages and other inflammatory cells. KS spindle cells do not represent a specific endothelial cell type. Some studies support lymphatic endothelial cell or smooth muscle cell origin; others have proposed origination from primitive pluripotent mesenchymal cells (3).

In the United States, the overall risk of KS amongst HIV-1 infected individuals is more than 20,000 times greater than in the uninfected population and 300 times greater than in immunosuppresed transplant recipients (4). Although immunosuppresion is the hallmark of HIV-1 infection, the analysis of the epidemiological data reported to the Centers for Disease Control (CDC) until 1989 indicated that neither immunosuppresion nor HIV-1 infection was sufficient to explain the markedly high prevalence of KS in HIV-1 infected individuals. There were differences in the incidence of KS within various HIV transmission groups, with the highest prevalence (21%) being found in homosexual or bisexual men. All other transmission groups had a significantly lower prevalence (4). KS was also found to be more common in women with AIDS who have had HIV-infected bisexual men as their sexual partners, rather than intravenous drug users (4). These epidemiologic data suggested that a sexually transmitted agent, distinct from HIV, might play a role in the development of KS, particularly in homosexual or bisexual men. Over the years, laboratory experiments have provided important insight into the roles of HIV and the proposed sexually transmitted agent in the pathogenesis of AIDS-KS.

KS - associated herpesvirus (KSHV)

Several studies have been carried out over the years to identify the sexually transmitted cofactors for KS. Although a number of viruses were implicated in various epidemiologic forms of KS, including cytomegalovirus, human papillomavirus, Epstein-Barr virus, hepatitis B virus (5), these findings lacked confirmation. In 1994, however, Chang *et al.* (6) found more convincing evidence for an infectious agent strongly linked to KS. Using a polymerase chain reaction (PCR) detection technique, they identified unique DNA sequences in more than 90% of KS lesions of AIDS patients. Sequence analysis of the PCR products and their cloned flanking regions showed partial homology to the genomes of 2 herpesviruses: Epstein-Barr virus and herpesvirus saimiri, members of the gammaherpesvirus subfamily. These data suggested the presence of a new human herpesvirus in the AIDS-associated KS, now referred to as KS-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV-8). Since its first detection, KSHV sequences have been found at a high frequency in all forms of KS including classic and endemic, as well as KS in iatrogenically immunosuppresed individuals (7), providing additional evidence that this virus contributes to the development of KS. The new herpesvirus, however, is not unique to KS. KSHV sequences have also been identified in AIDS-related body cavity based lymphomas, HIV-negative angiosarcomas, Castleman disease, and in non-KS skin lesions of immunosuppresed transplant recipients (5).

Now there is overwhelming epidemiological evidence that confirms close association between KSHV and the incidence of KS. KSHV-specific DNA has been detected in as much as 52% of the peripheral blood mononuclear cells (PBMC) of HIV-infected individuals with KS compared with only 8% without KS at the time of their blood sample (8). In the HIV-infected individuals, KSHV DNA or seropositivity has been shown several months to several years prior to the emergence of KS (5), clearly demonstrating a temporal association of KSHV infection with KS. The epidemiological studies are also consistent with the notion that KSHV is a sexually transmitted agent. Major differences in KSHV seroprevalence and KS risk exist among HIV-1 positive individuals who acquire HIV through homosexual activity versus those who acquire HIV-1 infection parenterally through contaminated blood and blood products. The incidence of KS in patients with parenterally-acquired HIV-1 infection is 1-3% (4). This range is close to the rates of KSHV seropositivity (3-5%) among these individuals (9). On the other hand, the high 15-30% risk of AIDS-KS in homosexual men (10) correlates with a high 35% seroprevalence of these individuals with KSHV (9). According to a recent study, 37.6% of the homosexually active men were found to be seropositive for KSHV compared with 0% in exclusively heterosexual men (11). The 10-year probability of developing KS in those who were positive for both KSHV and HIV-1 was as high as 49.6%. The KSHV seropositivity also correlated with other sexually transmitted diseases like gonorrhea or syphilis in these studies (9,11), providing further support to the epidemiological data that the etiologic cofactor of KS is transmitted mainly through sexual contact.

Although there is clear evidence for the presence of KSHV in KS tumors and within the endothelial cells, spindle cells, and monocytes present in these tumors (12-14), the precise role of the virus in KS pathogenesis still remains elusive. Since its discovery in 1994, much has been learned about its genomic structure and potential mechanisms by which it can induce KS and other tumors. There is now accumulating evidence that KSHV expresses a number of homologues to cellular genes such as bcl-2, interleukin-6 (IL-6), G-protein-coupled receptor (GPCR), cyclin D, and macrophage inflammatory protein type-chemokines (vMIP-I and vMIP-II) that have the potential to induce transformation, cell proliferation, and angiogenesis (5). These findings indicate that KSHV has both oncogenic and angiogenic potential but provide no insight into the direct role of the virus in the induction of KS.

A potential indirect role for KSHV in KS was suggested by the observations that none of the 3 malignant KS cell lines, 1 from an AIDS-associated KS, another from transplant-associated KS, and a third from a classical KS, had KSHV DNA sequences (15). More recently, an indirect role of KSHV has been shown in growth promotion of uninfected endothelial cells by a small subset of KSHV-infected endothelial cells through paracrine mechanisms (16). The growth-promoting effect was attributed to the upregulation of vascular endothelial growth factor (VEGF) receptor on uninfected cells causing these cells to respond to VEGF. VEGF is a major angiogenic and growth factor for KS cells (17,18), and it has been reported that signaling by KSHV-encoded GPCR induces a switch to an angiogenic phenotype through VEGF secretion which may ultimately lead to autocrine or paracrine stimulation of the angiogenic and proliferative responses necessary for the development of KS lesions (19). KSHV-infected monocytes and other inflammatory cells have been detected in the peripheral blood of patients with KS (5,14), lending support to the hypothesis that autocrine/paracrine mechanisms induced by KSHV might contribute to KS pathogenesis rather than direct infection of the endothelial cells.

HIV - 1

The high incidence and aggressive clinical course of KS in AIDS patients suggested a causative role of HIV-1 in the development of KS. The most encouraging evidence to support this hypothesis came from transgenic mouse studies showing that the HIV-1 Tat can produce KS-like lesions when introduced into the germ line of mice (20). Interestingly, these lesions were seen exclusively in male mice. On the basis of several lines of experimental evidence developed in their laboratory, Gallo and colleagues have suggested that HIV-1 plays an active role in the pathogenesis of AIDS-KS through the cooperative effects of inflammatory cytokines produced by infected or activated immune cells and the Tat protein (21).

The levels of these inflammatory cytokines such as interleukin-1 (IL - 1), tumor necrosis factor-α (TNF -α), interferon - γ (IFN - γ), and IL - 6, have been found to be elevated in the sera or culture supernatants of PBMC from HIV-1 infected individuals (21). There are also reports that treatment of AIDS-KS patients with these cytokines leads to an increase in the progression of KS. Laboratory studies have shown that HIV-1 infection can be directly implicated in the production of inflammatory cytokines through interaction of viral antigens with HIV-1-specific cytotoxic T lymphocytes (22), monocytic cells (23), and B cells (24). In addition, continuous stimulus for immune activation and inflammatory cytokine production is also provided by frequent challenge of the HIV-1 infected individuals to other infectious agents. This might be the case with homosexuals who are exposed to more sexually transmitted infections because of their life style. Studies have shown that these individuals often show signs of immune activation compared with other risk groups for AIDS and develop AIDS-KS even before developing HIV-1 infection-associated immunodeficiency (25,26).

Inflammatory cytokines present in the conditioned medium of activated T cells have been shown to support the growth of spindle cells derived from AIDS-KS lesions (21). These cytokines induced the release of basic fibroblast growth factor (bFGF) and VEGF, 2 highly angiogenic and proliferative cytokines. Subsequently, it was found that AIDS-KS cells themselves produced bFGF and VEGF in response to inflammatory cytokines, which promote their own growth in an autocrine fashion and, after release, growth of normal endothelial cells (17,27). Inoculation of these cells in nude mice results in KS-like lesions of mouse cell origin (28). The growth of AIDS-KS cells in culture and lesion formation in nude mice can be blocked by antisense oligonucleotides directed against bFGF mRNA (28). Similar results have been obtained with the use of antisense oligonucleotides directed against VEGF (17), suggesting that bFGF and VEGF play a critical role in the pathogenesis of AIDS-KS.

The HIV-1 Tat protein released into the extracellular medium during HIV-1 infection of T cells stimulates the growth of AIDS-KS-derived spindle cells (21,29). Tat also stimulates growth and induces spindle morphology in normal endothelial cells that have been exposed to inflammatory cytokines present in the conditioned medium from activated T cells (30). Tat-induced migration, invasion, proliferation, and collagenase IV expression in KS spindle cells and cytokine-activated endothelial cells in culture indicated that Tat has angiogenic potential (28,30). The *in vivo* angiogenic response of Tat has been demonstrated by subcutaneous injection of purified Tat in mice (28,31) as well as in transgenic mice expressing full-length Tat protein (20,32,33). Experimental evidence suggests that the angiogenic properties of Tat are mediated by the arginine-glycine-aspartate (RGD) sequence which is primarily a cell-attachment domain of extracellular matrix proteins and binds cell-surface integrin receptors (29). In addition, the angiogenic properties of Tat also appear to be mediated by the basic region which is similar to that of angiogenic growth factors and binds heparin complexes (34,35).

Heparin (35) and bFGF (28) have been shown to significantly enhance the *in vivo* angiogenic effects of Tat. Studies have shown that bFGF and Tat are present in AIDS-KS lesions, and integrin receptors that bind Tat are highly expressed by vessels and spindle cells (28). The integrin receptor-expression is also upregulated by inflammatory cytokines resulting in enhanced responsiveness to the Tat protein (36). Tat also upregulates the expression of endothelial leukocyte adhesion molecule (E-selectin), intracellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) proteins in human endothelial cells (37). These activation markers of endothelial cells mediate binding of inflammatory cells and regulate the extravasation of these cells into KS lesions.

Conclusion

The epidemiological and laboratory findings clearly indicate that both KSHV and HIV-1 play a crucial role in the pathogenesis of AIDS-KS. However, neither of them is sufficient to achieve the extraordinarily high incidence seen in HIV-1 infected individuals. Although, as shown in Table 1, KSHV-related DNA sequences have been detected in 100% of the KS biopsies from transplant patients undergoing immunosuppressive therapy (5), the incidence of this disease in these individuals is only 70-fold higher than in the general population compared with 20,000-fold in the HIV-1 infected individuals (Table 1) (4,38), suggesting that HIV-1 plays a more direct etiologic role in AIDS-KS. The role of HIV-1 in AIDS-KS is also supported by a recent study in Gambia showing that although KSHV infection is widespread, the risk of developing KS is 12.4 times higher in HIV-1-positive individuals than in HIV-2-positive individuals

(39). Now it is known that HIV-1 contributes to the pathogenesis of AIDS-KS through the induction of inflammatory cytokines and the HIV-1 Tat protein released from the infected cells in a biologically active form (Table 2) (15,21,29). The other infectious agent, KSHV, might have a multifaceted role in the pathogenesis of KS since its genome encodes a number of proteins that have growth-promoting, angiogenic, and transforming capabilities (Table 2) (5). One of the recently reported functions relevant to KS pathogenesis is the induction of growth and angiogenic response in KSHV-uninfected endothelial cells through the paracrine upregulation of VEGF receptors by the KSHV-infected cells (16,19). In conclusion, the risk of developing KS is much higher in individuals who are dually infected with HIV-1 and KSHV compared with those who are infected with either one of these viruses.

Table 1. Incidence of KS in HIV-1 and/or KSHV-infected individuals

	Immunosuppressive therapy-associated KS	AIDS-associated KS
(A) Viral infection		
KSHV	100%[a]	97%[a]
HIV-1	0%	100%
(B) Incidence of KS (folds) relative to general population	70[b]	20,000[c]

a (5); b (38); c (4)
KS = Kaposi's sarcoma;
HIV-1 = Human immunodeficiency virus Type 1
KSHV = Kaposi's sarcoma herpes virus

Table 2. Proposed role of HIV-1 and KSHV in the pathogenesis of AIDS-KS.

HIV-1	KSHV
• Release of inflammatory cytokines by infected or activated immune cells • Induction of angiogenic cytokines by inflammatory cytokines • Upregulation of adhesion molecules and integrin receptors on endothelial cells resulting in spindle cell morphology • HIV-1 Tat protein-induced proliferation, migration, and invasion of KS spindle cells and cytokine-activated endothelial cells	• Upregulation of vascular endothelial growth factor (VEGF) receptors on endothelial cells • Transformation of endothelial cells

See text for details and references.
HIV-1 = Human immunodeficiency virus Type 1; KSHV = Kaposi's sarcoma -associated herpesvirus
AIDS-KS = Acquired immunodeficiency syndrome-associated Kaposi's sarcoma

Acknowledgments:

We thank Dr. Felipe Samaniego of the Institute of Human Virology, Baltimore, MD, for critical reading of the manuscript and helpful comments.

We have included references to reviews and research articles that should help the reader to trace back the related work.

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