Current Perspectives on the Molecular Pathogenesis of Virus-Induced Cancers in Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

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A distinct group of cancers particularly threaten human immunodeficiency virus (HIV)-infected people. Most HIV/acquired immunodeficiency syndrome (AIDS)-associated cancers have a substantial component of viral etiology. Epstein-Barr virus (EBV), Kaposi’s sarcoma-associated herpesvirus (HHV8), human papillomavirus (HPV), and HIV have been implicated in the etiology of cancers in AIDS. The molecular mechanisms by which HPV, EBV, HHV8, and HIV persist and cause cancer are summarized. The viral etiology of AIDS-associated cancers is important because pharmacologic and immunologic strategies to prevent or attack persistent or latent virus infection and cell growth transformation may be useful in preventing and treating these cancers. Effective immune attack on latent and persistent virus infection will require enhanced cellular immune responses. Such responses may be achievable through active immunization or by in vitro expansion of viral and host specific cytotoxic and helper T lymphocytes. Enhanced knowledge of clinically applied T-cell immunology may also be useful in preventing and treating HIV infection and other opportunistic infections in HIV-infected people. [Monogr Natl Cancer Inst 1998;23:7–14]

Viruses are obligate intracellular parasites that are now recognized to be an etiologic factor in cancers in all human populations [reviewed in (1,2)]. These simple organisms have RNA or DNA genomes and have evolved to propagate themselves in human populations. Their causation of cancer is an unusual outcome of an infection that has gone awry in an individual host. Nevertheless, estimates of the frequency of virus-induced cancer worldwide are in the order of 15%–20% of all cancers. In human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS) (HIV/AIDS), viruses are estimated to cause a significantly higher fraction of cancer. The importance of viruses in HIV/AIDS-associated cancer is due to at least five factors. The first factor is the relatively young age of HIV/AIDS-infected people and the relatively low incidence of non-viral-associated cancers in young people. Second, the acquisition of HIV infection is associated with circumstances that place the individual at increased risk for acquiring other persistent virus infections. Third, some viruses that can cause persistent infections have evolved strategies for evading immune responses, for persisting in a latent state within cells, or for altering cell growth. These strategies can enable virus-infected cells to become malignant. Fourth, HIV infection alters cellular, cytokine, and humoral components of the immune response, decreases antiviral cellular immune responses, increases the burden of persistent virus infections, and thereby predisposes HIV-infected people to develop viral-associated cancers. In being at increased risk for viral-associated cancers because of decreased antiviral immune responses, HIV/AIDS-infected people are similar to other people with decreased cellular immune responses such as organ transplant recipients. Fifth, at the cellular level, HIV infection leads to provirus integration. Integrated proviral DNA can cause disruption or dysregulation of cellular gene expression and thereby initiate or promote oncogenesis. This brief overview will focus on the current status of knowledge of the molecular mechanisms by which viruses persist and effect changes in cell growth that result in cancer in patients with HIV/AIDS. Knowledge of these molecular mechanisms is highly relevant to strategies for the prevention and treatment of HIV/AIDS-associated cancers.

The viruses associated with cancer in HIV/AIDS-infected people are a subset of the viruses associated with cancer in non-HIV-infected people. In non-HIV-infected populations, human papillomaviruses (HPVs) are the initiating etiologic agents in most cervical and anogenital carcinomas, in many laryngeal carcinomas, and in a small fraction of cutaneous carcinomas. Hepatitis B and C viruses (HBV and HCV) are major factors in the etiology of hepatocellular carcinomas (HCC), a prevalent cancer worldwide. Epstein-Barr virus (EBV) is etiologically implicated in nasopharyngeal carcinoma (NPC), a cancer affecting specific populations, in B-cell proliferative disease in immune compromised patients, in lymphomas, in Hodgkin’s disease (HD), and in a small fraction of gastric carcinomas. Human T-cell leukemia virus (HTLV-1) is implicated in an uncommon tumor, adult T-cell leukemia (ATL). Human herpesvirus 8 (HHV8, also known as KSHV) is implicated in Kaposi’s sarcoma (KS), an unusual tumor in most human populations, but more common

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among some Mediterranean and African populations, in multicentric Castleman’s disease, and in body cavity B-cell lymphomas (BCBLs). HPV, HBV, HCV, HHV8, and perhaps, to a small extent, HTLV-1 are more common in people exposed to blood, blood products, or sexually transmitted diseases and are therefore more prevalent in HIV/AIDS, while EBV is highly prevalent in all populations. In HIV/AIDS, HHV8 is associated with KS and BCBL, and EBV is associated with central nervous system and peripheral lymphomas, HD, and leiomyomas, all of which occur with increased frequency. In contrast, despite attributable viral etiology, HCC, cervical cancer, and ATL appear to be no more common than in the general population. HPV infection appears to be intermediate in increased association with cancer in HIV/AIDS in that while cervical and anogenital cancers are not much increased in incidence, HPV-associated premalignant lesions of all types are more common in patients with HIV/AIDS. The failure to observe an increased frequency of cervical and anogenital invasive cancers in AIDS may be due to the previously short life span of HIV-infected people and the usual long interval between HPV infection and cancer. Similarly, the long interval between HBV or HCV infection and HCC or between HTLV-1 and ATL is likely to be a factor in the low incidence of HCC and ATL in HIV/AIDS. However, the low incidence of HCC and ATL in HIV/AIDS may also be due to specific effects of HIV infection. HIV infection depletes CD4+ T lymphocytes, which are critical for enhanced CD8+ T-cell immune responses, and changes in cytokine levels shift the immune responses away from natural killer (NK) and CD8+ lymphocytes and toward B lymphocytes. Since CD8+ lymphocyte-mediated liver injury and regeneration are important in HBV or HCV infection of HCC (3) and interleukin 2 secretion from CD4+ cells is critical for the proliferation of HTLV-1-infected cells (4), HIV infection may actually decrease the frequency of cancer associated with HBV, HCC, or HTLV-1 infection. Because HCC and ATL are not commonly associated with HIV/AIDS, they will not be further discussed in this review. Also, viral-associated benign proliferations that are important in HIV/AIDS, such as Mulluscum Contagiosum (due to the mulluscum viral-associated benign proliferations) and oral hairy leukoplakia (associated with EBV), are only mentioned at this point.

**Human Papillomavirus**

HPV 16, HPV 18, and other high-risk papillomaviruses are strongly associated with cervical, penile, and anal cancers and HPV 5 and HPV 8 have been associated with widespread epidermal, dysplastic lesions, and some carcinomas in HIV-infected and noninfected people (5–9). Important aspects of the molecular mechanisms through which HPVs cause persistent infection and cancer are now partially understood. At the cellular level, HPVs infect basal epithelial cells and persist as an episome in the latently infected cell (Fig. 1). It is uncertain whether any virus-encoded proteins are expressed in infected basal epithelial cells. Basal epithelial cells replicate and some progeny differentiate into nondividing parabasal cells that further differentiate into keratinocytes. Viral replication ensues in infected differentiating keratinocytes. The HPV early promoter governs transcription of the seven HPV early genes (E1–E7). The high-risk HPV E7 proteins bind to the cellular retinoblasota protein and thereby release the cellular transcription factor E2F. E2F is then free to up-regulate the expression of cellular genes that are important for viral and cellular DNA synthesis. High-risk HPV E6 proteins cause the degradation of p53. p53 would otherwise be induced by HPV infection, inhibit cellular cyclin dependent kinases, and cause apoptosis. The HPV E1 and E2 proteins enable viral DNA replication. When the E2 protein accumulates to high levels, the viral early promoter is down-regulated (10) and an as yet unidentified promoter that transcribes messenger RNAs (mRNAs) for the late viral structural proteins is presumed to be turned on.

HPV DNA integration into chromosomal DNA is not part of the normal strategy by which HPV persists in cells. Integration is the first major step away from the events of normal persistent HPV infection and toward cancer. In carcinoma tissue or carcinoma cell lines in culture, HPV DNA is usually integrated with the E2 open-reading frame interrupted so that the E2 protein is not expressed or it is unable to down-modulate the early promoter (10–13). Integrations that release the HPV early promoter from the repressive effects of E2 result in continuous high-level expression of E6 and E7. While normal levels of E6 and E7 expression in an infected, differentiating keratinocyte may enable HPV to synthesize its own DNA, high-level E6 and E7 expression from a high-risk HPV in a cell that is not fully committed to terminal differentiation has immortalizing effects (14). E7 has several functions, including an ability to associate with the part of the retinoblasota protein that would otherwise bind to and inactivate E2F transcription factors (15,16). The freeing of E2F results in activation of the many E2F-responsive cellular genes whose expression enables a resting cell to enter cycle, traverse G1, and enter S phase (17). In contrast to E7 that acts in the nucleus, HPV E6 acts in the cytoplasm where it binds to several cellular proteins. One important interaction is with a ubiquitin ligase (18). The E6-associated ubiquitin ligase catalyzes the ubiquitination of the p53 tumor suppressor gene and its subsequent destruction by cellular proteosomes. By inducing the degradation of p53, E6 prevents p53’s tumor-suppressive effects, including the induced expression of cyclin-dependent kinase (CDK) inhibitors and the induction of apoptosis. High-risk HPVs characteristically have E6 and E7 proteins that are significantly more active in targeting p53 and the retinoblasota protein than E6 and E7 of low-risk HPVs. Although HPV DNA appears to randomly integrate into cellular chromosomes, some integration events may promote tumorigenicity by enhancing transcription of neighboring cellular oncogenes. One example is the integration of HPV DNA near c-myc in a cervical cancer cell line. c-myc is also amplified in some tumors (11).
HPV infection, integration of the HPV genome into cellular DNA, and overexpression of high-risk and transforming E6 and E7 proteins are important steps in a multistep carcinogenic process. In most populations, 10%–30% of adults are infected with high-risk HPVs. HPV integration is unusual and an integration that results in HPV early promoter up-regulation is a key oncogenic event, since each tumor is marked by a single distinctive integration. Progression through low- and high-grade intraepithelial neoplasia to invasive carcinoma appears to usually require several decades. Most infected people never even progress to low-grade intraepithelial neoplasia, and invasive carcinoma is a very infrequent outcome. Many invasive carcinomas have a loss of heterozygosity at 3p, implicating this site and the FHIT gene as being important for progression (19,20). Chromosome 11 has also been implicated through its activity in suppressing HPV gene expression and oncogenicity (5).

One reason that most HPV-infected people never progress to invasive cervical cancer is that HPV infection can be cleared by immune mechanisms. Despite the somewhat inefficient overall immune surveillance in epithelial tissue, humoral and cell-mediated immune responses contain and eventually eliminate most HPV infections. Interferon appears to be an important component of the immune response because of its immune regulatory and antiviral activities. Nevertheless, the frequent persistence of even benign HPVs for months or years and the prolonged persistence of high-risk HPVs for decades in some people are compatible with the notion that HPV-encoded proteins may facilitate immune evasion. However, even high-risk HPVs are usually eventually cleared by the normal immune response. The immune-suppressive effects of HIV infection appear to enable longer term and higher level HPV persistence, placing HIV- and HPV-coinfected people at increased theoretic risk for cervical or anogenital cancer (10). If contemporary antiretroviral therapies have their expected effects in delaying HIV disease progression and in forestalling HIV-associated mortality, HPV infections are likely to have a greater cumulative effect on disease progression and in forestalling HIV-associated mortality, where retroviral therapies have their expected effects in delaying HIV disease progression and in forestalling HIV-associated mortality.

Epstein-Barr Virus

EBV infection [reviewed in (21,22)] is similar to HPV infection in that the viral genes have a direct role in oncogenesis, but it is strikingly different in three respects. First, while the EBV genes involved in oncogenesis perform the same functions in normal latent virus infection, HPV E6 and E7 are important for early stages of viral replication and are not known to be expressed in latently infected cells. Second, high-risk E6 and E7 genes only become oncogenic as a result of integration and overexpression in cells that are not terminally differentiated. In contrast, the EBV genes important for oncogenesis are normally expressed in latent infection. They enable EBV to expand the number of latently infected cells and establish persistence in the normal host. Third, since the EBV genes associated with oncogenesis are part of the normal life cycle of the virus, their ability to cause cell proliferation must be limited so as to not cause the premature demise of the host and the virus. This latter balance is struck by the reliance of the virus on the normal innate and immune response to viral infection. These responses strongly contain virus-infected cell proliferation. Because of its dependence on innate and acquired immunity, EBV infection is rarely oncogenic in people with normal immune function and is a more important cause of cancer in severely immune-compromised people, especially people with advanced HIV infection or AIDS.

EBV infection is usually spread by saliva and virus replicates in the oropharyngeal epithelium. The molecular events of intracellular replication are similar to those of other herpesviruses. In primary infection, EBV spreads from epithelial cells to B lymphocytes. In B lymphocytes, the EBV genome circularizes and expresses two nuclear proteins (EBNA-LP and -2). EBNA-LP and EBNA-2 turn on the expression of some cell genes and up-regulate their own promoter and an upstream promoter, thereby increasing transcription through the EBNA-2 polyadenylation site with downstream transcription of four additional nuclear protein (EBNA-3A, -3B, -3C, and -1) mRNAs. EBNA-LP and EBNA-2 also turn on the transcription of mRNAs encoding two integral membrane proteins (LMP-1 and -2). EBV latent infection-associated gene expression results in rather uniform progression of the infected B lymphocyte from G0 into G1 and S phase, the expression of small RNAs (EBERs) from the EBV genome, and continuous cell proliferation. The EBNA2 and LMPs are not related to genes of other herpesviruses.

In vitro, EBV infection of B lymphocytes results in the expression of the EBNA3B and LMPs and the establishment of long-term lymphoblastoid cell lines (LCLs). Similar EBV-infected LCLs result from the cultivation, in vitro, of peripheral blood B lymphocytes from EBV-seropositive people. Injection of EBV into cottontop tamarins causes an acute fatal polyclonal lymphoproliferative disease (23), similar to that seen in severely immune-compromised humans with primary or even latent EBV infection. In more normal people, cells expressing EBNA and LMPs engender natural killer and EBV-specific, major histocompatibility complex (MHC) class I-restricted, cytotoxic CD8+ T-cell responses (24–26). The EBNA3B, in particular, except for EBNA1, have multiple epitopes that are recognized in the context of common class I determinants. The EBNA3B and LMP1 also induce the expression of adhesion molecules, rendering the cell susceptible to T-cell adherence and cytoidal effects. As a consequence of immune responses by normal people to primary EBV infection, the number of proliferating virus-infected B lymphocytes in the peripheral blood rapidly declines to a level of one infected B lymphocyte in 10−5 or 10−6. However, cytotoxic T lymphocytes specific for epitopes from five of the EBNA3B and the two LMPs persist forever, indicating that cells expressing the EBNA3B and LMPs are at least intermittently present in the normal host.

Some EBV-infected B lymphocytes switch to a latent infection in which only EBNA1 is expressed. These cells are apparently not proliferating and EBNA1 is not usually recognized by immune CD8+ lymphocytes, so that cells expressing only EBNA1 are immunologically indistinguishable from normal B lymphocytes. EBNA1 appears to escape immune surveillance because it has a cis-acting glycine alanine repeat sequence that inhibits its processing through proteasomes (27). The expression of EBNA1 in the absence of other EBNA3B and LMPs was first described by Rickinson et al. (22) in studies of Burkitt’s lymphoma cell lines and tissue and has led to the working hypothesis.
that there may be immunologic selection against EBV gene expression in the later stages of lymphoma evolution.

Long after primary EBV infection, EBV is closely associated with endemic Burkitt’s lymphoma, sporadic Burkitt’s lymphoma, T-cell lymphomas, HD, and anaplastic NPC in normal hosts. In HD and NPC, EBNA-1 and LMPs are expressed in the absence of other EBNAs. LMP-1 appears to be a central effector of altered cell growth in lymphocytes or epithelial cells.

Recombinant EBV-based reverse genetic analyses have shown that EBNA-2, EBNA-LP, EBNA-3A, EBNA-3C, and LMP-1 are critical or essential for virus-mediated B-cell growth transformation (28). EBNA-1 is also presumed to be important, since EBNA-1 binds to a site upstream of the EBNA promoter, thereby enhancing transcription and creating a functional origin for replication of the EBV episome in cellular S phase (29). LMP-2, EBNA-3B, small non-polyadenylated RNAs (EBERs), and a long highly spliced mRNA (BARF0), which are also expressed in latent lymphocyte infection, are unimportant for primary B-lymphocyte growth transformation. Their role in latent EBV infection is uncertain except for LMP-2. LMP-2 has a critical role in rendering the transformed cell not susceptible to activation signals that would otherwise result in the activation of lytic EBV infection. Most of the rest of the viral genome, including EBV-encoded IL10 and Bcl2 analogues that are ordinarily expressed in lytic infection, have also formally been shown to be unimportant in the conversion of resting B lymphocytes into LCLs and subsequent growth into tumors in SCID mice.

Recent biochemical and reverse genetic analyses are compatible with the hypothesis that most of EBV’s effects on cell growth are mediated by virus-encoded proteins that usurp control of the notch and CD40 signaling pathways. Early biochemical work established EBNA-2 as a transactivator of expression of the B-lymphocyte activation marker, CD23, and of LMP-1 transcription. Subsequent recombinant EBV reverse genetic analyses defined three critical components of the EBNA-2 open-reading frame. The first is simply several codons that encode prolines near the amino terminus of EBNA-2. These codons are the core of a dimerization element. The second component is codons 280–337 that mediate interactions with PU.1, a B lymphocyte and macrophage-specific “ets” family transcription factor, and with RBP-Jk, a transcription factor that has been genetically implicated in notch-mediated neural development in Drosophila. EBNA-2 in an EBV-transformed lymphoblast is strongly associated with RBP-Jk and a substantial fraction of the cellular RBP-Jk is associated with EBNA-2 (30). The third essential EBNA-2 component is codons 424–464, which encode an acidic activator. This acidic activator can interact with TFIIIB, TAF40, and two subunits of TFIIH and extensively associates with a novel coactivating protein, p100, and with citric acid lyase, a major acetyl-CoA donor. P100 can interact with two subunits of TFIIIE. P100 also has a domain that is functionally equivalent to the major carboxyl terminal negative regulatory domain of the c-myb protein (31). Recent experiments indicate that EBNA-LP coactivates transcription along with EBNA-2. EBNA-LP is not an independent transactivator, but the localization of the EBNA-2 acidic domain near a promoter enables EBNA-LP to strongly coactivate transcription from that promoter (32).

Surprisingly, EBNA-3A and EBNA-3C (as well as EBNA-3B, which is not critical for growth transformation) also associate with RBP-Jk and regulate expression of specific virus and cell genes with RBP-Jk sites. The central role of RBP-Jk in EBV regulation of viral and cellular gene expression, evidence that notch activation is a cause of T-cell leukemias (33), and evidence that RBP-Jk mediates notch effects, are all compatible with the hypothesis that RBP-Jk may mediate the regulation of transcription of some cellular gene(s) that are important in lymphocyte growth control.

EBV uses the EBNAs and RBP-Jk to precisely regulate transcription of its oncogene, LMP-1 (Fig. 2). LMP-1 expression in Rat1 cells causes loss of contact inhibition, anchorage-independent growth, and tumorigenicity in nude mice. In lymphocytes, LMP-1 enhances bcl-2 expression, activates NF-kB and c-jun, and induces most of the activation and adhesion molecules that are activated by EBV infection or by antigen and T-cell help. T cells express CD40 ligand, and the T-cell CD40 ligand/B cell CD40 receptor complex is a key component of T-cell help.

LMP-1 has six hydrophobic transmembrane domains that enable it to constitutively aggregate in the plasma membrane and are essential for EBV transformation of B cells into LCLs (Fig. 3). The observation that a mutation in the LMP-1 transmembrane domain that results in diffuse expression in the plasma membrane has a nontransforming phenotype indicates that aggregation is linked to transformation, most likely because aggregation enables the cytoplasmic domains of several LMP1 molecules to locally concentrate next to the plasma membrane as though they were the cytoplasmic domains of a growth factor receptor that had encountered ligand. The LMP1 carboxyl terminal cytoplasmic domain is not critical, but the carboxyl terminal cytoplasmic domain has two important components: a proximal component that is sufficient for initial immortalization (transformation effector site 1, TES1) and a distal domain that enables efficient LCL outgrowth (TES2) (28,34,35). TES1 and TES2 mediate 30% and 70% of the NF-kB activation from LMP-1, respectively. Since TES1 only mediates a small component of NF-kB activation, but is both necessary and sufficient for initial lymphocyte immortalization, NF-kB can only be a component of TES1 LMP1-mediated growth transformation. Two proteins that interact with LMP1 were identified in a yeast-two hybrid screen and in a screen for proteins induced by EBV infection. These proteins are highly homologous to proteins identified as mouse tumor necrosis factor receptor II (TNFRII)-associated factors or protein 10.
TRAfs. LMP1 is similar to activated CD40 in its effects on B-lymphocyte growth and CD40 is also a TNFR that associates with TRAFs, implicating TRAFs in signaling from LMP1 (Fig. 3). In EBV-transformed B lymphocytes, LMP1 is constitutively strongly associated with TRAF1 and TRAF3 and is also associated to a lesser extent with TRAF2 (36). Such cells have a high level of TRAF1/2 heterodimers that mediate NF-kB activation from TES1. The pathway appears to be similar to TNFRII, LT-beta receptor, or CD40 ligand-dependent activation of NF-kB through TRAF2. Furthermore, like CD40, LMP1 activates SAPK and c-jun through TRAF2. Thus, the LMP-1 transmembrane domains mediate constitutive aggregation in the plasma membrane, enabling the LMP1 cytoplasmic domain to be associated with TRAFs and mediate NF-kB and c-jun activation, as well as other less well-characterized effects important for cell growth stimulation. Deletion of DNA encoding the TRAF binding site from the LMP1 gene in recombinant EBVs results in a null phenotype for resting B-lymphocyte growth transformation.

Recent experiments (35) indicate that TES2 maps to the last three residues of LMP1. High-level NF-kB activation is also mediated by this site, providing genetic evidence that high-level NF-kB activation is important in LMP1’s effects. The TNF receptor death domain protein (TRADD) uniquely interacts with TES2 and is constitutively associated with LMP1 in EBV-transformed B lymphocytes. These data implicate TRADD in LMP1-mediated growth transformation and NF-kB activation, underscoring the degree to which LMP1 mimics a constitutively activated TNFR. Curiously, LMP1 engagement of TRADD does not appear to effect cell death, whereas bcl-2 and NF-kB activation by LMP1 protect EBV-infected cells from cell death.

Most of the central nervous system lymphomas that occur late in AIDS and some of the peripheral lymphomas are characterized by the expression of the full range of EBNAls and LMPs. The occurrence of EBV-associated lymphomas is consistent with earlier studies, indicating that the number of lymphocytes latently infected with EBV increases during HIV infection. Waning cytotoxic T-cell immunity is likely to be a critical factor in the increased abundance of EBV-infected cells in the peripheral blood and the subsequent emergence of lymphoma. Changes in cytokine levels and the shift in cytokine balance toward B lymphocyte up-regulation may also have a role.

The fact that many of the EBV-associated lymphomas that occur late in AIDS are similar in viral and cellular gene expression to resting B lymphocytes transformed by EBV infection in vitro and to B lymphocytes in the EBV-associated oligoclonal lymphoproliferative disease that can occur with primary EBV infection in organ transplant recipients who are receiving high dose immune-suppressive therapy has two important implications. First, the lymphoma cells may still be critically dependent on EBV gene expression for their proliferation. Second, the ability of cytotoxic T lymphocytes from normal EBV-infected people to kill latently infected cells through EBNA- or LMP-derived epitopes in the context of common class I MHC molecules can be exploited for the prevention or treatment of EBV-induced lymphoproliferative diseases in which these viral proteins are expressed.

Clinical research with human T-cell immunotherapy has already achieved some promising results in post-transplant patients (37,38). For bone marrow transplant recipients, infusion of donor peripheral blood mononuclear cells or of donor T-cell lines derived through exposure of T lymphocytes to autologous EBV-transformed B lymphocytes has resulted in the regression or the prevention of EBV-induced lymphoproliferative disease. In initial studies, donor T cells appear able to survive, home to sites of proliferating EBV-infected cells, effect killing, and proliferate to a limited extent. Similar approaches might be beneficial in patients with AIDS. Since the majority of patients with AIDS escape EBV-associated lymphomas, efforts to increase cytotoxic T-cell responses by active immunization or by expansion of EBV-reactive autologous T lymphocytes, in vitro, and reinfusion might confer protection against EBV-associated lymphoproliferations.

Many EBV-associated cancers in patients with HIV/AIDS are likely to be EBV-initiated and to have subsequent chromosomal changes that advance the malignant phenotype. Subsequent chromosomal changes are well described in Burkitt’s lymphomas and also characterize much of the spectrum of lymphoproliferative disease that occurs in post-transplant lymphomas and HIV/AIDS (39). The most frequent change is a c-myc translocation leading to dysregulated c-myc expression. Activation of c-myc does not necessarily indicate independence of EBV gene expression for continued cell growth, and EBV gene expression...
in malignant cells provides a basis for immune attack on the tumor cell.

Other EBV-associated cancers that occur with a higher frequency in HIV/AIDS include HD and leiomyosarcomas. EBV-associated HD is characterized by EBNA-1, LMP2, and high-level LMP1 expression and by the absence of expression of other EBNA. With regard to HD, the similarities of LMP1’s biochemical effects to those of activated CD30, the high level of CD30 expression on EBV-associated as well as EBV non-associated HD tumor cells, and the presence of T cells that are likely to express CD30 ligand in HD tissues are compatible with the hypothesis that CD30 and LMP1 are parallel signaling pathways important in HD tumor cell growth. EBV-infected HD cells may also be subject to immune attack (40). While lymphomas and leiomyomas are substantially increased in patients with HIV/AIDS and HD is marginally increased, NPC, the most common EBV-associated cancer worldwide, is uncommon in patients with AIDS. As with cervical cancer and HCC, the failure to observe an increased incidence of NPC in HIV/AIDS may be related to the long interval between EBV infection and NPC.

**HHV8 (KSHV)**

The discovery of HHV8 (or KSHV), a second human gamma herpesvirus, in human KS tissue by Chang et al. (41) is a breakthrough in understanding the etiology of KS. Previously, cytokines and HIV TAT had been implicated in the genesis of KS (42). However, TAT levels have been difficult to measure in tissues and the epidemiology of KS in HIV-infected people is most compatible with a second sexually transmitted agent (43). Importantly, KS tumors from patients with HIV/AIDS or even from patients with familial KS almost always contain HHV8 DNA (44). Furthermore, KS spindle cells that express CD54, an endothelial cell marker, also have RNA from a restricted portion of the HHV8 genome, indicating that these cells are latently infected with HHV8 (45). A few spindle cells also contain RNA encoding a late viral structural protein, which is compatible with similar findings in EBV-associated lymphoproliferative lesions. Moreover, in patients with HIV/AIDS, antibody to KSHV is a predictor of subsequent KS. Although there has been considerable controversy about the frequency of HHV8 DNA in semen and peripheral blood and about the frequency of HHV8 antibody in various populations, more recent data are consistent with the notion that HHV8 is a sexually transmitted virus that may infect 10% of the general population and a higher fraction of the HIV/AIDS population (46,47). Thus, there is now considerable evidence implicating HHV8 in KS. However, relatively little is known about the mechanisms by which HHV8 might effect cell growth. Additional evidence that would strengthen the case for HHV8 in the etiology of KS and BCBLs (48) could come from studies demonstrating that HHV8 can transform cells in culture or induce tumors in heterologous species.

The etiology of BCBL may be complex. An HHV8 ORF73-encoded viral nuclear protein is expressed in BCBL cells (49). The ORF73 latency-associated HHV8 protein has been termed LANA for latency-associated nuclear antigen. However, EBV is also usually present in BCBLs and may have a role in oncogenicity.

BCBL cells can be grown in culture and EBV-free BCBLs are at the present time the best source of HHV8 DNA or proteins. HHV8 replication can be induced in BCBL cells and large quantities of HHV8 are produced from some cell lines (50). So far, only limited replication has been described in primary B lymphocytes and only abortive infection has been described in 293 cells.

Sequence analysis of the HHV8 genome has revealed homology to other herpesviruses, particularly to the rhadinovirus subgroup of the gamma herpesviruses. While EBV has been the most intensely studied gamma herpesvirus, much is also known about the somewhat more closely related rhadinovirus, herpesvirus saimiri (HVS). HVS is a T-lymphotropic gamma herpesvirus that can induce T-cell lymphomas in some heterologous species, but has no such effect on its natural host (51,52). HVS has been investigated using biochemical and molecular genetic approaches and may offer some instructive precedent for how HHV8 may cause persistent infection and effect cell growth (Fig. 4). HVS has two putative transforming genes near the left end of its genome. The H. saimiri transforming protein and tyrosine kinase interacting protein encoding genes, STP and TIP, vary among HVS strains. Recombinant HVS reverse genetic analyses indicate that STP and TIP are essential for HVS lymphomagenesis. STP up-regulates the Ras pathway (53), whereas TIP down-regulates Lck and activates NF-kB (54). TIP down-regulation of Lck appears to be unimportant for transformation, while TIP-mediated NF-kB activation is likely to be important in lymphomagenesis. Whether the HVS-encoded cyclin homolog, a superantigen encoding open-reading frame, a bcl-2 homolog, and an IL-17 homolog have any role in HVS-induced lymphomagenesis is uncertain. HVS also has several open-reading frames that are likely to be important in escape from immune surveillance and similar open-reading frames are present in HHV8.

HHV8 has 75 open-reading frames that are homologous to HVS, which have been designated ORF1-ORF75, and at least 15 novel open-reading frames that have been designated K1–15 (51). K1 is positioned at the site of HVS STP and TIP and is also variable in sequence among HHV8 strains. Two other novel HHV8 genes could also be important in transformation. HHV8 K2 encodes a functional IL-6 homolog that could be important in endothelial cell growth and HHV8 K13 encodes an inhibitor of FADD-activated apoptosis (52,55–58). Other HHV8 open-reading frames that have homologs in HVS of unproven significance for HVS-induced T-cell transformation are HHV8 ORF72, the cyclin homolog, and ORF16, a bcl-2 homolog. However, the only HHV8 protein known to be expressed in latently infected cells is the ORF73-encoded protein, LANA. A
lytic infection-associated protein such as the HHV8 IL-6 homolog could be important for KS endothelial cell growth if it is expressed in large amounts from lytically infected cells and such cells are sufficiently prevalent to sustain high cytokine levels. Persistent lytic poxvirus infections, for example, cause hypertrrophic cutaneous lesions through sequestration of epidermal cell growth factor-related and immune modulating factors. Poxviruses that cause hypertrrophic lesions appear to be unique in that infection is highly localized, all infected cells are persistently lytically infected, and there is almost a complete blockade of an effective immune response.

Human Immunodeficiency Virus

The frequency with which HIV itself causes cancer is uncertain and only limited data are available. The positive data are from analyses of non-B-cell lymphomas of all types that were HIV p24 positive (59). Of 22 specimens analyzed in two studies, 18 were positive for HIV proviral DNA by inverse polymerase chain reaction. Of the 18 cases in which proviral DNA was detected, 10 were integrations of HIV upstream of the c-fes gene. Proviral integration upstream of c-fes was found in one T-cell lymphoma. However, almost all of the other integrations were in macrophage-like cells and not in the tumor cells. These findings have led to the hypothesis that HIV integration near c-fes might result in up-regulation of a cytokine that might contribute to lymphomagenesis. Given the frequent finding of defective proviral integrations in HIV infection overall and the substantial activity of the HIV promoter, particularly in cells with high basal NF-kB activation, HIV may be a more significant factor in HIV/AIDS-associated cancer than is currently appreciated.

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