HIV and KSHV-Related Cancers: Diseases at the Crossroads of Virology, Immunology, and Cell Biology

Although combination antiretroviral therapy makes AIDS less deadly, HIV-infected individuals remain vulnerable to a variety of cancers

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The development of combination antiretroviral therapy (cART) converted AIDS to a manageable chronic disease by 1996, and led to marked changes in this epidemic. Patients receiving cART usually show a rapid decrease in HIV to essentially undetectable levels, an increase in CD4 cell counts, and substantial restoration of immune function. In the United States (US), the number of deaths from AIDS dropped dramatically and patients are living substantially longer.

However, AIDS has not gone away, and the number of persons newly infected with HIV each year in the US remains essentially unchanged. Thus, the number of persons living with HIV infection in the US nearly doubled since 1996 to more than 1.1 million (Fig. 1A). Globally, an estimated 35 million persons are infected with HIV.

When AIDS patients receive cART, there are fewer deaths from AIDS itself, from opportunistic infections, or from opportunistic tumors such as Kaposi sarcoma (KS) that appear among individuals with low CD4 levels. Although HIV-infected patients continue to develop AIDS-associated tumors such as KS or lymphoma, their incidence rates are lower than earlier in the epidemic. However, those HIV-infected individuals who are living longer have subtle defects in their immune systems and HIV-associated inflammation. Also, the population of HIV-infected persons is increasing in age. Among the unanticipated consequences of these trends, the number of cases of other tumors are increasing, including rates for anal cancer, lung cancer, hepatocellular carcinoma, Hodgkin lymphoma, and oropharyngeal cancer (Fig. 1B). In fact, cancer is the leading cause of death in HIV-infected patients.

Antiretroviral Therapy Early during the AIDS Epidemic

When AIDS appeared in 1981, it presented clinicians and researchers with a new and bewildering illness in which patients were susceptible to an array of infectious, malignant, and other conditions. In 1983, human immunodeficiency virus (HIV) was discovered by Françoise Barré-Sinoussi, Luc Montagnier, and colleagues in the Pasteur Institute in Paris. Several months later, this virus was shown to be the cause of AIDS by Robert Gallo and colleagues in the National Cancer Institute (NCI) in Bethesda, Md.

Gallo and colleagues also developed an effective test for antibodies to HIV, which were soon used in an assay to screen the blood supply for HIV.

SUMMARY

➤ Cancer is now the leading cause of death in HIV-infected patients taking combination antiretroviral therapy.
➤ By identifying several antiretroviral agents that proved effective against HIV, researchers at the National Cancer Institute helped to bring the AIDS epidemic under control.
➤ Most of the cancers associated with HIV or AIDS are caused by one of several viruses.
➤ Although combination antiretroviral therapy controls HIV, individuals living with this virus tend to develop some kinds of otherwise rare cancers but not other cancers.
➤ In a small clinical trial, ganciclovir and zidovudine were used successfully to treat patients with KSHV-induced multicentric Castleman disease; the drugs are activated by lytic KSHV genes.
HIV. At this time, there were fewer than 8,000 cases of AIDS in the US. However, blood testing showed this lethal virus already infected about 500,000 persons in the US, with most of them unaware of their infection.

About this time, I joined with Samuel Broder and Hiroaki Mitsuya at NCI to search for an effective HIV therapy. The dogma then was that developing antiviral drugs was a fool’s errand. However, over the next several years we developed and did the initial clinical testing of the nucleoside reverse transcriptase inhibitor zidovudine (AZT), working with collaborators at Burroughs Welcome Co. We then invented, developed, and conducted initial clinical studies on two other drugs—didanosine (ddI) and zalcitabine (ddC)—and using these drugs started the first trials of combination antiretroviral therapy.

In these and subsequent studies, nucleoside antiretroviral therapy was found to improve immune function, reduce AIDS-related complications, and extend the lives of persons with AIDS. Subsequently, when protease inhibitors were developed in the mid-1990s, clinical investigators learned that treating such patients with three drugs—two nucleoside analogs and a protease inhibitor—could reduce HIV replication to almost zero, prevent the development of drug resistance, and substantially restore immune functions.

Several Viruses Deemed Responsible for AIDS-Related Malignancies

With pharmaceutical companies developing additional antiretroviral agents, I turned my attention to AIDS-associated malignancies. One striking but puzzling feature of AIDS is its association with cancers that were rare in the general population. These cancers tended to occur in AIDS patients with very low CD4 levels. Several of these cancers—KS, certain types of aggressive lymphomas, and invasive cervical cancer—were considered “AIDS defining,” meaning that they confer a diagnosis of AIDS when they develop in an HIV-infected individual.

Before AIDS, KS was typically seen in elderly men in the Mediterranean region, in sub-Saharan Africa, and in organ transplant recipients. Among AIDS patients, KS was much more common in men who had sex with men than among injection drug users or blood transfusion recipients. This pattern led scientists to hypothesize that a second transmissible agent other than HIV was the cause of KS.

However, the identity of this agent remained elusive until 1994, when the husband-wife team of Patrick Moore and Yuan Chang, then at Columbia University, discovered a novel gamma-herpesvirus closely related to Epstein Barr virus and demonstrated that it causes KS. This virus, called Kaposi sarcoma-associated herpesvirus...
(KSHV) or human herpesvirus-8 (HHV-8), also was found to cause a rare form of lymphoma called primary effusion lymphoma (PEL) and a form of multicentric Castleman disease (KSHV-MCD). With this discovery, it became apparent that most of the cancers closely associated with AIDS are caused by other viruses, especially Epstein Barr virus, human papillomavirus, hepatitis B and C viruses, and the more recently discovered Merkel cell virus.

With widespread use of cART, the number of tumors that occur with very low CD4 counts declined, highlighting the importance of deficient immune control in their pathogenesis. However, as patients are living longer, the number of non-AIDS-defining tumors increased. The factors driving the development of these non-AIDS-defining tumors vary. Oncogenic viruses are the cause of a number of these HIV-associated cancers, including anal cancer, nasopharyngeal cancer, Hodgkin lymphoma, hepatocellular carcinoma, and Merkel cell tumor. Immunodeficiency and chronic inflammation caused by HIV is believed to play a role in nearly all these tumors. The increase of lung cancer may be due in part to increased smoking among those at risk for HIV. Meanwhile the incidence of other cancers, including those affecting the breast, colon, or prostate gland, is not increased among HIV-infected individuals. Learning what leads to these differences should help us to understand better how the immune system affects the development of tumors.

**Role of Hypoxia in KS and Other KSHV-Associated Tumors**

While treating patients with KS, I was struck that the tumor often involved their feet, which have a relatively poor oxygen supply. PEL also usually develops in a hypoxic environment. These observations led me to think that hypoxia might be contributing to the pathogenesis of these tumors. David Davis and Andrea Rinderknight in my group soon showed that, upon exposure to hypoxia, KSHV in PEL cells replicates lytically. Cells exposed to hypoxia produce hypoxia-inducible factors (HIFs), which upregulate specific genes with HIF-responsive elements (HRE) in their promoter regions.

Then Muzammel Haque in my group showed that RTA, the KSHV lytic switch gene, had a potential HRE in its promoter region that is upregulated by hypoxia. Further, HIFs directly upregulate certain KSHV lytic genes, including a cluster spanning open reading frames (ORF) 34–37.

**Virus-Activated Therapy for KSHV-MCD**

Open reading frame (ORF) 36, a lytic gene of KSHV that is upregulated by hypoxia, encodes a phosphotransferase. ORF36 phosphorylates the anti-herpes drug ganciclovir, making it toxic to human cells, according to Jennifer Cannon, Richard Ambinder, and colleagues at Johns Hopkins University and David Davis in my group. Also, the protein encoded by ORF21 of KSHV, another lytic gene, phosphorylates zidovudine as well as ganciclovir, making both of them toxic to cells, according to Jennifer Cannon in Johns Hopkins University, Baltimore, Md., Erik A. Gustafson in Beth Israel Hospital in Boston, Mass. and their collaborators.

Following these observations, our group showed that when PEL cell lines are cultured with gancyclovir or zidovudine and exposed to hypoxic conditions, the toxicity of these drugs increases, killing the cells (Fig. 2). These experiments suggested that KSHV-induced tumors might be treatable by ganciclovir and zidovudine if the lytic KSHV genes could be activated. To test this approach, we turned our attention to KSHV-MCD, a lymphoproliferative disease characterized by severe inflammatory symptoms, including fevers, weight loss, and respiratory distress. In the absence of effective treatment, patients typically die within two years.

A number of the cells in the lymph nodes and spleen of individuals with KSHV-MCD are infected with KSHV and express latency-associated nuclear antigen (LANA), while others also express KSHV-encoded viral interleukin-6 (vIL6), an analog of human IL-6, and still others express other lytic KSHV genes. Other cells express an excess of human cytokines. Cytokines, and especially viral or human IL-6, are believed to play a major role in causing the inflammatory symptoms in patients with KSHV-MCD. Mark Polizotto in my group showed that an excess of either human or viral IL-6 could lead to symptomatic MCD.

We hypothesized that a combination of ganci-
clovir and zidovudine might be effective in treating this condition. To make the regimen easier to administer, we substituted valganciclovir, an orally available pro-drug for ganciclovir. In a small-scale clinical trial spearheaded by Thomas Uldrick of NCI along with Richard A. Little, Mark Polizzotto, Karen Alemen, and Kathleen Wyvill, we treated 14 HIV-infected patients with symptomatic KSHV-MCD with high-dose zidovudine and valganciclovir. Patients were also maintained on their anti-HIV therapy.

Twelve of these 14 patients (86%) had a major clinical response to this therapy, and 7 of the patients (50%) had a major biochemical response. In addition, patients had a decrease in viral and inflammatory parameters, including KSHV viral load, KSHV vIL-6, human IL-6, and human IL-10, and C-reactive protein (Fig. 3). While a number of patients subsequently relapsed, this trial showed that KSHV-MCD could be treated by drugs activated by viral-encoded lytic genes. This small trial was the first time that a tumor or hyperproliferative condition was treated using this approach. Rituximab, a monoclonal antibody to CD20, is also effective against KSHV-MCD, either alone or, as Uldrick and colleagues later found, in combination with the anti-cancer drug liposomal doxorubicin, further broadening the armamentarium against KSHV-MCD.

**KSHV-Associated Inflammatory Cytokine Syndrome**

A few HIV/AIDS patients developed inflammatory symptoms very similar to those with KSHV-MCD but did not appear to have the pathological changes of KSHV-MCD or other known causes. Reviewing our records, Thomas Uldrick, Richard Little, and others in my group identified six such patients with KS or with serological evidence of KSHV infection. These patients had unexplained fevers and other symptoms, including wasting, effusions, and inflammatory markers. In addition, these patients had increased KSHV viral loads, vIL-6, human IL-6, and human IL-10 comparable to patients with KSHV-MCD, but greater than other patients with severe or mild KS. Four of the six patients also had KS. We were not able to find pathologic evidence of KSHV-MCD, however, and none of the patients subsequently developed KSHV-MCD.

We postulated that these patients had a syndrome related to KSHV-MCD, in which their severe inflammatory symptoms were driven by high levels of KSHV replication and associated production of KSHV-encoded and human cytokines. We now refer to this syndrome as KSHV-associated inflammatory cytokine syndrome (KICS), and Mark Polizzotto is spearheading an
effort to study additional patients and evaluate possible therapies.

KSHV-MCD is still considered rare, and we are only now beginning to explore KICS as a separate entity. At first glance, it would seem that these diseases are of relatively minor importance in the scope of the AIDS epidemic. However, it is likely that they are more common than now thought. KSHV-MCD can easily be missed. Also, it is not known how much of the AIDS symptomatology during the early years of the AIDS epidemic might have been due to KSHV-MCD or KICS.

KSHV-Related Diseases in Africa

KSHV-MCD also might be more common in Africa than is recognized. Unlike most herpesviruses, the prevalence of KSHV varies widely among different populations, and generally parallels the incidence of KS. While as many as 40 to 50% of men who have sex with men are infected with KSHV in the US, the prevalence in the general population is much lower, about 5 to 10%. The prevalence is higher elsewhere, particularly in sub-Saharan Africa, where up to 80% of individuals are infected in some regions.

Even before the AIDS epidemic, KS was relatively common in these areas, and in some countries, KS is now the most common tumor in men. Even so, very few cases of KSHV-MCD have been reported in Africa. By contrast, in our NCI clinic, we have seen five cases of KSHV-MCD in African immigrants over the past several years. Patients with KSHV-MCD-like symptoms in Africa rarely undergo appropriate diagnostic evaluations. While KSHV-MCD is usually fatal if untreated, effective treatments are now available, and it will be important to identify and treat infected individuals.

Conclusion

Although antiretroviral therapy has extended the life of HIV-infected individuals and reduced the incidence of some AIDS-defining tumors, these
individuals are aging with somewhat compromised immune systems and are developing other tumors. Many, but not all, of these HIV-associated tumors are caused by oncogenic viruses, which offer potential targets for prevention. Moreover, there are substantial opportunities for research to enable improved care in Africa and other regions where many of these tumors remain undiagnosed and where affordable therapies are often not available.

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