4. KSHV-ASSOCIATED DISEASE IN THE AIDS PATIENT

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INTRODUCTION

Twenty five to thirty percent of all human cancers are etiologically linked to an infectious agent, such as a virus or bacterium. These microbes are normally kept in check by the host immune system. However, in individuals that are immunodeficient, such as acquired immunodeficiency syndrome (AIDS) patients or those receiving immunosuppressive drugs following organ transplantation, this checkpoint fails and there is a correspondingly higher risk for the development of cancers associated with infectious agents. Viruses contribute to the development of neoplasia either cell autonomously through the activities of viral oncogenes, or through paracrine mechanisms that modulate the transformed cell as well as the supporting microenvironment.

Prior to the onset of the AIDS epidemic and the emergence of HIV, Kaposi's sarcoma (KS) was described in 1872 by Moritz Kaposi, the then head of the Vienna Dermatology clinic, as "*idiopathisches multiples Pigmentsarkom*," a rare angiosarcoma in elderly men of Mediterranean descent.[1] In the early and mid-1980s, the HIV epidemic lead to a dramatic increase in the incidence of KS. KS remains the most common neoplasm seen in individuals with AIDS today. In 1994, KSHV (also known as human herpesvirus 8; HHV-8) was initially identified in KS lesions of AIDS patients by Chang et al.[2] using representational difference analysis.

KSHV AND THE DEVELOPMENT OF KS

KS is divided into four subtypes with distinct clinical manifestations: classic, endemic, epidemic or AIDS associated, and iatrogenic. Classic KS is a disease of elderly Mediterranean and Eastern European men, while endemic KS is found in parts of equatorial Africa such as Uganda and Zambia[3] where it is responsible for an estimated 1% of all tumors. Hence, in these regions, transmission of KSHV is thought to occur vertically. Endemic KS tends to be more aggressive than classic KS.

Widespread HIV-1 infection has resulted in an epidemic of KS. Prevalence levels for KSHV antibodies reach 30% in black South African HIV patients, and childhood KS has become the most common neoplasm in many regions of sub-Saharan Africa that are ravaged by HIV infection. In 1981, KS was recognized as a defining pathology for the diagnosis of AIDS. Highly active antiretroviral therapy (HAART) has led to a substantial decline of AIDS-related KS in the United States. However, even in the current post-HAART era, standardized incidence rates for KS are higher than that of any other AIDS-defining or AIDS-associated cancers.[4] This suggests that KS will remain a permanent health problem for years to come.

Iatrogenic KS occurs after solid-organ transplantation in patients receiving immunosuppressive therapy[5] and KS comprises an estimated 3% of all tumors associated with transplantation.[6] This is seen particularly in regions of high KSHV prevalence, such as Southern Italy, Turkey, and Saudi Arabia. KSHV present in the recipient may be acquired during iatrogenically induced immunodeficiency after transplantation or may be transmitted through the graft itself.[7] The frequency of KS in AIDS patients is 20,000 times higher than in the general population and the frequency of KS in transplant recipients is 500 times higher than in healthy individuals.[8] It is important to note that 95% of all KS lesions, regardless of clinical type or HIV status, contain KSHV viral DNA thus strongly linking KS to KSHV infection.

In the mid-1980s, incidence rates for KS showed a greater exponential increase. At that time KS was only observed in AIDS patients with a history of men who had sex with men, but not in individuals who became HIV infected through blood transfusion.[9] In AIDS-associated KS, incidence rates correlated significantly with the lifetime number of male sexual partners,[3] which established KSHV as a sexually transmitted agent responsible for the development of this cancer. Today, more women have become infected with HIV and consequently KS has now also been reported in this group. African KS also affects both genders; while classic (Mediterranean) KS affects predominantly elder men. The reason for the gender bias in classic KS is unknown. In the US, KS incidence rates per age group follow a bimodal distribution that peaks at ages 30–36 and again at ages >70. Since incidence rates for most spontaneously occurring cancers increase exponentially with age due to the accumulation of spontaneous mutations in tumor suppressor and oncogenes, the bimodal distribution of KS posits an infectious agent with oncogenic potential as the cause of the disease.

The KS lesion itself is highly angiogenic and comprises spindle-shaped cells, slit-like endothelium-lined vasculature, and infiltrating blood cells. The spindle cells

form the majority of the cell population, and are thought to arise from lymphatic endothelial cells.[10] In fact experimental KSHV infection can reprogram the endothelial gene expression profile into that of the lymphatic endothelium.[11,12]

KS lesions are classified as plaque, patched, or nodular. As the KS tumor progresses clinically, the number of KSHV-infected cells increase and the endothelial cell population within the lesion expands. KS lesions range from patches or plaques, to nodules and there is evidence for both polyclonality and monoclonality of the lesions.[13,14] It is thought that KS probably initiates as a polyclonal hyperplasia and develops into a clonal neoplasia. KS not only affects the skin but also involves multiple organs such as the liver, lung, spleen, and gastrointestinal tract. Very aggressive types of KS can lead to foci formation in the visceral organs and ultimately result in hemorrhage and death.

KSHV viral load in PBMC rises in the one-to-six months that precede lesion formation.[15] A rise in viral load predicts imminent clinical lesions independent of HIV or immune status[16] (also Dittmer and Martin, unpublished). KSHV is found in circulating B cells as well as macrophages and endothelial cells.[17–19] The presence of anti-KSHV antibodies documents prior exposure but does not allow a prediction of KS development, since in HIV-positive individuals the median time from seroconversion to disease is 7 years or greater.[3,9]

KS-tumor explants lose the virus after serial passage in tissue culture over time. This suggests that ex vivo passage selects for cells that no longer depend on the virus for survival and that the cells previously infected with KSHV have undergone epigenetic mutations or changes that allow the cells to persist without the virus. KSHV-infected endothelial cell preparations in culture generally also lose the virus over time,[20,21] though KSHV-positive tumor cell lines have recently been derived.[22]

KSHV AND THE DEVELOPMENT OF LYMPHOMAS

In addition to KS, KSHV is also found in B lymphoproliferative diseases; primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). In fact, the first association of KS and a B-cell lymphoproliferative disorder, MCD, was reported in a patient who presented with both diseases.[23] Greater than 50% of KSHV-positive transplant recipients develop lymphoproliferative disease[24] and KSHV transmission can occur from organ donor to organ recipient[7].

The association between KSHV and MCD and PEL has been firmly established.[25,26] MCD is a B-cell lymphoproliferative disorder that is sometimes referred to as multicentric angiofollicular hyperplasia. As the name implies, patients usually present with diffuse lymphadenopathy and a series of constitutional symptoms. The disease is characterized by vascular proliferation of the germinal centers of the lymph node. There are two forms of MCD: (1) a plasmablastic variant form that is associated with lymphadenopathy and immune dysregulation and (2) a hyaline vascular form, which presents as a solid mass. Nearly 100% of AIDS-associated MCD is associated with KSHV, while approximately 50% of non-AIDS-associated MCD contains KSHV DNA. AIDS-associated MCD is usually accompanied by the development of KS in the affected individual.

MCD is a polyclonal tumor and is highly dependent on cytokines such as interleukin-6 (IL-6). KSHV itself encodes a viral IL-6 that is also expressed in these lesions.[27] Viral antigens can be detected in the immunoblastic B cells in the mantle zone of the lymph node. The plasmablasts in MCD express monotypic IgM light chains[28] and MCD is a polyclonal disorder with MCD patients frequently developing cytopenia, autoimmune disease, and other malignancies such as KS and non-Hodgkin's lymphoma.[29]

PELs, sometimes referred to as body cavity based lymphomas, represent a specific subset of non-Hodgkin's B-cell lymphomas that involve body cavities and form a distinct clinicopathologic group.[30] Most PELs are KSHV-positive, and are often coinfected with EBV as well. PELs may involve the peritoneal, pleural, or pericardial cavities. These tumors are typically large-cell immunoblastic or anaplastic large-cell lymphomas that express CD45, clonal immunoglobulin gene rearrangements, and lack of c-myc, bcl-2, ras, and p53 gene alterations.[29,30]

PELs have the characteristics of a preterminal stage of B-cell differentiation. Since PELs have mutations in their immunoglobulin genes, they are thought to arise from post-germinal center B cells. Most PELs express CD138/syndecan-1 antigen, which is normally expressed by a subset of plasma cells, but they do not express immunoglobulins.

Although KSHV has been associated with PEL and MCD in HIV patients, other reports describe cases of KSHV-positive lymphomas that do not fit the classic PEL phenotypes. For instance, KSHV has been linked to cases of germinotropic lymphoproliferative disease (GLD).[31] This disease also involves plasmablasts but unlike plasmablastic lymphomas, the GLD lymphomas contain polyclonal immunoglobulin receptors. Another report suggests a high incidence of KSHV infection in solid HIV-associated immunoblastic/plasmablastic non-Hodgkin's lymphomas that developed in patients lacking PEL and MCD[32] and yet others have found KSHV associated with solid lymphomas, which resemble PEL cell morphology but do not present as effusions.[33] This suggests a model in which KSHV infects an early germinal center B cell that can still differentiate into multiple lymphoma phenotypes dependent on secondary mutations to the cellular genome.

The evidence linking KSHV to KS, PEL, and MCD is overwhelming and has been confirmed by multiple laboratories. KSHV DNA has also been detected in multiple myeloma, primary pulmonary hypertension, angiosarcomas, as well as malignant skin tumors in post-transplant patients such as Bowen's disease, squamous cell carcinomas, actinic keratosis, and extramammary Paget's disease. However, these disease associations are at present controversial.[29]

PREVALENCE OF VIRAL INFECTION

Several serology studies have suggested that KSHV infection is widespread in Africa with 30–60% of people being KSHV-positive, but is uncommon in the United States and Western Europe with seropositivity ranging from 3 to 10% in these areas.[34] Regions such as Italy and Greece show a higher prevalence of KSHV at

about 4–35%,[35] which correlates with correspondingly higher incidence rates for classical or transplant-associated KS. Transmission routes include sexual transmission, mother-to-child transmission, as well as salivary transmission.[3,36,37]

THE KSHV GENOME

Herpesviruses are a diverse group of DNA viruses that differ in their biology and disease induction. A hallmark of herpesviruses is their ability to establish a latent infection for the lifetime of their host. Pathogenesis caused by these viruses is usually seen in the context of host immune suppression. All herpesviruses share a common evolutionary origin, which is highly evident from the homology seen among a substantial number of herpesviral genes.[38] Based on biological characteristics and genomic organization, herpesviruses are classified into three subfamilies: alpha, beta, and gamma. The gamma herpesviruses are lymphotropic and some are capable of undergoing lytic replication in epithelial, endothelial, or fibroblast cells. The gammaherpesvirinae are grouped into two classes: lymphocryptoviruses (gamma-1) and rhadinoviruses (gamma-2). Epstein-Barr virus (EBV) or human herpesvirus 4 (HHV4) is a lymphocryptovirus while KSHV (HHV8) is a rhadinovirus.

During latent infection, viral gene expression is highly attenuated and the viral genome remains stably associated with the cell. In the lytic phase of infection, viral gene expression and DNA replication ensue, leading to the production of progeny virions and eventual lysis of the cell. The KSHV viral genome comprises a ~140 kb long unique region flanked by multiple terminal repeat sequences with the total genomic size being ~160–170 kb. KSHV has at least 80 open-reading frames (ORFs) that encode for proteins greater than 100 amino acids.[39] The viral genes encoded by KSHV can be divided into three classes: (1) genes common to all herpesviruses, (2) genes unique to KSHV (these are generally given a "K" designation followed by the number of the open reading frame (ORF), and (3) KSHV-encoded genes that are homologous to cellular genes (these may be unique to KSHV or shared with other herpesviruses), and are likely to have been usurped from the host genome during the course of evolution. It is likely that several viral genes contribute to the neoplastic process[38] (Wong, 2005, p. 6849). [Au1]

MOLECULAR BIOLOGY OF KSHV-ASSOCIATED DISEASE

A relatively recent concept in understanding KSHV gene expression in human KS, PEL, and MCD disease has involved the use of microarrays to profile viral gene expression. Since the KSHV genome is orders of magnitude smaller than the human genome, it has been feasible to develop whole genome arrays based upon real-time quantitative RT-PCR for all individual viral genes and to analyze primary KS biopsy samples and KSHV-infected lymphomas.[40,41] Conventional microarray-based viral gene expression in KSHV-infected lymphomas has also been performed.[42,43] These new techniques generate a viral signature for each disease state and offer a chance to classify KS beyond Moritz Kaposi's observational diagnosis. High-throughput genomic profiling offers the chance to accelerate our

investigations into KSHV-associated cancers as much as it has benefited research into nonviral cancers. Microarray analyses of host cell transcription[44–46] proved that KSHV-positive PEL differ from other types of B-cell lymphomas. This is consistent with the idea that KSHV reprograms the tumor cell. Recently it was shown that KSHV infection reprograms endothelial cells toward a specialized cell fate; that of lymphatic endothelium, which expresses characteristic lineage markers, such as LYVE-1.[11,12] Several studies have ascertained the host transcription profile in tissue culture models of KSHV infection.[47–50] Potentially interesting drug targets emerged in each of these studies but a consensus among the different models has yet to be found. KS will almost certainly have a cellular transcription signature that is distinct from other cancers and tied to the unique pathology of this disease, as an angioproliferative, cytokine driven disease. For instance, c-Kit and other growth factor receptors in microarray studies of KSHV-infected endothelial cells led to a successful pilot study using the kinase inhibitor gleevec (imatinib).[51] Another recent phase II study found a significance response rate of KS to a matrix metalloproteinase inhibitor.[52]

Every KS tumor transcribed high levels of the canonical KSHV latency genes LANA, vFLIP, vCyclin, and Kaposin. These genes are under control of the same promoter and are expressed in every KS tumor cell.[53,54] Kaposin is located immediately downstream of these three genes and, in addition to the common promoter, can be regulated by a promoter located between LANA and cyclin[55] and during lytic reactivation yet another, ORF-proximal promoter.[56] Like LANA, Kaposin too is expressed in every tumor cell[18] and has recently been shown to stabilize cellular cytokine mRNAs.[57] In addition to these latent proteins, many KS tumors as well as PEL engrafts[58,59] express an extended set of proteins that were initially classified as lytic viral genes, but in the context of the tumor may be the result of abortive or incomplete viral reactivation. These include the KSHV interferon regulatory factor (vIRF-1) and G-coupled receptor (vGPCR) homologs[41] and the K1 constitutive signal protein,[59,60] which suggests that a subset of KS phenotypes may be attributable to these genes and the paracrine mechanisms that they invoke.[61–63] Interestingly, vIRF-3, a duplicated KSHV IRF homolog, is constitutively transcribed in KSHV-infected PEL,[64] but not KS. Thus we speculate that KSHV has to interfere with the host cell's innate interferon response in every infected cell regardless of cell lineage or mode of infection and has thus placed multiple copies of the vIRFs, all of which interfere with normal interferon signaling, under different control elements, e.g., vIRF-3 is specific for B cells while vIRF-1 is specific for endothelial cells. Thus, both latent and select lytic genes can be considered tumor-specific therapy targets for KS.

THERAPIES TO TREAT KS, PEL, AND MCD

Currently, treatment modalities for KS include observation, local therapy, or systemic chemotherapy such as paclitaxel and liopsomal anthracycline,[65] depending on the severity of the disease. Interferon alpha is also used to treat KS. KS is a

highly angiogenic tumor but a clinical trial targeting the angiogenic nature of KS using IM862 proved ineffective in obliterating KS.[66] However, a clinical trial involving daily doses of imatinib mesylate (Gleevec), which targets c-kit and platelet derived growth factor receptor signaling, resulted in clinical and histologic regression of cutaneous KS,[51] as did a trial of a matrix metalloproteinase inhibitor.[52]

A recent report suggested that recipients of organ transplants, who were susceptible to KS due to immunosuppressive therapy, benefited from treatment with sirolimus (also called rapamycin) since sirolimus displayed both immunosuppressive and antineoplastic properties.[8] Sirolimus likely acts via an antiangiogenic mechanism ultimately reducing the levels of VEGF and of VEGF receptor on endothelial cells.

Currently many clinical investigations are underway for treating KS. These include (1) bevacizumab, a recombinant human anti-VEGF antibody, (2) valproic acid, which can activate KSHV lytic replication in vitro leading to lysis of the infected cells,[67] (3) halofuginone, an inhibitor of matrix metalloproteinases, and (4) IL-12 therapy, with and without liposomal doxorubicin.[68]

Risk for KS and virally associated lymphomas increases rapidly as the $CD4^+$ cell counts of HIV-infected individuals diminish,[69] and the risk of developing AIDS-associated cancers is lower for individuals who are less severely immune suppressed. Since the prevalence of KS in AIDS patients is very high, and HIV coinfection is thought to be an important factor in the development of KS, attempts to control KS by improving the immune system of HIV-infected individuals through HAART is recommended. Indeed, the incidence of KS has declined considerably following the introduction of HAART therapy and often HAART alone will lead to KS regression in AIDS patients. However, it is important to note that even in the face of HAART therapy, the likelihood of an HIV-positive individual developing KS is still 20 times higher than uninfected individuals.[69]

Current treatments for MCD, PEL, and other AIDS lymphomas include standard chemotherapy such as CHOP, which contains four drugs – prednisone, vincristine, cyclophosphamide, and doxorubicin, or EPOCH, which in addition contains etoposide. These can be given coincidentally with HAART.[70,71] Case reports in the literature also suggest that rituximab (rituxan) is effective against PEL and MCD. Rituximab is an anti-CD20 antibody, but because rituximab targets normal B cells as well, it can be associated with an increased risk of infection when used in AIDS patients.[72] Scott et al. have reported on two MCD patients that went into sustained remission with just oral etoposide.[71] Another line of thinking has lead to exploratory studies using antiherpesviral drugs that inhibit herpesviral replication such as ganciclovir or AZT[73–75] in patients, suggesting a mechanism of action that suppresses viral reactivation and dissemination rather than direct tumor toxicity. Cidofovir, another herpesvirus polymerase inhibitor, did not show a clinical benefit.[76] HAART therapy has resulted in varying degrees of success with respect to decline in the incidence of non-Hodgkin lymphoma. It is estimated that HAART therapy decreases the incidence of non-Hodgkin lymphoma anywhere in the range

of 40–76%. Moreover, there is emerging, but as of yet controversial evidence that protease inhibitors such as indinavir, which also inhibit matrix metalloproteinase may have direct anti-KS activity in addition to HAART-associated reconstitution of the immune system.[77]

More information on current trials that are underway to treat KS, PEL, and MCD can be gleaned by visiting the National Cancer Institute (NCI) website: http://www-dcs.nci.nih.gov/branches/aidstrials/adlist.html or http://www.amcoperations.com.

CONCLUSIONS

As a consequence of HAART therapy, the life expectancy of HIV-infected individuals has increased tremendously. It is likely that as these HIV-infected patients continue to age, there will be a corresponding increase in the incidence of AIDS-defining as well as non-AIDS defining cancers. Most of the current therapies with the exception of antiherpesviral drugs do not take advantage of the unique viral etiology of KSHV-associated cancers, and antiherpesviral drugs themselves are not effective against latent virus. Thus it will be important to show that "traditional" anticancer therapies are safe in the context of HAART and HIV infection, and to develop future therapies that directly impact upon, and obliterate, the function of viral genes.

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