AIDS Virology: A Battle on Many Fronts

Proof that a retrovirus causes AIDS put research on a new footing, but who should get the credit has become a matter of dispute

In the war on AIDS, "the spring of 1984 was the functional equivalent of Stalingrad or El Alamein," says Samuel Broder, chief of clinical oncology at the National Cancer Institute (NCI). "The war is not over by any means and it is still possible that we will not have a happy outcome, but a turning point occurred."

What put the war on a new footing was the publication of research by Robert C. Gallo of the NCI and his many co-workers, firmly implicating a new virus as the cause of AIDS. Equally important, Gallo's work enabled large quantities of the virus to be grown for further studies and it led to mass production of a test to detect viral antibodies in human blood.

The publication of Gallo's work also marked a turning point in hostilities of a different kind. It crystallized a dispute between Gallo and Luc Montagnier of the Pasteur Institute in Paris over who should be given credit for discovering and identifying the cause of AIDS. Montagnier's group had been working for more than a year on a virus isolated from a patient with chronic lymphadenopathy and other symptoms of AIDS. Their virus also turned out to be the AIDS virus.

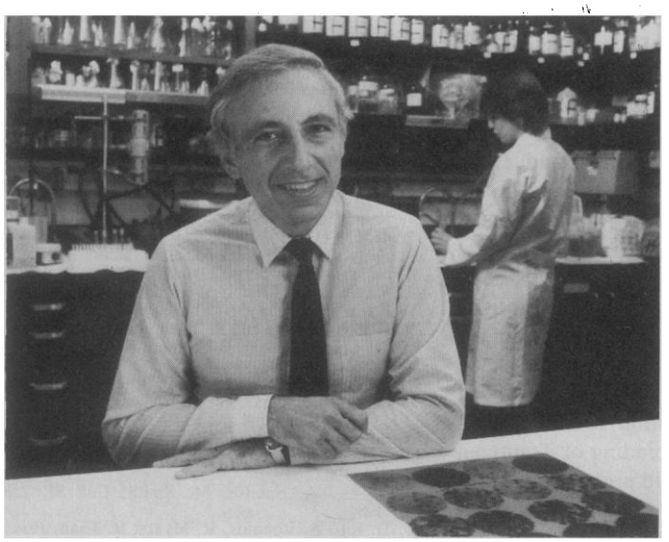
This priority dispute, which has been fanned by gossip and rumors circulating around parts of the scientific community, has since led to a battle between the Pasteur Institute and the U.S. government over patents governing the use of kits for testing blood donations.

The War on AIDS

This is the second article in a series about research on AIDS. Next: scientific disagreements and a dispute over patents.

The patent fight, which involves millions of dollars a year in royalties, is likely to come to a head in the next few weeks. A blood test kit, made under license to the Pasteur Institute by Genetic Systems Corporation of Seattle, Washington, is expected to be approved soon by the Food and Drug Administration. When it hits the market in the United States, it will be in violation of a U.S. patent that is held by the federal government and is based on Gallo's work.

Pasteur officials, in meetings with officials of the U.S. Department of Health and Human Services (HHS), have argued that Montagnier should be declared the true inventor of the blood test and have demanded that the Pasteur Institute be included on the U.S. patent. They have also said that the institute should receive half the royalties—estimated to amount to at least $5 million a year—from sales of test kits in the United States. In an interview with *Science*, Raymond Dedonder, the head of the Pasteur Institute, said that "if there is no solution, then we will have to go to court. That will not be with pleasure, but we are prepared to do it." The U.S. government has so far refused to give way.



Robert Gallo

His previous work on cancer viruses led him to search for a retrovirus as the cause of AIDS.

The Pasteur Institute's legal case rests in part on the fact that it filed for a U.S. patent on a blood test 4 months before the U.S. government applied for its patent. The government's patent was awarded on 25 May 1985, but the Pasteur Institute's application has not yet been dealt with. At the heart of the dispute, however, are different perceptions of the events that led to the identification of the AIDS virus and different interpretations of what constituted proof that the virus is indeed the cause of AIDS.

The following account, based on interviews with dozens of scientists on both sides of the Atlantic, describes the work of both groups prior to Gallo's publications in the spring of 1984. A second article will examine the subsequent patent contest and allegations that have arisen following publication of the genetic sequences of viruses isolated by each group.

For Gallo, his work in unraveling the cause of AIDS was a natural outgrowth of previous studies of human cancer viruses. In 1978, his lab isolated a virus from patients suffering from a rare type of leukemia characterized by proliferative growth of a class of lymphocytes known as T cells. He subsequently proved that the virus, which he called human T-cell leukemia virus, or HTLV, was the cause of the disease. The discovery broke fresh ground in two respects. It was the first time that a virus was linked to cancer in humans, and HTLV was also the first human retrovirus to be isolated and identified.

Although retroviruses were known to cause a variety of cancers and other diseases in animals, they had never before been seen in people. They are an insidious class of viruses whose genetic material consists of RNA rather than the DNA that constitutes the genetic blueprint of virtually every other known organism.

When they enter a cell, retroviruses employ an enzyme known as reverse transcriptase to copy their RNA into DNA, which is then spliced into and becomes part of the DNA of the host

cell. The integrated virus, known in this form as a provirus, eventually commandeers the cell's reproductive machinery to replicate its own genes. Virus particles then emerge from the host cell and go on to infect new cells.

Gallo's group, along with a group headed by David Golde at the University of California at Los Angeles, subsequently isolated a second human retrovirus, which he called HTLV-II (the first one then became known as HTLV-I), from cells of patients with hairy cell leukemia, another rare form of cancer. And, in another development that would have importance for work on AIDS, researchers in his lab identified a hormone that plays a key role in the growth of T cells. Originally called T-cell growth factor, it has since become known as interleukin-2, or IL-2.

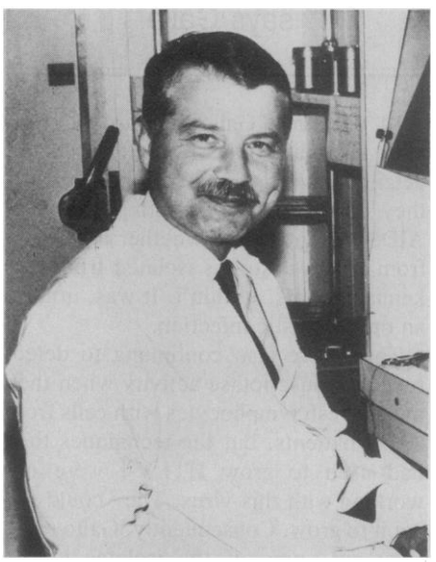
By early 1982, many researchers were coming to the conclusion that AIDS was caused by an infectious agent, probably a virus. The chief clinical symptoms were severe suppression of the immune system, opening up the victim to attack by a range of opportunistic infections. Underlying the collapse of the immune system was a sharp drop in the number of T cells, particularly a subclass known as T4 cells, which play a central role in orchestrating the body's response to invasion by infectious agents.

Gallo, influenced by his work with retroviruses that infect T cells, speculated at a meeting at Cold Spring Harbor in February 1982 that a retrovirus might be involved in AIDS. This view was shared early on by Max Essex of the Harvard School of Public Health. "Gallo and I had endless conversations [in 1982] about looking for HTLV-related viruses" as a cause of AIDS, Essex recalls.

The idea began to pick up momentum in the fall of 1982, when an ad hoc task force was assembled at NCI by Peter Fischinger, the institute's associate director. The core group consisted of about 20 people, including members of Gallo's lab and virologists and clinicians from hospitals and universities on the East Coast. Gallo was the scientific director. His leading bet, supported by other task force members, was that a variant of HTLV-I was causing AIDS.

The bet relied on several tantalizing pieces of evidence. First, HTLV-I itself selectively infects T4 cells, the very cells depleted in AIDS. Second, Essex had found that a T-cell retrovirus that causes leukemia in cats also causes immunosuppression. Third, a high incidence of AIDS was being reported in Haiti and Africa, two regions in which HTLV-I is endemic. And fourth, Essex, in collaboration with researchers in Japan, was finding that some people infected with HTLV-I were coming down with relatively high rates of opportunistic infections. "It looked and smelled like HTLV-I," says Flossie Wong-Staal, a molecular biologist in Gallo's lab.

This theory was not universally shared. Because AIDS patients are prone to a range of infections as a result of the crippling of their immune systems, it was not easy to separate infections that were a possible cause of AIDS from those that were a consequence of it. A wide variety of potential culprits were



Luc Montagnier

First publication was greeted with skepticism but was proved correct.

therefore being touted as the AIDS agent, and clinicians were forced to make priority choices in the distribution of serum samples among laboratories searching for the etiology of the disease.

According to Broder, who handles NCI patients admitted to the National Institutes of Health's Clinical Center, "we basically made a decision—not a popular decision, I might add—that we would give Gallo absolute priority over samples from the cancer institute." Several other clinicians on the ad hoc task force made a similar choice.

By the end of 1982, Gallo, using the same techniques he employed to isolate HTLV-I, had some intriguing glimpses of the presence of retroviruses. When patients' cells were mixed with fresh lymphocytes, reverse transcriptase activity was detected, but after a few days the activity dropped off and he could not get any virus to grow. Gallo says he tested the samples with probes for HTLV-I and HTLV-II, but they were negative.

In retrospect, it is clear that Gallo was finding footprints of a virus that was not HTLV-I or HTLV-II. These findings have become an issue in the subsequent dispute with the Pasteur group. According to Gallo, the evidence is documented in lab notebooks that have been copied and sent over to Fischinger's office for a defense against any potential suit from the Pasteur Institute.

Other members of the ad hoc working group say that Gallo told them at the time that he was getting results that were difficult to interpret. "He said he was getting RT [reverse transcriptase] activity but he couldn't do the molecular biology because he couldn't get the damn things to grow," recalls Dani Bolognesi, head of the Department of Virology at Duke Medical Center. Gallo did not mention these findings in his first publications on AIDS, however.

In any case, results obtained over the next few months steered Gallo's thinking more toward something closely related to HTLV-I. Essex, using a test based on antigens derived from HTLV-I, detected antibodies in at least 25 percent of patients with AIDS and chronic lymphadenopathy, but not in healthy, matched controls. This was the first strong evidence that infection with a retrovirus was a possible cause, and not a consequence, of the disease. But because the antibodies were reacting with antigens from HTLV-I itself, Essex's results suggested that the AIDS virus was closely related to HTLV-I.

Gallo was also getting results pointing in that direction. Using probes designed to detect specific genetic sequences, he found HTLV-I genes integrated in the DNA of T cells from two out of 33 AIDS patients. Moreover, he isolated HTLV-I itself from cells of one patient. Essex and Gallo submitted their findings to *Science* in March and April 1983.

Meanwhile, Montagnier and his coworkers were also beginning to work on the AIDS problem. In March 1982, a group of physicians, epidemiologists, and immunologists was formed in France to try to understand the cause and transmission of the disease. According to Montagnier, he received a call early in January 1983 from one of his former students, Françoise Brun-Vézinet, who was a member of this group. She said she had a biopsy from the lymph node of a patient with lymphadenopathy, and "asked if we wanted to look at it." Montagnier, a virologist who had been studying mammary tumors in mice, told her to bring it over.

The Pasteur group, led by Montagnier, Jean Claude Chermann, and Françoise

Barré-Sinoussi, put the cells into culture with fresh lymphocytes and IL-2. According to Chermann, the sample arrived on 4 January, and they isolated a virus by 23 January. Like Gallo, they found that reverse transcriptase activity peaked after a few days and then dropped off, but they propagated the virus by adding fresh lymphocytes to the culture.

Chermann then called Gallo, whom he describes as "a good friend," to ask for reagents to test whether the virus they had isolated was HTLV-I. Gallo "sent the reagents immediately." The virus did not react with monoclonal antibodies to the core proteins of HTLV-I, indicating that it was a different virus. However, serum from the patient did react with cells chronically infected with HTLV-I, indicating that the serum contained antibodies to HTLV-I and suggesting that the two viruses might be related. Montagnier would later conclude that this reaction was an artifact, but it caused confusion at the time.

The Pasteur group did some initial characterization of their virus, determining that it had a core protein roughly similar in size to that of HTLV-I. They also obtained electron micrographs of virus particles budding from the surface of infected cells.

Montagnier wrote up the results and sent a copy of the paper to Gallo in April "to ask his opinion and, if he thought it was OK, to transmit it to *Science*." Gallo called to say he thought it was OK, but suggested some modifications, including adding a phrase stating that the new virus appears to be a member of the HTLV family. Gallo says he made the suggestion because the virus had several features in common with HTLV-I, especially the cross-reaction with the infected cell line. Montagnier agreed to the change but the phrase has since come back to haunt him in a subsequent dispute over the naming of the virus.

Montagnier's paper was published alongside those of Gallo and Essex in the 20 May 1983 issue of *Science*. It was immediately greeted with skepticism. "At the time, and for a year afterwards, I was not convinced they had the right virus," says William Haseltine of Harvard University's Dana-Farber Cancer Institute. One problem was that, because the paper reported only a single virus isolation, there was no way to tell whether the virus was a cause of the disease or an opportunistic infection. Another problem was that the electron micrographs were not of high quality, and some scientists suggested that they did not even depict a retrovirus.

Undeterred, the Pasteur group became more and more convinced that what they had was very different from HTLV-I. To distinguish their virus from the HTLV family, they began calling it lymphadenopathy-associated virus, or LAV. The French researchers were also able to isolate virus from AIDS and lymphadenopathy patients from all the known risk groups.

"By January [1984] we had solved the problem" of the cause of AIDS, says Gallo.

At this time, Gallo's group was running into difficulties. They conducted detailed investigations of the HTLV-I they had reported isolating from an AIDS patient to see whether it differed from HTLV-I strains isolated from leukemia patients. It didn't. It was, in fact, an opportunistic infection.

They were also continuing to detect reverse transcriptase activity when they mixed fresh lymphocytes with cells from AIDS patients, but the techniques they had used to grow HTLV-I were not working with this virus. They could not get it to grow. Consequently, Gallo says, they put some of the isolates in the freezer at the peak of reverse transcriptase activity until they could figure out what was going on.

The problem was that, because they expected the AIDS virus to be a variant of HTLV-I, they assumed it would *act* like HTLV-I. Once the leukemia virus infects T cells, the cells can be maintained in culture with IL-2, continuously producing virus. What Gallo's group had not realized was that the AIDS virus was killing the T cells. "We just didn't believe that that is what this kind of virus could do," says Gallo.

"It is certainly true that in that period of time in summer and certainly by early fall, Chermann had recognized the cytopathic effect of that virus and I had not," Gallo concedes. "As I look back now, I could bang my head against a wall that we were so stubborn in trying to grow those cells long term in IL-2. . . . We went through loss of months with that problem." Chermann and Barré-Sinoussi were continuing to propagate LAV on fresh lymphocytes, getting low levels of virus production.

One reason why the American scientists were still thinking in terms of a variant of HTLV-I was the data coming from Essex's lab. Essex had refined his test, based on antigens derived from HTLV-I, to the point where he was able to detect for the first time antibodies in people who were infected but not showing symptoms of AIDS.

The data came from a study, conducted in collaboration with the Centers for Disease Control (CDC), of 12 transfusion-associated AIDS cases. In each case, the patient had received blood from multiple donors from whom CDC had subsequently collected serum samples. In 9 of the 12 donor sets, Essex detected antibodies in a single serum sample. It is now clear that, by pushing the sensitivity of the technique to its limits, he was picking up distant cross-reactions between the HTLV-I antigens and antibodies to the AIDS virus.

Around this time, in the summer of 1983, an exchange took place between Gallo and Montagnier that would later figure prominently in the patent dispute and the rumors circulating in the scientific community. On 17 July, Montagnier attended a meeting of the NCI ad hoc AIDS task force and he brought with him a sample of supernatant from his LAV culture. Gallo says no reverse transcriptase activity could be detected in the sample, and Mikulas Popovic, a cell biologist in Gallo's lab, says he tried twice to infect fresh lymphocytes but failed. Popovic says he called Montagnier at home to tell him.

Montagnier sent a second sample of supernatant on 23 September. This time there was detectable reverse transcriptase activity and Popovic transiently infected some fresh lymphocytes with the virus. According to Gallo, the reverse transcriptase activity was very low, and they could not get continuous virus production, so they put the material in the freezer.

The French group, meanwhile, began developing a test to detect antibodies to LAV in human sera. The test, developed in collaboration with Brun-Vézinet at the Hôpital Claude Bernard in Paris, was similar to that used by Essex, except that LAV itself, rather than antigens from HTLV-I, was used.

Montagnier presented some preliminary results from this test at a meeting at Cold Spring Harbor in September. He reported that antibodies were detected in 22 of 35 lymphadenopathy patients, 7 of 40 healthy homosexuals, and 1 of 54 controls. These results strengthened the link between LAV and AIDS, but many American scientists remained unconvinced. For one thing, Essex was getting better results using HTLV-I antigens.

The Pasteur Institute quietly filed for a

patent on the test in Europe on 16 September 1983 and in the United States in December. The patent application recounts the same results as Montagnier presented at Cold Spring Harbor, but also notes that antibodies could be detected in only 20 percent of patients with frank AIDS.

The Pasteur group was still hampered by the fact that the virus could not be grown in quantity, which made it difficult to do some of the molecular biology. One indication of this, for example, is that the patent application states categorically that there is no immune reaction to LAV's envelope protein. It is now known that the envelope protein evokes the most powerful immune response.

Gallo was also having problem's getting the virus to grow, but he was about to get his big break. When he finally realized that the virus was killing off the cells it infected, he began to look for cells that would resist its cytopathic effects. He found what he was looking for in early November.

Popovic discovered that clones developed from a line of T cells established from a leukemia patient could be infected with virus from the cells of AIDS patients and go on producing virus indefinitely. For the first time, the virus could be grown in quantity.

Because virus from some patients appeared to infect the cell line more readily than others, Popovic did an unconventional thing. He pooled virus isolates from 10 patients and used the mixture to infect the cells. By December, Gallo's lab was mass-producing virus from a cell line, called H9, infected with virus from the pooled samples.

This breakthrough enabled Gallo's group to characterize the virus. They established that it was different from HTLV-I and HTLV-II, but until its precise genetic sequence was determined several months later, it was not known just how different the new virus was. Gallo called it HTLV-III.

Equally important, mass production of the virus opened the way for development of a sensitive test, similar in principle to those of Essex and Montagnier, to detect antibodies to the virus in blood samples. The test provided the means by which Gallo nailed down, to almost everybody's satisfaction, that the virus was the cause of AIDS. It also provided the basis for the screening test that would later be used to monitor blood donations. "The data poured in in December and by January we had solved the problem [of the cause of AIDS]," says Gallo.

Gallo's results, submitted to *Science* on 30 March 1984 and published in a series of papers in the 4 May issue, stated that antibodies were detected in almost 90 percent of AIDS patients and 80 percent of patients with what were then called pre-AIDS conditions. Only one out of 186 controls tested positive. Gallo also reported that he had isolated virus from a total of 48 patients. Patents on the method of mass producing the virus and the test itself were filed by the U.S. government on 23 April 1984.

The French researchers, unaware of Gallo's breakthrough, continued to refine their blood test. At a meeting in Park City, Utah, in February 1984, Chermann reported finding antibodies in 74.5 percent of patients with lymphadenopathy syndrome, 37.5 percent of AIDS patients, 18 percent of healthy homosexuals, and 0.8 percent of healthy controls.

Jean Claude Chermann

Recognized the cytopathic effect of the virus well before Gallo did.

By this time, CDC was cooperating closely with the French group and had confirmed many of their results. CDC researchers had also developed an antibody test using a sample of LAV sent from the Pasteur Institute in February, and in the spring of 1984 the three tests were indirectly compared. According to Donald Francis, a CDC virologist, some 170 serum samples were sent blind to all three labs between January and April. Gallo's group, which reported first, detected antibodies in 81 percent of the samples from AIDS patients and 100 percent of pre-AIDS patients. The CDC group detected antibodies in 70 percent and 100 percent of these samples, and the Pasteur group reported 66 percent and 78 percent. Although the Pasteur group's test could not be mass produced because LAV was not available in quantity, its sensitivity was improving fast. also close to solving the problem of growing the virus in quantity. In February, Montagnier sent a sample of LAV to Robin Weiss at the Chester Beatty Laboratory's Institute of Cancer Research in London. Weiss and his co-workers managed to infect an established T-cell line known as CEM with the virus. The cells produced substantial quantities of the virus but were partially resistant to its cytopathic effect. The Pasteur group had in fact tried to infect a CEM cell line with their virus early in 1983, but the attempt failed.

Weiss sent the line to Montagnier in April and the Pasteur Institute and the Institute of Cancer Research came to an agreement that any commercial development would be a joint venture of the two organizations. Montagnier says, however, that he tested the line for contamination with mycoplasma and it tested positive. He then discarded the line Weiss developed and established LAV in another clone of the CEM line obtained from a different source. The Pasteur Institute maintains that this negated the agreement with the Institute of Cancer Research, but Weiss holds that the agreement covered the whole principle of propagating LAV in CEM lines. This could complicate the dispute over commercial use of blood tests, for both the Pasteur Institute and Genetic Systems are using CEM lines to mass produce LAV.

Shortly before this, Montagnier and Chermann received the news that Gallo was about to publish. Gallo went to the Pasteur Institute to lecture on HTLV-I and HTLV-II, and in a private meeting afterwards told Montagnier and Chermann of his results. "I came back to the United States happy, confident, and feeling one of the best feelings I have had," Gallo recalls.

Within a short time, however, those good feelings were soured by the mounting controversy over patents and priority.—**COLIN NORMAN**