How DNA Viruses May Cause Cancer

DNA tumor viruses contribute to the development of 20% of world cancers. Researchers are beginning to understand how

"TEN YEARS AGO we couldn't have said that viruses cause cancer. Now we know that 20% of world cancers are virus-induced," says Palmer Beasley of the University of Texas Health Science Center in Houston. Three viruses in particular, all of which have DNA as their genetic material, have been linked to common cancers: hepatitis B virus to liver cancer, Epstein-Barr virus to lymphomas and nasopharyngeal cancer, and certain strains of human papilloma virus to cervical and other genital cancers.

Last month, many of the principal researchers who have been investigating the links between the DNA viruses and cancer gathered in San Diego to describe their recent findings at a conference on "The Role of DNA Viruses in Human Tumors."* Their presentations showed that they are beginning to learn how these viruses help make cells malignant, although in no case is the viral action completely understood. What was clear, however, is that efforts to use vaccination to prevent the viral infections—and presumably the cancers with which they are linked—can proceed in the absence of a full understanding of the viral mechanisms of action.

The hepatitis B virus provides a case in point. As many as 300 million people, most of them concentrated in Southeast Asia and especially in southern China, have become long-term carriers of this virus. A 10-year epidemiological study conducted in Taiwan by Beasley and his colleagues has shown that the carriers' risk of getting liver cancer is at least 100 times greater than that of noncarriers. For comparison, Beasley notes that cigarette smoking increases the risk of getting lung cancer about 20-fold. "It's absolutely clear that hepatitis B virus is the major cause of hepatocarcinoma," he concludes.

Effective vaccines to protect against hepatitis B virus infections have been available since the early 1980s. The major route of the virus transmission is from a carrier mother to her infant children at birth. "The vaccine will interrupt infection from the mother to the infant in the vast majority [95%] of the cases," Beasley says.

Several Asian countries, including Taiwan, Indonesia, Thailand, and China, have already embarked on infant vaccination programs. Many years will be required to establish that vaccination reduces the toll taken by liver cancer in those countries. But, Beasley maintains, all available evidence indicates that it will.

Somehow latently infected cells escape surveillance by the immune system.

A vaccine to protect against Epstein-Barr virus infections may also be available soon, according to M. A. Epstein of Oxford University. The Epstein group has shown that a vaccine containing gp340, a large surface glycoprotein from the virus, works in cottontop tamarins.

Ordinarily these monkeys develop a malignant lymphoma within weeks of being infected with Epstein-Barr virus. The animals do not get sick, however, if they are first vaccinated with the gp340 preparation. "It protects tamarins against a dose of virus that will otherwise produce tumors in 100% of the animals," Epstein says.

Epstein has approval from the appropriate committees in the United Kingdom to begin preliminary clinical trials of the gp340 vaccine in human volunteers to see whether it will elicit appropriate immune responses without producing unacceptable toxicity. If the vaccine passes muster in this trial, then a more extensive study will be undertaken to determine whether it will protect humans against Epstein-Barr virus.

Most of the presentations at the tumor virus conference focused, however, on efforts to understand how the viruses put the cells they infect on the path to malignancy, rather than on efforts to prevent those infections. The hope is that a better understanding of how the viruses induce cancers will pay off in a better understanding of the origins of cancer in general.

Recent results suggest that the Epstein-Barr and human papilloma viruses carry genes that immortalize infected cells and cause them to divide continuously. A variety of evidence points to two genes, designated E6 and E7, as the likely transforming genes of the cancer-associated papilloma viruses. Both genes are consistently found in the DNA of cervical cancer cells, for example, and are active there.

At the meeting, Peter Howley of the National Cancer Institute (NCI) in Bethesda, Maryland, presented new data that may help to explain how the protein encoded by the E7 gene transforms cells. Howley and Karl Münger of the NCI, with Nicholas Dyson and Ed Harlow of Cold Spring Harbor Laboratory on Long Island, have found that the E7 protein forms stable complexes with a cellular protein, the product of the retinoblastoma (RB) gene. The E7 protein resembles transforming proteins from two other viruses, adenovirus and simian virus 40, that also bind to the RB protein.

Because the RB gene is missing or inactivated in certain cancers, including retinoblastoma tumors of the eye, researchers think that the RB protein normally acts to inhibit cell growth. By binding to the RB product, E7 and the other transforming proteins may prevent it from acting, thereby allowing cells to grow out of control. At present, there is little information about the E6 gene's contribution to cell transformation.

The Epstein-Barr virus also appears to carry transforming genes. Work by Elliott Kieff's group at Harvard Medical School indicates that the viral LMP (for latent membrane protein) gene has transforming activity. How the LMP gene product acts is unknown. It structure indicates, however, that it may be a receptor for a growth factor or an ion channel.

In addition, William Sugden of the University of Wisconsin in Madison has used a genetic approach to show that another gene, designated EBNA-2 (for Epstein-Barr virus nuclear antigen 2), immortalizes cells. The EBNA-2 product may work at least partly by stimulating the expression of other viral and cellular genes, including the LMP gene.

So far as is known, hepatitis B virus does not carry transforming genes. Pierre Tiollais of the Pasteur Institute in Paris described one way in which hepatitis B virus infection may lead to liver cancer—the viral DNA can insert itself into the genome of infected cells. Tiollais has found that sometimes these insertions can cause the activation of a cellular oncogene. In their normal state, oncogenes regulate cell division and differentiation, but when abnormally activated they can cause

*The conference, which was held on 22 to 26 January, was sponsored by the American Association for Cancer Research.

cells to become cancerous.

This may not be the main way that hepatitis B virus works, however. More often, it apparently paves the way for malignant transformation by establishing a persistent infection in the liver that causes chronic damage to the organ. Francis Chisari of the Research Institute of Scripps Clinic in La Jolla, California, reported results with an animal model that supports this hypothesis.

Chisari and his colleagues introduced the gene encoding the surface antigen of hepatitis B virus into mice. As a result, the animals' liver cells were damaged by the accumulation of large deposits of the antigen protein. Liver cells divide to regenerate damaged tissue, however. After many months of liver injury and regeneration, cancerous nodules developed in the animals' livers.

Something similar may happen in human carriers of hepatitis B virus, although in people most of the liver damage is caused by an immune attack on virus-infected cells, rather than by a buildup of surface antigen deposits in the cells. The repeated cycles of liver cell regeneration may foster cancerous transformation by increasing opportunities for carcinogenic mutations to occur.

Mutations, perhaps in oncogenes, might also occur more readily in cells that divide abnormally because they were immortalized by Epstein-Barr or human papilloma viruses. Burkitt's lymphoma cells have an activated *myc* oncogene, for example, in addition to carrying Epstein-Barr virus genes.

In fact, the conference participants stressed that none of the DNA tumor viruses can cause cancer by itself. Other changes, perhaps several, are also required in infected cells.

The need for additional changes is indicated by the long time between the initial viral infection and the eventual emergence of cancer. Usually this takes years or even decades, during which the viruses are maintained in the body.

Moreover, the viruses are common, but only a small percentage of those who are infected get cancer. For example, Ethel-Michele de Villiers of the German Cancer Research Center in Heidelberg reported on one study in which 80 to 90% of the women under 50 years of age showed signs of infection with cancer-associated strains of human papilloma virus. "It's not only papilloma virus that plays a role in causing cervical carcinoma, but other factors are also involved," de Villiers says.

Most of these other factors are currently unidentified, although, as already mentioned, one might be the activation of cellular oncogenes. And the loss of the cell's normal growth inhibitory and tumor-suppressing mechanisms may be another.

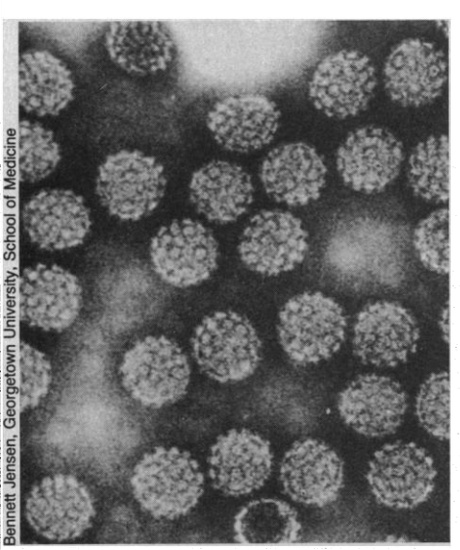
Harald zur Hausen and Elisabeth Schwarz of the German Cancer Research Center have found that cells in living animals normally make a factor, which they call cellular interfering factor, that inhibits the expression of the transforming genes of human papilloma virus. Only when the production of that factor is shut down in some way can the viral genes become active and drive the cells toward tumorigenicity. Zur Hausen also has evidence for the existence of cellular factors that suppress the tumorigenicity of Burkitt's lymphoma cells.

In addition, George Miller of Yale University School of Medicine in New Haven notes that, to participate in cancer development, the DNA tumor viruses have to be maintained in infected cells for long periods without reproducing infectious viral particles. Production of such particles will kill the cells, causing an acute infection but obviating any long-term progression to cancer.

Miller and his colleagues have identified a gene in the Epstein-Barr virus that must be turned on for the virus to replicate. "It's the first gene that we have identified in any eukaryotic virus that controls the switch between latency and replication," Miller says.

Ordinarily this gene, called the "zebra" gene by the Yale group, is not expressed in cells infected with Epstein-Barr virus, a circumstance that allows the virus to remain in the latent state. Prevention of the zebra gene expression gives the Epstein-Barr virus the opportunity to transform cells.

Researchers have identified some ten Epstein-Barr virus genes that may be expressed in latently infected cells. These include the transforming genes EBNA-2 and LMP. The products of both of these genes are targets for cytotoxic immune cells, yet somehow latently infected cells can escape surveillance by the immune system.



Bennett Jensen, Georgetown University, School of Medicine

Human papilloma virus particles. *There are approximately 60 strains of human papilloma virus. Several have been linked to cervical and other genital cancers.*

Epstein-Barr virus infects epithelial cells in the mouth and throat and also the B cells of the blood. Either type of cell could serve as the reservoir for maintaining latent infections, but evidence presented by George Klein of the Karolinska Institute in Stockholm now suggests that B cells are the reservoir. Both Klein and Alan Rickinson of the University of Birmingham in England propose that Epstein-Barr virus is maintained in a type of small resting B cell that does not express EBNA-2 and LMP or certain of the cellular molecules needed for interactions with cytotoxic immune cells. The cells can therefore escape immune surveillance.

The viral transforming genes apparently can come on at some point, however. Epstein-Barr virus sometimes induces B cell lymphomas in immunosuppressed patients. These tumor cells express both the viral transforming genes and the cellular adhesion molecules that bind T cells. The cells should be subject to immune attack in normal individuals, but they may grow out of control when the immune system is defective.

Such cells should also be subject to immune surveillance in Burkitt's lymphoma patients, who have functional immune systems. Burkitt's lymphoma cells do not make products of the viral transforming genes and the adhesion molecules, however. The assumption is that they once did, Rickinson says, and subsequently stopped.

Cervical cells that have been infected with human papilloma virus are also subject to immune surveillance. "Natural killer cells are the most important mediators of cellular immunity against HPV-induced anogenital cancers," says Stefania Jablonska of the Warsaw School of Medicine.

The development of the ability to circumvent that cellular immunity may be one of the additional changes that help infected cervical cells to become cancerous. Jablonska finds that natural killer cell activity is reduced in patients with cervical carcinoma, apparently because the cancer cells produce some factor that prevents the natural killers' attack.

If researchers can learn how the DNA viruses contribute to cancer development, they may be able to devise ways of blocking the viral activities that lead to cancer. Such treatments are needed. Vaccination to prevent infections by the DNA tumor viruses may one day be possible, but many people around the world are already infected, and many more will become infected before that happens. ■ JEAN L. MARX