SPECIAL ARTICLE

Human Immunodeficiency Virus and Malignancy

Thoughts on Viral Oncogenesis

William P. Schecter, MD

In the spring of 1981, as a newly minted assistant professor of surgery at the University of California–San Francisco, I was called to see a 33-year-old man with bilateral interstitial pulmonary infiltrates who was dying of acute respiratory failure at San Francisco General Hospital. My medical colleagues requested an open-lung biopsy. To our mutual amazement, this previously healthy gay man had *Pneumocystis carinii* pneumonia, despite the fact that there was no history of lymphoma or organ transplantation. The first cluster of cases of this pneumonia was reported the same year.[1]

At approximately the same time, a previously rare tumor, Kaposi sarcoma (KS), began to appear in epidemic numbers, first reported in Los Angeles, Calif, and New York, NY.[2] By 1983, Luc Montagnier had discovered an RNA retrovirus associated with the disease complex occurring primarily in gay men, which had been termed the *acquired immune deficiency syndrome* (AIDS).[3] The viral etiology of AIDS was not generally accepted in the United States until 1984, when Gallo et al[4] confirmed Montagnier's work with specimens obtained from Montagnier. In 1986, the virus was named the *human immunodeficiency virus* (HIV). Later that year, the original virus was named HIV-1 after Montagnier's group discovered a similar virus (HIV-2) in West Africa.[5]

The severity of the epidemic was initially underestimated. By the end of 1989, 100 000 cases had been reported in the United States.[6] From the beginning of the epidemic to the end of calendar year 1999, 16.7 million people worldwide had died, with 5.6 million new cases of HIV infection occurring during 1999. A total of 33.6 million people were living with AIDS, including 23.3 million in sub-Saharan Africa, where the disease is decimating an entire generation. Almost 1 million people are living with AIDS in the United States.[7]

In the early years of the epidemic, before we became familiar with the disease, surgeons in San Francisco and elsewhere spent a lot of time performing biopsies on HIV-infected gay men with generalized lymphadenopathy. It was not long before B-cell lymphomas, located in the gut, brain, liver, and other relatively unusual sites, became common devastating problems.[8]

One of my early patients was a previously healthy 29-year-old gay man with no known history of HIV infection who presented with a small-bowel obstruction and computed tomographic evidence of a large soft tissue mass involving the proximal jejunum (**Figure 1** A). An en bloc resection of the ascending duodenum, jejunum, splenic flexure, and omentum was required to remove the mass, which proved to be a B-cell lymphoma (Figure 1B-C). Although the patient left the hospital 7 days after surgery eating and smiling, within 4 months he died of recurrent lymphoma that was unresponsive to chemotherapy.

Kaposi sarcoma and B-cell lymphoma are the 2 malignancies that helped define AIDS. With the spread of HIV infection via heterosexual transmission, particularly in Africa and Asia, squamous cell carcinoma of the cervix (SCCC) was added to the list of AIDS-defining malignancies in 1993.[9] This decision is controversial, since there is no evidence that the risk for cervical cancer is greater for HIV-infected women.[10,11]

We experienced a dramatic rise in the number of cases of anal condylomata and

From the Department of Surgery, University of California–San Francisco.

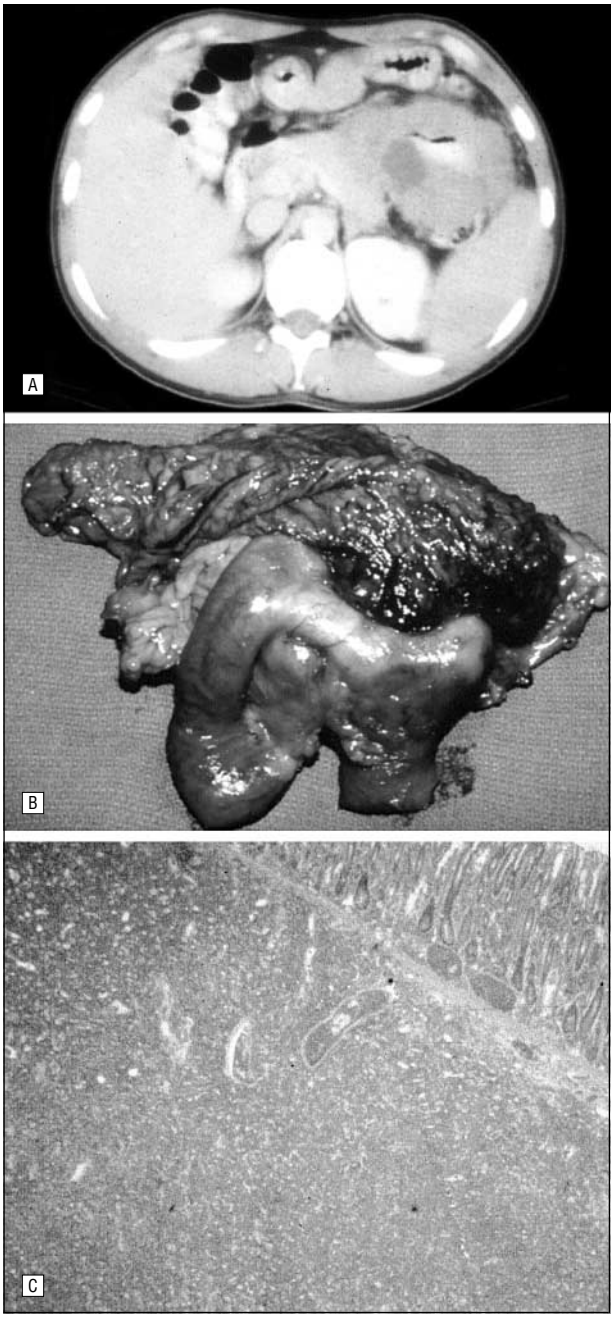

Figure 1. Proximal small-bowel obstruction caused by B-cell lymphoma. A, Abdominal computed tomographic scan showing mass surrounding jejunum. B, En bloc resection of the ascending duodenum, proximal jejunum, omentum, and splenic flexure of the colon. C, Histological section showing lymphoma of the jejunum (original magnification ×10).

squamous cell carcinoma of the anus (SCCA) in gay men in San Francisco in the 1980s paralleling the rise in cases of AIDS. The prevalence of SCCA in HIV-infected patients raises the questions of a possible connection between HIV and SCCC/SCCA.[12]

All of the tumors mentioned so far are associated with other viruses besides HIV, ie, KS with human herpesvirus 8 (HHV-8),[13] B-cell lymphoma with Epstein-Barr virus (EBV),[14] and SCCC/SCCA with human papillomaviruses 16 (HPV-16) and 18 (HPV-18).[15,16] In addition, other tumors not associated with HIV have close associations with viral infection, ie, hepatocellular carcinoma with hepatitis B and C viruses,[17,18] nasopharyngeal carcinoma with EBV,[19] and T-cell leukemia with human T-cell leukemia virus 1.[20]

Developments in Molecular Virology and Oncology*

Year	Investigator	Contribution
1898	Beijerinck[32]	Coined the term *virus*; discovered TMV
1911	Rous[21]	Identified Rous sarcoma virus
1933	Shope[22]	Malignant tumors in rabbits caused by a papillomavirus
1935	Stanley[23]	TMV composed almost entirely of protein
1936	Bawden et al[24]	A small component of TMV is nucleic acid
1944	Avery et al[25]	Genetic traits transmitted by nucleic acids
1950	Chargaff[26]	Identified the 4 base pairs of DNA and described the rules of base pairing (adenine with thymine, guanine with cytosine)
1953	Watson and Crick[27]	Double helix structure of DNA
1961	Jacob and Monod[28]	DNA regulation of protein synthesis
1970	Temin and Mizutani[29]; Baltimore[30]	Discovered the viral enzyme reverse transcriptase allowing conversion of viral RNA to DNA within the host cell
1976	Bishop and Varmus†	Discovered oncogenes

*TMV indicates tobacco mosaic virus.
†Reported in Stehelin et al.[31]

The close association of certain viruses with specific tumors raises the following questions: What is the relationship between viral infection and oncogenesis in general and between HIV and malignancy in particular? How do specific viral infections, including HIV, affect control of cell cycle progression and cell death? Can we prove that HIV, HHV-8, EBV, and HPV-16 and -18 cause cancer? To answer these questions, I offer a historical review of developments in molecular virology and oncology before addressing the specifics of AIDS-associated malignancies (**Table**).

HISTORY

In 1898, the Dutch botanist Martinus Wilhelm Beijerinck[32] studied tobacco plants with tobacco mosaic disease. He rubbed healthy plants with the juice of ground-up diseased tobacco leaves that had been passed through an unglazed porcelain filter that was fine enough to extract the smallest known microorganisms. These healthy plants quickly acquired the disease. Beijerinck coined the term *virus* (Latin for *poison*) to describe the submicroscopic cause of the disease (termed *tobacco mosaic virus*).

Working at the Rockefeller Institute in New York in 1911, Rous[21] discovered that he could induce cancer in chickens by injecting sarcoma tumor cells from a diseased to a healthy chicken. In a manner similar to Beijerinck's experiments, he then made an ultrafiltrate of an extract of the tumor cells, injected it into healthy chickens, and so induced sarcomas in the healthy chickens.[21] This infectious agent came to be called the *Rous sarcoma virus*.

In 1933, Shope,[22] again at the Rockefeller Institute, demonstrated that malignant tumors in rabbits were caused by a papillomavirus. In 1935, working with to-

bacco mosaic virus, Stanley[23] showed that the virus was composed almost entirely of protein. One year later, Bawden et al[24] made a fateful discovery; a very small component of the tobacco mosaic virus was composed of nucleic acid.

At this time, almost all scientists thought that genetic traits were transmitted by proteins. However, in 1944, in a series of elegant experiments with bacteriophages, Avery et al[25] proved that genetic information is transmitted by DNA. This discovery electrified the scientific world still in the grip of World War II. By 1950, Chargaff[26] had described the 4 bases of DNA and the rules of base pairing (adenine with thymine; guanine with cytosine).

After viewing Rosalind Franklin's x-ray diffraction pictures of DNA, Watson and Crick[27] postulated the double-helix structure of DNA in 1953. A decade later, Jacob and Monod[28] linked DNA to protein synthesis via messenger RNA (mRNA), using the triplet base pair code in the mRNA specific for each amino acid.

ANATOMY OF HIV

The outer shell of HIV is known as the viral envelope. Embedded in the envelope is a complex protein consisting of an outer protruding cap–glycoprotein (gp120) and an inner stem (gp41). Within the viral envelope is an HIV protein called p17, the matrix protein, and within the matrix is the viral core or capsid composed of another viral protein p24, the core antigen. The components of the viral core are 2 stable strands of HIV RNA, and the following 3 critical enzyme proteins: p51, also known as reverse transcriptase, p11, also known as protease, and p32, or integrase (**Figure 2**).

The gp120 envelope protein of HIV binds to the CD4 cell membrane protein of the helper T lymphocytes, inducing a series of protein interactions that allow fusion of the viral envelope and cell membrane. The capsid is then injected, as it were, into the cytoplasm of the lymphocyte.[33]

Following entry of the viral core into the cytoplasm, p51 (discovered by Temin and Mizutani[29] and Baltimore[30] in 1970) converts the viral RNA to DNA, which then travels to the nucleus, where it integrates into the host chromosome with the aid of viral p32. There, the integrated viral DNA transcribes mRNA via the host-cell RNA polymerase, leading to the translation of viral proteins and ultimately to the budding of new HIV progeny. Ten new virions are created by each infected cell. The process of HIV budding results in destruction of the host cell, leading to immune deficiency by loss of CD4 helper T lymphocytes. Immunodeficiency, then, is a potential mechanism of oncogenesis in HIV infection, due to the failure of CD4–helper T cells to recognize clones of abnormal proliferating cells. However, what causes the abnormal proliferation of cells in the first place, and what is the connection with viral infection?

CELL-CYCLE PROGRESSION

The cell cycle is composed of 4 segments. In the G_1 phase, the cells commit to division, usually after stimulation by

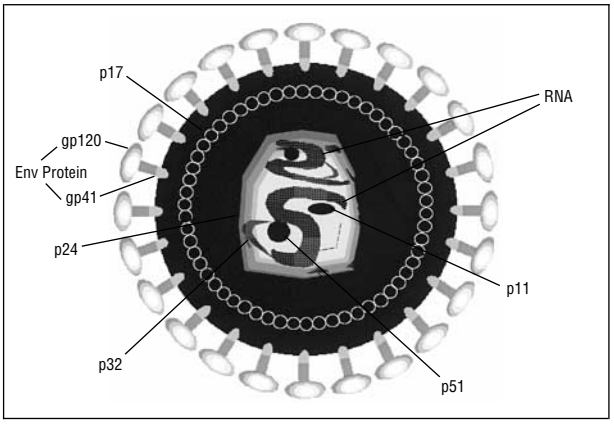

Figure 2. Anatomy of the human immunodeficiency virus. Env indicates the viral envelope; p17, matrix protein (an HIV protein); gp120, protruding cap–glycoprotein; gp41, inner stem; p24, core antigen (a viral protein); p32, integrase; p51, reverse transcriptase; and p11, protease.

a specific growth factor. The growth factor may be a protein expressed as a product of the cell's own genome, such as the oncogene first described by Bishop and Varmus in 1976,[31] or may be one of a multitude of protein signals expressed by other cells (eg, platelet-derived growth factor).

In the S phase, a precise doubling of the amount of DNA in the cell occurs so that chromosomes are replicated. This process requires an accurate copy of the approximately 3 billion base pairs in the human genome. The process of DNA replication is extremely accurate but not perfect. Errors in nucleotide sequencing occur only once in every 1 billion to 10 billion nucleotide sequences encoded. Still, errors in copying the DNA template catalyzed by the enzyme DNA polymerase occur as often as once in every 10 000 nucleotide sequences. What accounts for the difference in the relatively frequent number of copy errors in the DNA template and the low overall incidence of copy errors in new cells?

The answer is molecular quality control mechanisms built into DNA polymerase, and other enzymes, which examine the DNA copy and enzymatically excise and repair nucleotide sequence errors.[34] If the DNA cannot be repaired, there are tumor suppressor genes, chief among them *TP53*, that can recognize the abnormal DNA, halt the cell cycle progression, and induce apoptosis or programmed cell death.[35]

In the G_2 phase, the DNA has been replicated, and additional protein synthesis occurs in preparation for mitosis. The trigger for passing from one state of the cell cycle to the next is the cyclin-dependent kinases (CDK), which are composed of 2 proteins, a cyclin or structural protein and a kinase or enzyme. A cyclin joins with a kinase to form a CDK complex.[36]

If a problem such as defective DNA is identified, *TP53* is activated, up-regulating another protein (p21) that eventually blocks the formation of the CDK complex. Cell-cycle progression is thereby halted. If no defect is identified, the CDK complex is activated by phosphorylation, allowing the activated CDK to block inhibition of a transcription factor for the next step in the cell cycle, including transcription of the next cyclin and

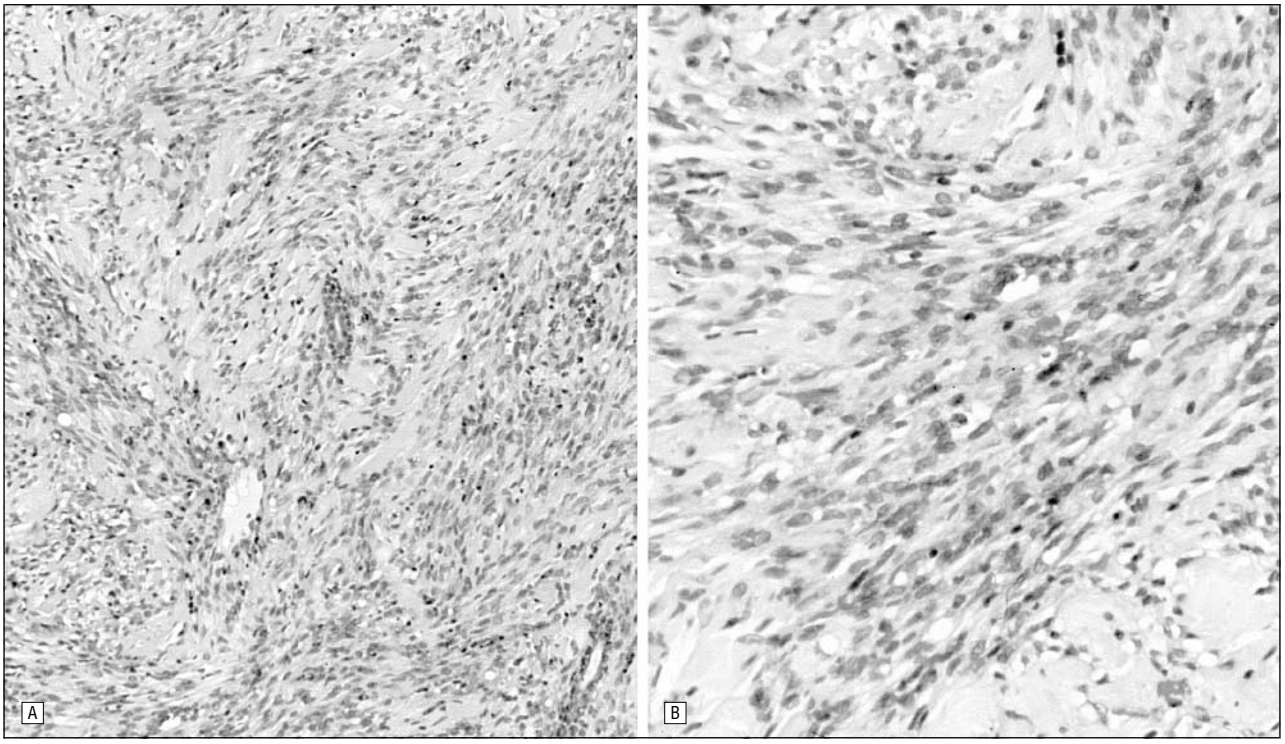

Figure 3. Kaposi sarcoma. A, Low-power view of proliferation of spindle cells arising from vascular or lymphatic endothelium (original magnification ×10). B, High-power view of spindle cell proliferation with infiltration of plasma cells and extravasation of red blood cells (original magnification ×20).

kinase genes for new CDK synthesis. In other words, a cascade of CDKs is necessary to complete the cell cycle.

Inhibition of phosphorylation of the cyclin-CDK complex by tumor suppressor genes at the checkpoints between the stages of the cell cycle is one of the main mechanisms of control of cell proliferation. The other is the induction of apoptosis or programmed cell death.

Apoptosis is a process induced by a proapoptotic signal (eg, *TP53*) that activates a cascade of proteolytic enzymes called caspases, resulting in the dismantling of the cell architecture.[37]

Uncontrolled cell growth occurs under the following circumstances:

1. Less than the usual inhibition of the cell cycle progression by the tumor suppressor genes;
2. Increased stimuli to cell division by oncogenes and other growth factors;
3. Increased antiapoptosis signals; and
4. Decreased proapoptosis signals.

The major proteins regulating the checkpoints of cell-cycle progression are the retinoblastoma protein (pRb), which inhibits progression from the G_1 to the S phase of the cell-cycle; the p53 protein, which inhibits progression from the G_1 to to the S phase and induces apoptosis; bcl-2, which prevents apoptosis; and c-*myc*, which induces cell proliferation.[38]

EFFECT OF VIRAL INFECTION ON CELL-CYCLE CONTROL

As stated above, 3 tumors are associated with HIV infections—KS, squamous cell carcinoma of the anogenital region, and B-cell lymphoma. Kaposi sarcoma, first described by Moritz Kaposi in 1872,[39] is a proliferative disorder of spindle cells arising from lymphatic or vascular endothelium with infiltration of plasma cells and extravasation of red blood cells (**Figure 3**). Four clinical presentations have been described.[40] The classic type occurs in older men of Mediterranean origin, ie, Jews, Italians, and North Africans. The lesions usually occur on the lower extremities and follow an indolent course. The endemic type occurs in sub-Saharan Africans and is associated with a broad range of presentations and severity. I can only speculate whether the African endemic KS is related to immunodeficiency due to HIV or to some other chronic infection. The third type is associated with immunosuppression for organ transplantation, which began to appear in the 1970s.[41] Finally, the fourth type is AIDS-associated KS. When I left San Francisco in the summer of 1981 to spend 3 years overseas, I had never seen a case of KS. I saw 1 case, a single lesion on the sole of the foot of a Bantu man, during a year's work in South Africa. When I returned to San Francisco in 1984, a second-year medical student could diagnose KS from across the room. The rapid rise in the number of KS cases was dramatic and frightening.

Strong epidemiological evidence suggests that KS is a disease transmitted by anoreceptive intercourse in gay men.[42] There is also very strong virological evidence that KS is caused by a virus, the KS-associated virus discovered by Chang et al[13] in 1994 and now called HHV-8. To date, more than 95% of KS tumors studied have been infected with HHV-8.

Strong molecular evidence also suggests that HHV-8 causes cancer. Like many small DNA viruses, HHV-8 in-

hibits both the retinoblastoma protein and the p53 proteins. In addition, HHV-8 DNA transcribes a protein called interferon-regulating factor, which prevents interferon from suppressing the c-*myc* oncogene.[40] Kaposi sarcoma cells are known to express and respond to cytokines.[43]

Another piece of epidemiological evidence is critical to our understanding of the pathogenesis of KS. Since the introduction of highly active antiretroviral therapy in 1996, the number of new cases at San Francisco General Hospital has fallen dramatically.[44] Kaposi sarcoma has again become unusual. Clearly, HHV-8 and immunosuppression from HIV appear to be important for KS oncogenesis.

Squamous cell carcinoma of the anus is a malignancy arising in the epithelium of the anal canal. The anus and the cervix arise from the transitional epithelium of the developing fetus. As with SCCC, extensive epidemiological evidence suggests that SCCA is a sexually transmitted disease.[45-47]

Strong evidence suggests an association of SCCA with HPV infection (anogenital warts), a history of anoreceptive intercourse, a history of sexually transmitted diseases, and/or more than 10 sexual partners. In addition, a history of cervical, vulvar, or vaginal cancer and a history of immunosuppression after solid organ transplantation are strong risk factors. Moderately strong epidemiological evidence suggests an association between SCCA and HIV infection, chronic use of corticosteroids, and cigarette smoking.[48]

Epidemiological and molecular virological evidence support the hypothesis that the HPV-16 and -18 cause SCCA. Human papillomavirus is a virus similar to the papillomavirus proven to cause skin tumors in cottontail rabbits in 1932. Human papillomavirus is a small DNA virus with a closed circular double-stranded genome. More than 70 different types have been described, but only types 16 and 18 have a strong association with SCCA and SCCC.[49]

The HPV genome contains 3 known regions. The early region transcribes proteins that govern viral DNA replication, the process of transcription of the viral DNA code to mRNA and cellular transformation. The late region transcribes 2 viral capsid proteins. The long control region governs viral DNA replication. The early region of the HPV genome transcribes 2 proteins, called E-6 and E-7, which transform and immortalize cultured genital keratinocytes.[50] In other words, these cells reproduce indefinitely.

To understand the molecular mechanism of HPV E-6 and E-7 protein oncogenesis, let us return briefly to the 2 tumor suppressor genes, *RB* and *TP53*, discussed earlier. Through transcriptional factor p21, *TP53* blocks the cyclin-CDK system, thereby preventing phosphorylation of the pRb protein that in its unphosphorylated form is bound to the transcribing protein E2F. E2F is an essential transcription factor allowing progression of the CDK cascade, ultimately resulting in cell division. Without the removal of the pRb protein from E2F, the cell cycle cannot progress.

The HPV oncoproteins E-6 and E-7 have direct effects on *TP53* and *RB*. Oncoprotein E-6 binds to E-6–associated protein, which unfolds and degrades the p53 protein, thereby removing the inhibition of cyclin and CDK. Oncoprotein E-7 binds and inhibits p21, the p53-associated protein, thereby further blocking inhibition of cyclin and CDK. It also binds and degrades pRb, thereby removing inhibition of the transcribing protein E2F and allowing cell cycle progression to mitosis. The end result is loss of inhibition of cell-cycle progression.[50] The histological changes in the anal epithelium due to HPV infection range from anal condylomata to dysplasia, in situ SCCA, and invasive SCCA (**Figure 4**). A continuum of histological change is caused by HPV, but only HPV-16 and -18 are associated with the development of malignancy.

To prove that an infectious organism or agent causes a particular disease, we have to satisfy the following 3 postulates of Koch[51]:

1. Isolate the infectious agent from the body;
2. Grow it in pure culture; and
3. By administering the isolated infectious agent to animals, reproduce the same morbid condition.

Have we definitely proven that HHV-8 causes KS, that HPV-16 and -18 cause SCCA, or that HIV infection is critical to this process? The answer to all 3 questions is no. Satisfying Koch's postulates when studying viral disease is extremely difficult, because it is so difficult to grow these viruses in the laboratory.

In 1965, Hill[52] outlined the following specific criteria for epidemiological assessment of causality:

1. Temporality
2. Strength of statistical association
3. Consistency
4. Plausibility
5. Dose-response relationship
6. Coherence
7. Analogy
8. Specificity
9. Experimental evidence

Very strong epidemiological evidence based on the strength of statistical association, consistency, plausibility, and experimental evidence suggests that HHV-8 causes KS. However, this hypothesis is not definitely proven. The epidemiological evidence that HPV-16 and -18 cause SCCC/SCCA is less strong because squamous cell malignancies do not develop in many infected patients.

I would now like to make a few comments about B-cell lymphoma, a disease that remains poorly understood despite advances in the fields of virology, molecular oncology, and chemotherapy. Burkitt lymphoma and B-cell lymphoproliferative disorders occur in 3% to 4% of HIV-infected patients.[44] However, the incidence of B-cell lymphoma is 60 times greater in HIV-infected patients than in the general population. Almost all HIV-associated lymphomas are B-cell lymphomas, and almost all are due to polyclonal rather than monoclonal proliferation, suggesting a major defect in the recognition and destruction of cells with abnormal protein receptors on the cell membrane.[53] Only 40% of cases of systemic AIDS lymphoma are associated with EBV infection, as op-

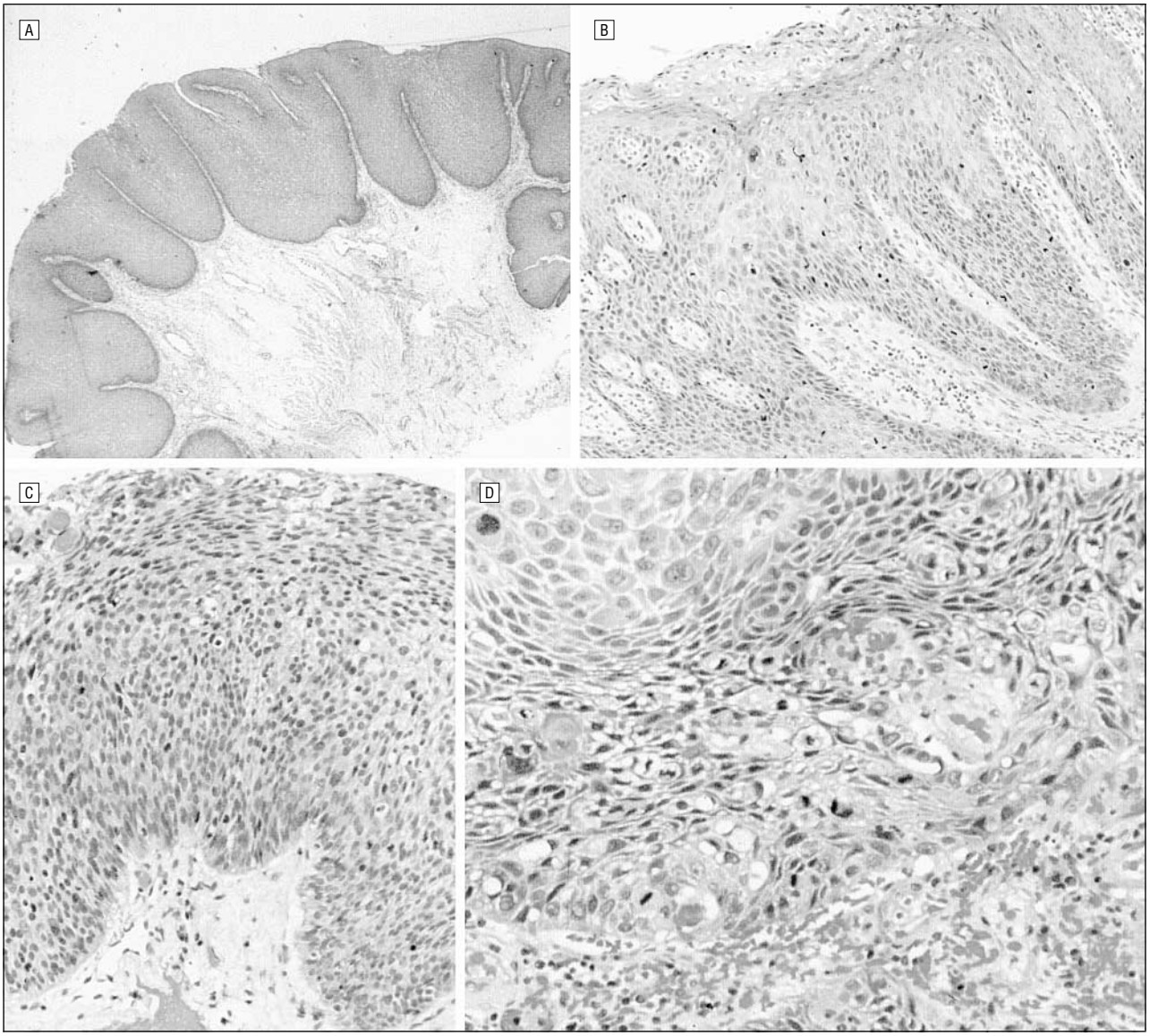

Figure 4. Human papillomavirus infection of anal epithelium. A, Anal condyloma (original magnification ×20). B, Anal condyloma with severe dysplasia (original magnification ×20). C, Squamous cell carcinoma of the anus in situ (original magnification ×20). D, Invasive squamous cell carcinoma of the anus (original magnification ×40).

posed to 100% of central nervous system lymphomas. The reason for the discrepancy is unknown.[54,55] A variety of genetic molecular lesions have been observed in HIV-associated B-cell lymphoma. However, the importance and mechanism of oncogenesis in each case is poorly understood at this time.

Cytokines seem to play an important part in the genesis of B-cell lymphoma. It is well established that the level of circulating cytokines is elevated in patients with HIV infection. Interleukin 6 and its receptor may be pivotal in the genesis of B-cell lymphoma.[56] Although the mechanism of oncogenesis in lymphoma remains unclear, one can speculate that the increased levels of circulating cytokines seen in HIV infection lead to massive proliferation of B cells with a variety of copy errors, tumor suppressor gene deletions, etc. In the presence of profound immunosupression due to CD4–helper T-cell destruction, these abnormal cells are not recognized and destroyed but go on to proliferate and form multiple clones of malignant B lymphocytes.

CONCLUSIONS

None of the viruses discussed herein—HIV, HHV-8, HPV, and EBV—satisfy Koch's postulates proving a viral cause of malignancy. Weak epidemiological evidence links EBV and HIV-associated B-cell lymphoma. The molecular mechanisms are poorly understood. Strong epidemiological and molecular evidence suggests that SCCC and SCCA are sexually transmitted diseases caused by infection with HPV-16 and -18. Very strong epidemiological evidence links HHV-8 and KS. The DNA of HHV-8 contains multiple eukaryotic genes that enhance cell proliferation and inhibit apoptosis.

However, EBV, HHV-8, and HPV all appear to induce oncogenesis by altering cell-cycle control, either by

deactivating the tumor suppressor gene *TP53*, the tumor suppressor gene *RB*, or the cyclin inhibitor p21 or by up-regulating oncogenes such as c-*myc* and *bcl-2*. The overall result is down-regulation of apoptosis and up-regulation of growth factors. Immunosuppression due to CD4 helper T-cell depletion leading to decreased ability to identify and kill abnormal cells appears to be an important factor.

Presented at the Eighth Annual Charles Eckert Lecture in Surgery, the Albany Medical College, Albany, NY, September 7, 2000.

I thank Edward Howes, MD, for the photomicrographs, Jan Horn, MD, for the computer-generated cartoon of HIV anatomy, Chen Lee, MD, for help preparing the figures, and Sonia Silva for help preparing the manuscript.

Corresponding author and reprints: William P. Schecter, MD, UCSF Department of Surgery, San Francisco General Hospital, 1001 Potrero Ave, Ward 3A, San Francisco, CA 94110 (e-mail: bschect@sfghsurg.ucsf.edu).

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