New Reports of a Human Leukemia Virus

Researchers in the United States and Japan independently report the association of a retrovirus with human leukemia

In January 1975 Robert Gallo, of the National Cancer Institute, Bethesda, thought he had isolated a human leukemia virus. As was the fate of all such reports of "the first identified human cancer virus," Gallo's was not substantiated. He and his colleagues appeared to have isolated an already known monkey leukemia virus.

Now, half-a-dozen years after that cautionary experience, Gallo is making a similar claim. "I know a lot of people will be skeptical, but, as you can imagine, we have been extremely careful about this whole thing," he says, "and it now looks pretty good."

The virus, which is associated with a rather rare form of leukemia, was discovered as a result of Gallo's persistent search and a piece of good fortune. Adding weight to Gallo's new evidence is parallel work by Yorio Hinuma and his colleagues at Kyoto University, Japan. Although the Japanese group started working on the problem more recently than Gallo, they too say they have isolated a virus similar, if not identical, to Gallo's. More intriguing, however, Hinuma says that he has transformed normal human lymphocytes with the virus. If confirmed, this will be an important "first" in the field of cancer research.

A principal requirement for anyone looking for cancer viruses is the establishment of a vigorously growing cell culture. For this reason Gallo's laboratory devoted a good deal of effort from 1975 onward to isolating natural substances that promote the growth of human T lymphocytes, which are involved in a range of leukemias. "When I went to Gallo's lab in the summer of 1978, T cell growth factor was everyone's main thought," recalls Bernard Poiesz, who is now at the Veterans Administration Medical Center, Syracuse.

Gallo and his colleagues, particularly Francis Ruscetti, had been working with crude extracts that would stimulate the growth of T cells, once they had been appropriately triggered. At the time of Poiesz's arrival, James Mier had been purifying the growth factor with Gallo and had thereby fashioned the tool that was to be so crucial in the discovery of the virus.

Poiesz had gone to Bethesda for a 3-year program, 1 year of which was to be spent as a clinical associate taking care of patients, and 2 years doing research in Gallo's laboratory. During part of the clinical associateship Poiesz was involved with patients suffering from various forms of cutaneous T cell malignancies, a clinical and research program that Paul Bunn had been running since the mid-1970's. It was therefore fortuitous that Poiesz took with him experience of clinical care of T cell malignancies to a laboratory that was interested in growing T cells in culture using the newly isolated growth factor.

Meanwhile, back at the VA laboratory, Adi Gazdar, using conventional growth techniques, had established a T cell culture from a patient, C.R., with mycosis fungoides. With Poiesz now transferred to the NCI, the two labs began discussing the use of T cell growth factor for setting up such lines of malignant T cells. The aims and facilities of the two laboratories complemented each other very neatly. Eventually, the use of Gallo's growth factor led to the establishment of a number of malignant T cell lines. "Gallo then asked me to check the cell line I had set up from patient C.R.," says Poiesz. "It was something we routinely did whenever we had a cell line going," explains Gallo. "It was just part of our continual search."

This time the routine turned up the unexpected. Very quickly Poiesz detected the presence of the enzyme reverse transcriptase, an essential part of the molecular equipment of a type of RNA virus known as a retrovirus. When these viruses infect tissues, they make DNA copies of their genetic material, and this then inserts into the genome of the host. The reverse transcriptase assembles the DNA copy, the provirus.

Soon after detecting the reverse transcriptase, Gallo and his colleagues turned to electron microscopy for confirmation of the presence of a virus. They picked up pictures typical of retroviruses, showing the virus particle "budding" from the cell membrane. "We all maintained a healthy amount of skepticism about it," recalls Poiesz. For more than a year the lab worked hard to characterize the virus further. The reverse transcriptase, the RNA genome, and the core proteins of the virus were analyzed and compared with their counterparts from other known retroviruses. "We wanted to take it all slowly, step by step," says Gallo. "We had to be sure we were right before we published anything at all."

The first paper on the virus, published in the *Proceedings of the National Academy of Sciences* in December 1980 (p. 7415) revealed only a fraction of what the lab already knew about it. None of the key characteristics coincided with known viruses: it did appear to be a novel retrovirus. Gallo's group isolated the virus from fresh blood from C.R. as well as from cultivated cell lines, thus strengthening the supposed association between virus and disease. The virus, or at least one very similar to it, was then isolated from a second patient, M.B., with the cutaneous T cell malignancy Sézary syndrome. "This helped to convince us we were dealing with a potential cancer virus," says Poiesz.

The NCI group has shown the virus to be integrated in the genome of diseased cells, whereas it is absent from normal cells in these patients. No sign of the virus has been seen in healthy people. "It looks to be an exogenous virus," says Gallo, "which is what you'd expect of a disease-causing agent." Although the virus is distinct from all known retroviruses, it does have affinities with bovine leukemia virus, both in the structure of one of its proteins and in some characteristics of the associated pathology.

Another important step in the story was to look for antibodies against the virus in patients with T cell malignancies. "We sent coded samples of serum to Gallo's lab," says Bunn, "and he picked out a small number of positives." These were patients C.R. and M.B., another Sézary syndrome patient, and the wife of the first patient. Later another relative, the sister of M.B., also turned out to have a high antibody titer against the virus. "This kind of epidemiology is indicative of an infectious agent," suggests Gallo.

To date the virus has been discovered in four cutaneous T cell leukemia or

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lymphoma patients in the United States. Antibodies have been detected in these four patients (although many patients with the disease have neither virus nor antibody), in the two relatives mentioned, but not in healthy people. Although this is not much to go on, epidemiologist William Blattner, at the National Institutes of Health, is very excited about the prospects. "This is a unique opportunity for an epidemiologist to become involved in a new disease," he says.

So far, one intriguing lead has emerged: three of the four patients detected in the United States have been black, as has the only one yet confirmed from the United Kingdom. "We are establishing contacts with physicians all over the United States," says Blattner, "and we are hoping to get cooperation from the Pan American Health Organization. We would like to test people in the Caribbean and Central America."

The United Kingdom is already well set up to help push this investigation further. "There is a very widespread referral system here," says Mel Greaves of the Imperial Cancer Research Fund, London. "Gallo's work looks very good," he comments. "The molecular biology, the immunology, and the epidemiology all look very interesting, and so we have agreed to collaborate with Gallo, mainly by sending samples of serum for testing."

These collaborative connections will be very important. But meanwhile, Gallo has turned his attention to Japan. Gallo heard that in the southwestern part of Japan, on the islands of Kyushu and Shikoku, the incidence of adult T cell leukemia, which is in the same class as the virus-associated malignancies in the United States, was extraordinarily high. About a year ago he began a collaboration with Yohei Ito of Kyoto University. Ito supplies the Gallo laboratory with serums, where they are analyzed for viral antibodies.

The collaboration has already been productive. For instance, six of seven patients with adult T cell leukemia were positive for the antibody (the seventh was in remission from chemotherapy). A smattering of other patients with T cell malignancies of different types were also positive. All healthy people tested, including those living in the area where the disease is endemic, were negative. "It is difficult to say if the disease is infectious," comments Ito. "We will have to start testing relatives before we can say much about this."

Inevitably there is considerable interest in Japan over this disease and its possible viral association, an interest that has been sharpened since Gallo's collaboration with Ito. Hinuma leads the work on the virology, with his team at the Institute of Virology at Kyoto University. The Kyoto team has isolated a virus from cultures from patients with adult T cell leukemia, pictures of which it will publish in a forthcoming issue of the *Proceedings of the National Academy of Sciences*. "I'm not sure it is the same virus as Gallo's," Hinuma told *Science*. "There are some unusual characteristics about it too," he says. "The size of the particle varies between 100 and 180 nanometers in diameter, and so far we haven't seen the typical budding we'd expect."

If there is some uncertainty on this front, it is more than compensated for by the kind of result that every cancer virologist seeks; Hinuma and his colleagues have managed to transform normal human T cells by exposing them to the virus, a result that Hinuma revealed to participants at the International Congress of Leukemia and Lymphoma held recently in Los Angeles.

"We have tried all kinds of ways to infect normal cells with the virus," laments Gallo, "but Hinuma did something we didn't." The trick was to cocultivate infected cells with blood taken from umbilical cord. "The virus was probably transmitted to the lymphocytes by cell fusion," guesses Hinuma. Like Gallo, Hinuma had failed to get infection by simply exposing the target cells to the virus. Nevertheless, it is an important result; the cells were infected, and, as far as can be determined, they were transformed. As Hinuma comments, "This has never been done before with human cells."

Hinuma's group now has a tremendous momentum, and it has already done extensive antibody tests on serums, both from patients with T cell malignancies and healthy people. "We have found antiviral antibodies in 100 percent of patients with adult T cell leukemia," he says. More interesting, in the area where the disease is endemic, "between 20 and 40 percent of healthy people also have the antibodies." The epidemiology gives the strong impression of an infection, but, says Hinuma, "We don't know the mode of transmission of the virus."

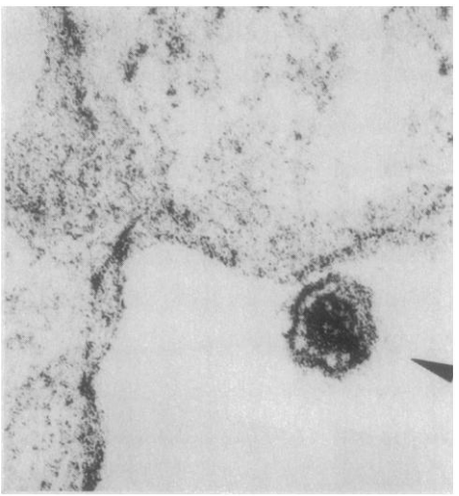
The Kyoto group already has ambitious plans to begin clinical trials with viral proteins as a vaccine. "We are also planning to see if we can use the vaccine for immunotherapy in patients with the cancer," says Hinuma.

Robin Weiss, a virologist and director of the Chester Beatty Institute for Cancer Research, London, is impressed with Gallo's current work and is intrigued by the reports coming from the Japanese group, none of which is yet published. He regards Hinuma's cocultivation experiment as extremely interesting, but warns that there are many potential problems with it. "You would have to look at the data very critically to be certain about it," he says.

"Gallo's virus is a new retrovirus; there's no doubt about that," Weiss comments. "The evidence for it is very persuasive. It clearly comes from leukemia cells, although this is not necessarily to say that it causes the disease."

With the vast amount of money that has been poured into human cancer virology, especially in the United States, it would be gratifying if at last a true candidate as a human cancer retrovirus were to be confirmed. But it would be no great surprise: retroviruses cause leukemia in chickens, cats, rodents, cattle, and gibbons. The cutaneous T cell leukemias and lymphomas are rare, so the discovery will have little impact on clinical oncology. And in any case, Gallo's virus would have to take its place alongside other strong candidates for cancer-associated viruses: Epstein-Barr virus and Burkitt's lymphoma, and hepatitis B virus and hepatoma, for instance (these are DNA viruses, not retroviruses). Nevertheless, the virus, if it is confirmed, will be the first human retrovirus to be discovered.

Will the discovery be confirmed? "Gallo has been extremely cautious," says Greaves. "He's done everything extremely carefully." Weiss's comment is this: "The virus deserves serious attention."—ROGER LEWIN



Portrait of a human cancer virus

The virus is seen here (arrowed) near the surface of a cultured T cell lymphoma cell (× 55,000).