Post-Transplant Kaposi’s Sarcoma

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The once rare cancer called Kaposi’s sarcoma (KS) chiefly causes purple cutaneous macules, plaques, or nodular infiltrates, although visceral involvement may occur without skin lesions, and primarily affects elderly men in the Mediterranean region and in Eastern Europe. Today, it is no longer a rare disease, and is in fact quite frequent in acquired immunodeficiency syndrome (AIDS) patients (1) and in patients given immunosuppressants after an organ transplant (2). In this latter condition, the clinical course of KS can be very aggressive, with a mortality rate of 34% within 32 ± 32.6 months after diagnosis (2). KS is normally more common in men than in women, with a ratio of 4:1 (3), suggesting that sex hormones are protective for women. The recent discovery of a new sequence of a novel herpes virus (4), isolated from KS lesions in patients with and without AIDS, has considerably advanced current knowledge of this disease.

In some 60% of post-transplant patients, KS manifests with nodular lesions limited to the skin or oropharynx, but as many as 40% show more invasive malignant lesions with ulcerating skin, lymphadenopathy, and/or organ involvement (5). Organ localization is a marker of severity of the disease, as in the case of aggressive forms (5–8) with gastrointestinal involvement observed by upper endoscopy but no cutaneous lesions.

Epidemiology

Until 1980, the transplant literature reported few cases of KS in recipients of organ grafts (9), but the frequency has risen in the last 15 yr, coinciding with the use of new, more potent antirejection medications. Right now KS accounts for 0.52% of de novo cancers in organ transplant recipients (2) and is more common in patients on cyclosporine immunosuppression than on non-cyclosporine-based regimens (2). Thus the mean time of appearance of the tumor was shorter—12 months post-transplant (range, 2 to 33 months)—in patients on cyclosporine than in those given azathioprine and steroids alone (10) (25 months; range, 2 to 220 months) (10).

This presumably reflects the fact that cyclosporine inhibits the normal mechanisms of tumor immunosurveillance depending on suppressor T cells more effectively than did older drugs (11). This also suggests that the association of cyclosporine and KS in organ transplant recipients might reflect a state of overimmunosuppression (11–14) rather than a specific effect of the molecule. It is possible, but far from being proven so far, that cyclosporine allows the agent that is responsible for this unique tumor to flourish. A further argument that appears to give credit to the association between cyclosporine and development of KS is that patients with an history of KS before transplantation who were in apparent clinical remission all recurred after transplantation and introduction or reintroduction of cyclosporine and azathioprine (15).

The tumor is more frequent among transplant recipients of Mediterranean, Arabic, Jewish, or black origin (16), and is relatively rare among Anglo-Saxons (17). In the Johannesburg Hospital series, four of six patients who developed KS were black, an extremely high frequency considering that blacks only account for 30% of the transplant recipients in that hospital (18).

The Cell of Origin and Putative Mediator of Cell Growth

Doubts persist about the cells of origin of KS. Recent phenotypic and functional studies favor the hypothesis that the tumor originates from vascular or lymphatic endothelial cells (19,20) but other cell types have also been implicated, including smooth muscle cells, pericytes, and dermal dendrocytes (21–25).

KS spindle cells and vascular endothelial cells in areas within and adjacent to the tumor, but not in normal skin, from human immunodeficiency virus (HIV)-1 seropositive and seronegative patients, expressed CD40 antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily involved in the regulation of cell survival, proliferation, and differentiation (25) (Figure 1). CD40 has been recently implicated in Epstein-Barr virus (EBV)–associated growth transformation, as suggested by findings that this cell-surface molecule associates with a newly identified cellular protein interacting with the EBV latent infection membrane protein (LMP-1) that promotes cell growth (26). Because intracellular signals through CD40 allow cell proliferation and increase cell survival, the expression of the antigen by KS tumor cells might have an important role in the pathogenesis of this tumor.

Identification of the Causative Virus and Advances on Pathophysiology

Clinical and epidemiological observations indicate a possible infectious etiology for KS. Microorganisms suspected of causing the disease include hepatitis B virus, HIV, and Mycoplasma penetrans (27–29). Detection of human papilloma vi-
Integrins

Cells, protein findings from the possible codes suggested polymerase genesis indicate ru5

Figure 1. Schematic representation of pathways involved in the formation of Kaposi’s sarcoma (KS) lesions. Infection by HHV-8, a new member of the γ-herpes virus family, activates and transforms endothelial cells. This leads to overexpression of integrins and proteins that prolong survival of transformed KS tumor cells and endothelial cells and promote their proliferation. Among these Bcl-2, Bcl-xL, and CD40 are the most relevant. Activated KS cells and endothelial cells produce chemokines and growth factors that recruit and stimulate other tumor cells, ultimately sustaining the formation of the angioproliferative lesion.

Epstein-Barr virus (HPV)-16–related DNA in KS lesions (30) was taken to indicate that HPV-16 might somehow play a role in the pathogenesis of the tumor, although this remains unlikely in the absence of positive findings with other techniques, including polymerase chain reaction (PCR) or in situ hybridization (31).

More recent evidence that the tumor may arise in concomitance with cytomegalovirus (CMV) infections or re-infections suggested that CMV—which inhibits T cell function (32) and codes for growth factor–like substances (33)—might actually promote the development of KS (34,35). Substantiating the possible causal role of CMV were clinical findings of antibodies to CMV in nine of nine homosexual men with KS, although the significance of these findings has to be weighed against the fact that 90% of homosexual men have antibodies to CMV (36).

Even findings of CMV nuclear antigen in KS lesions are far from definite, because they may simply reflect reactivation of a latent virus in tumor tissue or a special affinity of CMV for tumor cells. The latter would be consistent with ultrastructural findings and dot-blot hybridization studies, which identified CMV in cultures of KS cells (37). Findings of CMV RNA in KS specimens (38) suggest that the virus can at least direct protein synthesis in these cells, but evidence of KS cell proliferation in nude mice in the absence of CMV DNA (35,39) strongly argues against a central role of CMV in tumor formation.

Researchers were impressed by the geographical distribution of KS in equatorial Africa, which paralleled that of Burkitt’s lymphoma, and this raised the question of whether EBV—the virus most often associated with Burkitt’s lymphoma (40,41)—was also a potential cause of KS (1). Chang and coworkers (42) found unique herpes-like DNA sequences in KS, reinforcing the possibility of a specific association of herpes viruses. Analysis of DNA from 20 KS biopsies (from classic and endemic forms) showed human herpes virus 6 (HHV-6) in 30% of cases (43).

In a retrospective case-control study (42), unique DNA sequences were identified by a representational difference analysis technique in KS tissue but not in non-KS tissue specimens from the same individual (Table 1). Four KS-associated bands were identified; two—KS390 Bam and KS480 Bam—hybridized nonspecifically to both KS and non-KS tissues, and the other two—KS330 Bam and KS631 Bam—that coded for amino acid sequences with homology to herpes viral polypeptides, were unique sequences found in over 90% of KS biopsies. Additional studies on polypeptide homology established that proteins encoded by KS-associated DNA were slightly different from the ones encoded by previously known
sequences, which can be taken to suggest a viral genome in the tumor related to but distinct from other members of the herpes virus family. On the other hand, findings of similar PCR product sequences in different forms of KS (44) suggest a highly conserved herpes-like virus DNA sequence.

All biopsies taken from Mediterranean forms of KS contained the novel herpes virus-like sequence provisionally called KS-associated herpes virus (KSHV). Samples tested blindly by PCR with specific primers to amplify KSHV showed the same herpes virus-like DNA sequences in the classic and AIDS-associated form and in HIV-seronegative male homosexuals (45). DNA from other human herpes viruses, including EBV and cytomegalovirus, never amplify KSHV. In another study, KSHV DNA was invariably found in classic, HIV, and post-transplant KS (46), definitely suggesting that the new agent—KSHV or human herpesvirus 8 (HHV-8), a member of the herpes virus family—is the most plausible candidate in the etiology of KS.

This is reinforced by recent reports of transcription of HHV-8 RNA by reverse transcription (RT)-PCR in 26 of 29 specimens (89%) of KS, although HHV-8 RNA was never found in eight biopsies of normal skin from healthy donors (47). The same investigations also showed (by in situ hybridization) that the RNA expression of HHV-8 was localized to the endothelial cells lining vascular structures and perivascular spindle-shaped cells in KS lesions.

A cell culture system has now been developed (4) for the growth of the virus, and has provided the first ultrastructural evidence of virus visualization. The fact that a very large number of HIV-related forms of KS had antibodies to a KSHV antigenic peptide (48) provides additional evidence of an etiologic role for KSHV and may, in the near future, offer an early diagnostic tool.

This virus preferentially colonizes KS cells, particularly in immunocompromised hosts. DNA testing of 193 lymphomas from 42 patients with AIDS and 151 patients who did not have AIDS (49) showed KSHV sequences in eight lymphomas of AIDS patients; all were body-cavity-based lymphomas, with pleural, pericardial, or peritoneal lymphomatous effusions (Table 2). None of the samples from the other 185 lymphomas contained KSHV sequences.

**Table 1.** PCR amplification for KS330,233 in human tissues from individual patients [from Chang et al. (42)]

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>AIDS/KS</td>
<td>27</td>
</tr>
<tr>
<td>AIDS Lymphomas</td>
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</tr>
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</tr>
<tr>
<td>Opportunistic Infections</td>
<td>13</td>
</tr>
<tr>
<td>Consecutive Surgical Biopsies</td>
<td>40</td>
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</table>

* PCR, polymerase chain reaction; KS, Kaposi's sarcoma.

**Immunity**

Although the high degree of conservation of KSHV sequences in KS and in the eight lymphomas implies that the same virus is involved in both lesions, a subsequent study (50) suggests that KSHV can also play a role in skin lesions other than those of KS. Thus in renal transplant recipients (50), KSHV was found in 78% of biopsies of actinic keratosis and 93% of cases of squamous cell carcinoma. Interestingly, in that study, KSHV sequences—admittedly in low copy numbers—were also found in a sample of apparently normal skin from an organ transplant recipient. It therefore appears that KSHV, as reported for the related EBV, is a quite common latent virus, possibly reactivated by immunosuppression (either AIDS-related or drug-induced), which causes neoplastic transformation and proliferation of cells normally hosting the virus in a quiescent state.

Whether other viruses are activated by immunosuppression and act synergistically with KSHV in promoting the development of tumoral skin lesions has to be further explored. In a recent study (6), KSHV was detected by PCR in situ hybrid-
ization in endothelial cells lining the different types of vessels in all forms of KS, including post-transplant KS and in KS spindle cells. KSHV was never found in normal vessels in the surrounding dermis.

Taken together, these findings certainly suggest that the agent that causes KS is actually a new human herpesvirus. This possibility has received strong support from very recent findings that the KS330 Bam sequence is part of a longer herpesvirus genome (51). Using an infected B lymphocyte cell line derived from an AIDS lymphoma and a genomic library obtained from a KS lesion, a 20.7-kilobase region of the genome was recently sequenced, finding positional homology with herpes virus genes (51). Phylogenetic analysis of single genes and combined gene sets led to identification of the agent as a gamma-2 herpes virus (genus *Radinovirus*), which can now be better referred to as HHV-8 (Figure 2).

Sera from patients with KS have antibodies specifically directed against antigens of infected cells, none of which was found in sera from control subjects or patients with AIDS who did not have KS. Thus HHV-8 may be a passenger in KS cells, its replication being induced by various cytokines recently found to be synthesized and secreted in excess by these cells. Cultured KS synthesize and secrete interleukin (IL)-6 and platelet-derived growth factor (PDGF) and express mRNA for the PDGF-β receptor. In addition, KS cell growth and proliferation in culture depends on a series of cytokines (52–55), including IL-6 and PDGF as well as basic fibroblast growth factor. KS cells cultured from human lesions express high levels of hepatocyte growth factor (HGF), transforming growth factor β, and HGF receptor (c-MET) (56). It is tempting to speculate that these cytokines synergize with the other ones (discussed earlier) in initiating and maintaining an autocrine stimulatory loop indispensable for KS cell growth.

Data that estrogens downregulate IL-6 (57), which could be an indirect mechanism by which female hormones inhibit KS lesions, are of some interest. KSHV was detected in peripheral blood mononuclear cells of KS patients, but not in blood donors or hospital control subjects (58). This KSHV detection in peripheral blood cells correlated with CD4-positive T cells and predicted the subsequent manifestation of KS.

Extrapolated to post-transplant cases, the above data obtained in AIDS KS would by analogy reinforce the concept that overimmunosuppression may favor proliferation of cells infected with KSHV, thus supporting a causative role for KSHV in KS. Findings of KSHV in peripheral blood mononuclear cells also suggests that growth of KS is somehow controlled by cytokines and growth factors, produced by infected peripheral cells, that may trigger proliferation of endothelial cells.

Evidence of KSHV DNA sequences in semen from otherwise healthy persons (59), together with the fact that KS is 10 to 20 times more frequent in AIDS-infected homosexuals than in cases of AIDS related to intravenous drugs (60), suggests that KSHV, while producing an occult infection in healthy

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**CLASSIFICATIONS OF HERPESVIRUS GROUP (HERPESVIRIDAE)**

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<tr>
<th>Subfamily</th>
<th>Genus</th>
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<td><em>(Simplexviridae)</em></td>
<td>Herpes simplex virus -2 (HHV-2)</td>
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<td><em>(Varicellazoober virus)</em></td>
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<td>Human cytomegalovirus group</td>
<td>Herpesvirus 5 (HHV-5)</td>
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<td>Kaposi's virus (?)</td>
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</table>

*Figure 2. Classifications of herpesvirus group (Herpesviridae).*
people, can subsequently reactivate in concomitance with other triggers to cause overt disease.

Clinical Manifestations

KS manifests with multiple skin lesions (plaques, nodules, and tumors) of a red or purple color with a bluish tinge in blacks. Aged or regressing lesions become brownish-blue. Lesions may be covered by atrophic skin and may ulcerate. Involvement of the lower limbs is frequently associated with variable degrees of edema that sometimes may even limit walking. Internal organs can be affected even in the absence of cutaneous lesions, and in severe cases this may lead to internal hemorrhages.

Organ transplant recipients who develop KS commonly present the first lesions soon after surgery (2,18), and sooner with liver than with kidney transplants. Clinical manifestations of post-transplant KS are generally more severe than in the general population, in which KS primarily manifests as a slowly progressive multifocal tumor (61). Almost 40% of post-transplant KS patients (62) have visceral disease commonly involving the gastrointestinal tract (63) and lungs (64), but the bladder or oropharynx is occasionally involved (65,66). In most patients, the disease progresses rapidly, with extensive involvement of the upper extremities, trunk, head, and legs (67).

That KS in organ transplant recipients may be a feature of overimmunosuppression agrees with clinical observations that other cancers, often of the reticuloendothelial system, develop in concomitance with KS or after it has been diagnosed (68,69). Clinical suspicion is based on the color and location of cutaneous lesions, which may resemble angiomas, malignant melanoma, or lymphomatous nodules, so a skin biopsy is mandatory for clinical diagnosis (70). Laboratory findings usually include elevated monocyte counts in the peripheral blood, with eosinophilia in occasional patients. In rare instances, patients may have microangiopathic anemia (71).

Treatment

Most authorities agree that the first line of approach to treating post-transplant KS is to progressively reduce and eventually stop antirejection medications (17,24,72). Data from the Cincinnati Transplant Tumor Registry (73) show that reduction or elimination of immunosuppressants was followed by complete remission of the disease in 30% of patients. Those patients who rejected their graft after immunosuppression was reduced may have benefited from reinstitution of antirejection therapy, but there were cases in which immunosuppression had to be abandoned (24). The decision to stop antirejection therapy completely is always difficult and should take into account the severity and extent of the cutaneous lesions, degree of visceral involvement, and particularly whether the transplanted organ is life-supporting.

With regard to the specific therapy of KS, despite the lack of control trials, the cumulative experience presented in a recent review (13) indicates that surgical excision or local radiation is enough for localized lesions. Widespread lesions that fail to respond to reduction of immunosuppressants, possibly supplemented with local radiation, have been treated with chemotherapy, i.e., vincristine, vinblastine, and bleomycin as single agents or in combination(s) (73,74,13). Guidelines to therapy still rest however, on studies in AIDS and epidemic KS, because there are no data on post-transplant forms.

Early treatments of AIDS-associated KS were largely based on a consensus from the U.S. National Cancer Institute workshop of 1981 (75). Patients with minimally extensive disease were recommended for single-agent chemotherapy, and those with advanced forms for combined chemotherapy. Three molecules were suggested as single agents: etoposide, vinblastine, and bleomycin (76,77). The effectiveness of doxorubicin and related anthracyclins appears to be confined to isolated cases (78). Several uncontrolled studies in patients with pulmonary KS or extensive mucocutaneous lesions showed a better response to combined treatments than to single agents. More severe cases with organ involvement clearly require combination chemotherapy, which consists of doxorubicin, bleomycin, and vincristine, or bleomycin and vincristine (79). A new formulation of liposomal doxorubicin is now being compared with conventional combined therapy in a controlled trial (80).

Because chemotherapy increases the risk of opportunistic infections, these multidrug protocols are normally used in association with nystatin and/or amphotericin and combined with Pneumocystis carinii chemophrophyaxis (81). The severity and duration of neutropenia after chemotherapy for AIDS-related KS can be reduced by using granulocyte-macrophage-colony stimulating factor (82). In theory, the anti-retroviral agent zidovudine in combination with cytotoxic chemotherapy (bleomycin and vincristine) should help prevent opportunistic infections while increasing the chances of the tumor responding to chemotherapy, but a conclusive answer awaits the results of prospective trials now being planned (83).

Recombinant interferon alpha-2a was studied in patients with KS and AIDS and appeared to be relatively well tolerated (84). Results were conflicting (13,85) and apparently confined to patients with higher CD4+ T cell counts (86). The dose-limiting toxic effects were fever, myalgia, and bone marrow depression. The combination of reduced immunosuppression and interferon could precipitate rejection (13).

Intral esional interferon-α injection was also attempted recently in epidemic forms of KS, with some encouraging results (87). Other agents, including interferons beta and gamma, tumor necrosis factor, and IL-2, have been tested less widely, and data so far are inconclusive despite occasional claims of benefit for systemic interferon beta and intral esional tumor necrosis factor injections (88).

In general terms, however, a major question concerns the application of chemotherapy protocols originally developed for AIDS to post-transplant cases, particularly to those in whom KS is a consequence of overimmunosuppression (68,89,90).

Whether radiation actually has a role in the treatment of KS has not been clearly defined. There is at least one study in nontransplant cases that shows that radiotherapy effectively and completely controlled local disease without side effects (91). Painful and incapacitating skin lesions can benefit from local radiotherapy (92).

A completely different approach is based on the rationale that KS cell growth can be limited by specific antiviral agents.
Morfeldt and Torssander (93) noted regression of KS in two HIV patients who also had CMV disease with organ localization, and who had bee treated with foscarnet, a new agent with documented antiviral properties in herpes and retrovirus infections (94). This observation led to a pilot study in AIDS patients with a recent onset of KS. Three of five patients treated with foscarnet had a long-term remission of KS. Despite the small number of patients, findings appear to warrant a controlled trial to establish the real value of antiviral drugs in KS.

Human β-chorionic gonadotropin (βHCG) and serum from pregnant mice and women inhibit the growth of KS cell lines in vitro, and pregnant mice inoculated with KS cell lines do not develop the disease, unlike control mice (95). Pregnancy slowed the progression or abolished the lesions in two KS patients (96). On the basis of these observations, six HIV patients with KS were given human βHCG, achieving remarkable tumor regression in one report (97) and no substantial side effects, whereas in another trial (98) enrolling only five patients, treatment was stopped prematurely for lack of demonstrable effect and because of toxicity.

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