

Allegations of Cancer

Does a herpesvirus cause an AIDS-related tumor?

By KATHLEEN FACKELMANN

Since 1981, when the first cases of AIDS were identified, epidemiologists have intensively tracked Kaposi's sarcoma. They knew the disease, a cancer marked by purple tumors on the skin, had cut a wide swath among gay men with AIDS, and they embraced a variety of theories in their search for the cause.

At first, some researchers blamed the disfiguring tumors on the use of recreational drugs called poppers. Later, many epidemiologists believed that some infectious agent, probably a virus, was at the root of Kaposi's sarcoma.

In 1994, a team at Columbia University reported an intriguing lead. Epidemiologist Patrick S. Moore and pathologist Yuan Chang described a new herpesvirus they had found in tumor specimens taken from AIDS patients (SN: 12/17/94, p. 405). They called it Kaposi's-sarcoma-associated herpesvirus.

Because it is the eighth herpesvirus known to cause disease in humans, it is also called human herpesvirus 8 (HHV8).

"HHV8 is at the scene of the crime," remarks Don Ganem, a Howard Hughes Medical Institute researcher at the University of California, San Francisco (UCSF). "But is it the criminal? That is the question." The 1994 report started laboratories, including the one headed by Ganem, scrambling for the answer.

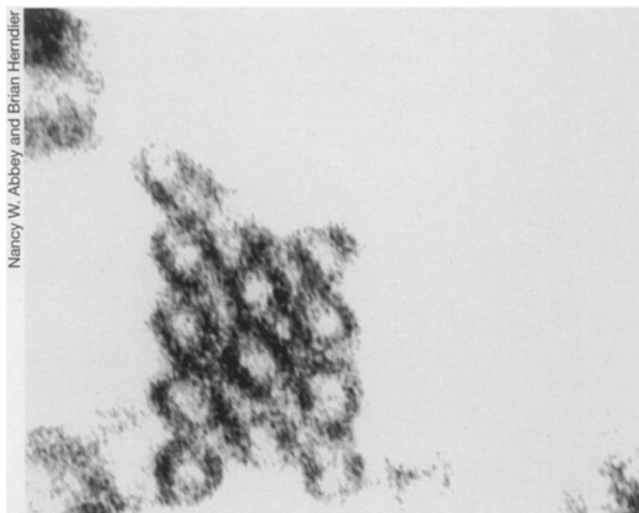
A trio of recently published reports adds to the evidence against HHV8. "There's no question that [the virus] causes Kaposi's sarcoma," Moore says.

Other HHV8 researchers, such as Ganem, also suspect HHV8 is the infectious agent but stop short of Moore's certainty. "We are very far along on the scale of causality," he says.

A few skeptics remain, among them AIDS researcher Robert C. Gallo of the Institute of Human Virology in Baltimore. Although he says that the new work "adds to the arguments that this virus is playing a role in the development of

Kaposi's sarcoma," he holds that there are still significant holes in the case. "Is it conclusive that [HHV8] plays an etiologic role? Not in my mind."

Right from the start of the AIDS epidemic, epidemiologists noticed a curious phenomenon: Among people infected with the AIDS virus, Kaposi's



This photograph of a human cell zooms in on a cluster of HHV8 viruses.

sarcoma disproportionately afflicts gay men. About 25 percent of such men develop the disease in the course of infection with HIV, the AIDS virus. In contrast, the cancer seldom surfaces in HIV-infected hemophiliacs—people with blood-clotting disorders who require infusions of blood or blood products.

Researchers knew that HIV can be transmitted through contact with tainted blood or through sexual contact. They wondered if Kaposi's sarcoma was caused by a sexually transmitted virus that rarely circulates in the bloodstream.

Moore and Chang's discovery, reported in the Dec. 16, 1994 SCIENCE, was the first laboratory evidence of such a virus.

It electrified the field.

Some researchers countered that HHV8 had no role at all in causing the purplish tumors. They said the virus, like

some other herpesviruses, is probably benign and ubiquitous. HHV8 could be like herpes simplex 1, the cold sore virus that infects almost everyone.

If HHV8 is a universal virus, the argument went, then finding it in Kaposi's sarcoma tumors might not represent cause and effect. Rather, the virus might be an innocent bystander.

That's precisely the conclusion many scientists drew from a May 2 NEW ENGLAND JOURNAL OF MEDICINE report. In that study, Italian researchers analyzed tissue and semen samples from 143 healthy adults. Paolo Monini of the University of Ferrara in Italy and his colleagues used polymerase chain reaction (PCR), a molecular technique that generates many copies of genetic material. They found DNA from HHV8 in a large number of samples, including 91 percent of the semen samples. The team concluded that HHV8 infects many healthy adults.

Moore doesn't buy the innocent bystander theory. Several other teams have failed to confirm the Italian team's results, he points out. Moore thinks that a slight contamination of the samples, magnified by PCR, may explain them.

Rather than focus on PCR, Moore's team tried a different strategy for detecting HHV8 infection. In the July 25 NEW ENGLAND JOURNAL OF MEDICINE, Moore, Chang, and their colleagues describe their use of a blood test that looks for antibodies to HHV8. The immune system manufactures these specialized proteins when it encounters HHV8.

Initially, the researchers tested blood that they had collected and stored at regular intervals from the time a man entered their study until he developed Kaposi's sarcoma. They discovered that among 40 HIV-infected gay men with the cancer, 80 percent had antibodies to HHV8. The presence of such antibodies in the bloodstream means the body has been infected

with the HHV8 virus, Moore says.

Next, the team compared that group to 40 gay men without Kaposi's sarcoma. Only 18 percent had signs of HHV8 infection. Moore suggests that some of these men may eventually develop the cancer or that the virus might cause cancer in most, but not all, infected people.

So far, so good, Moore reasoned.

Next, the researchers examined the timing of HHV8 infection. They discovered that 11 of the 40 gay men with Kaposi's sarcoma had been infected with HHV8 at the start of the study.

More important, 21 of the 40 had not. These men seem to have become infected with HHV8 during the course of the study. Signs of the virus in their blood appeared months before the men developed the tumors.

The findings indicate that infection with HHV8 precedes the development of Kaposi's sarcoma, Moore says. They also show that the men were infected with HHV8 as adults, which fits with the idea of the virus being passed from person to person sexually.

Adult transmission of HHV8 doesn't support the notion of a ubiquitous virus. Such a virus, like herpes simplex 1, usually gets passed on in childhood, Moore notes.

He and his team wanted to take a closer look at a group that is representative of the general population. In the August *NATURE MEDICINE*, they report results of giving an antibody test to U.S. blood donors. Out of 122 donors, none had evidence of HHV8 infection, a finding which argues that HHV8 is rare in healthy adults.

The researchers also studied 107 Italian blood donors and found that 4 had signs of infection with HHV8. That's still low, but it's higher than the zero infection rate for the U.S. donors.

The case file on HHV8 and Kaposi's sarcoma also contains data from a group led by Ganem. He, Dean H. Kedes, also a Howard Hughes Medical Institute researcher at UCSF, and their colleagues looked at the distribution of HHV8 in gay men and others at risk of Kaposi's sarcoma. They detail their findings in the August *NATURE MEDICINE*.

The team independently developed a blood test that would flag antibodies to HHV8 in the bloodstream.

Because HHV8 is thought to be transmitted sexually, the researchers then turned to a group they thought would be free of this virus. They secured blood samples from 18 college women who said they had never had sexual intercourse and who tested negative for common sexually transmitted diseases. None of the 18 had antibodies to HHV8.

Next, the team tried the test out on 46 men with Kaposi's sarcoma. Again, they got a result consistent with the theory that HHV8 causes this cancer: 83 percent

of the men were positive for HHV8 antibodies.

Kedes felt confident that the test worked, so the team began a systematic testing of groups at risk of the cancer. Of 141 HIV-negative blood donors, just 1 percent tested positive for HHV8, a finding supporting the view that the virus is not common in the general population.

The scientists reasoned that people attending a health clinic to treat sexually transmitted diseases would demonstrate a moderate prevalence of HHV8 infection. That's just what they found. Of 176 patients with a history of syphilis, 13 percent had evidence of infection with the virus. Only 46 of the 176 were HIV-positive. That group had the highest HHV8 infection rate of all—28 percent.

Next, the researchers turned their attention to hemophiliacs and surgical patients who had been infected with HIV through transfusions of tainted blood. From 1 to 3 percent of these people eventually develop Kaposi's sarcoma. The risk of HHV8 infection appears to be similar. The UCSF team tested 300 hemophiliacs and found that 3 percent had antibodies to this virus.

In every case, the pattern of HHV8 infection matched the risk of Kaposi's sarcoma. Groups deemed to be at high risk fell out on the high end of the HHV8 infection spectrum. Those at low risk had a low rate of infection.

"It all fit together extraordinarily well," Kedes says.

Much remains to be learned about HHV8. For example, scientists have yet to document a mechanism by which it causes cancer. They know that Kaposi's sarcoma results when certain blood vessel cells proliferate—a process that creates the hallmark tumors on the skin. Moore's team has uncovered several potential cancer-causing genes in the HHV8 genome. Moore presented those data this summer at the 21st Herpesvirus Workshop, held at Northern Illinois University in De Kalb.

Even if additional research confirms these findings, no one argues that HHV8 infection alone causes the cancer. Researchers believe that a damaged immune system is also necessary for Kaposi's sarcoma to develop. In healthy people, the immune system probably keeps a tight rein on HHV8, Moore says. When something like the AIDS virus injures the system, HHV8 may flourish—and somehow trigger the tumor.

That hypothesis fits with parts of the Kaposi's sarcoma story told by epidemiologists. They know that AIDS patients aren't the only ones with a higher than average risk. This cancer is also found in transplant patients, who must take immune-dampening drugs to prevent rejection of a donor organ.

Ganem believes gender may also play a role in Kaposi's sarcoma. It is surprisingly prevalent among elderly men of Mediterranean origin. It's "largely a disease of men," Ganem says. He wonders whether male sex hormones in some way fuel the growth of this tumor. Or whether female sex hormones protect women from the disease.

There remains the puzzle of Africa, where an extraordinarily high rate of Kaposi's sarcoma fells men and some women, including many people who are HIV-negative. Is something else damaging the immune system and allowing HHV8 a foothold?

Furthermore, researchers have yet to sort out how this virus is passed from one person to the next. "What are the things that people do that actually put them at risk?" Moore asks. The answer may help efforts to prevent this cancer.

Do the researchers have enough proof to convict HHV8 of causing Kaposi's sarcoma? Although Moore says yes, others are not as certain. "Conclusive proof is always extraordinarily difficult in these circumstances," Kedes says. He adds that antibody tests alone never prove anything.

"The trouble is, if you want to be rigorous, few things are proof of a causal link between the virus and cancer," Ganem says.

Gallo says the researchers have never shown that HHV8 injected into animals causes Kaposi's sarcoma. Humans are the only known host of HHV8, Ganem states. His team is now trying to infect monkeys with the virus.

Gallo also notes that HHV8 researchers have yet to produce the cancer in the laboratory. Some cancer-causing viruses enable human cells growing in the lab to divide indefinitely rather than die, as they normally would. Such evidence would add to the case against HHV8, Gallo says.

The researchers counter that they've built their case on a series of facts, all pointing to HHV8's link to Kaposi's sarcoma. "We know that infection precedes KS [Kaposi's sarcoma] development; that infection is most common in groups most at risk for KS; and that infection predicts an increased probability of KS development," Ganem says. "All together, that's a pretty strong set of findings."

For people with Kaposi's sarcoma, confirmation of HHV8's involvement may eventually bring a tangible payoff. If an antiviral drug were to block HHV8 replication, it might slow down the tumors, says Robin A. Weiss, an HHV8 researcher at the Institute of Cancer Research in London. Such results would also provide convincing evidence of HHV8's causal relationship to the tumor, he adds.

For now, however, that payoff remains just out of reach. □