Strong New Candidate for AIDS Agent

A newly discovered member of the human T-cell leukemia virus family is very closely linked to the immunodeficiency disease

In the 3 years since AIDS (acquired immunodeficiency syndrome) was first identified, the cause of the disease has been intensely sought and often just as intensely debated. Most investigators expected that an infectious agent, probably a virus, would turn out to cause AIDS, but this was by no means a universal view. This issue of *Science* contains four reports (beginning on page 497) from Robert Gallo of the National Cancer Institute and his many collaborators that appear to settle the matter.

According to these workers, a newly discovered subgroup of the human T-cell leukemia virus family, designated HTLV-III, is closely linked to the disease. The virus itself has been isolated from more than one-third of patients with full-blown AIDS and from nearly 90 percent of individuals with symptoms indicating that they may have an early form of the disease. Antibodies to HTLV-III have been found in 90 to 100 percent of AIDS patients, a finding that indicates that they have been infected with the agent. "I think that Dr. Gallo has identified the cause of AIDS," says Jerome Groopman of New England Deaconess Hospital and Harvard Medical School, "and I am a very cautious, skeptical person who has been involved with AIDS from the start."

In addition, Luc Montagnier of the Pasteur Institute in Paris and his colleagues have also been finding a virus in patients with AIDS and early AIDS. The Pasteur virus may be the same as HTLV-III, although this has not yet been definitively established.

Identification of the AIDS agent has enormous clinical implications. At the very least, it should be possible to develop a specific assay to identify blood that might be contaminated with the virus. The 10,000 to 15,000 hemophiliacs in this country are at high risk of developing the disease because the clotting factor preparations they must take to prevent uncontrollable bleeding are usually prepared from blood contributed by thousands of donors. According to figures compiled by the Centers for Disease Control in Atlanta, nearly 30 hemophiliacs have developed AIDS.

People who receive whole blood are at much lower risk, but the potential number of people who might be exposed is very large. Some 4 million people are transfused every year. So far, 46 people with no other risk factors have developed AIDS, apparently as a result of transfusions. A means of testing for the AIDS agent would do much to allay fears of contracting a dread disease as a result of a needed medical treatment.

The identification should also help to define the natural history and clinical course of the disease. At present the definition of AIDS is descriptive. It involves, among other things, a finding of severe immune depression, with depletion of helper T cells in particular, in patients for whom no other causes of an immune deficiency, such as cancer or organ transplantation, can be identified. They must also have either life-threatening opportunistic infections or Kaposi's sarcoma, a cancer that was rare and found only in older men of Mediterranean ancestry before it began turning up in AIDS patients. Full-blown AIDS has a high mortality rate. About 1750 of the more than 4000 victims have died.

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But this severe form of AIDS may represent just the tip of the iceberg. Many additional individuals show such symptoms as lymphadenopathy (a condition characterized by swollen lymph nodes, fever, and malaise) and depressed helper T-cell counts without having the devastating illness characteristic of the full-blown disease. These people may be classified as having early AIDS (pre-AIDS as it is sometimes called). About 10 percent eventually develop the more serious symptoms, but at present there is no way to predict whose condition will worsen. Without a definitive test for AIDS it is difficult to tell whether their symptoms are related to AIDS at all. A number of viral infections cause a transient immune suppression that resembles that seen in AIDS.

The test for the HTLV-III antibodies may help to clear up some of these uncertainties. "It is tremendously exciting. To really have the possibility of a diagnostic test is a breakthrough," Groopman says. In some cases the result may alleviate the fears of a high-risk individual who has been having symptoms of AIDS. Groopman cites the case of a homosexual male who showed evidence of immune abnormalities although HTLV-III antibodies could not be detected in his blood. He turned out to have a case of mononucleosis from which he is now recovering.

In contrast, individuals who learn that they have a positive antibody test will hardly be comforted. The ambiguous symptoms make it difficult to differentiate between a mild case and one that will progress. However, as Bijan Safai of Memorial Sloan-Kettering Cancer Center points out, earlier detection of AIDS may have clinical benefits. "If you treat early, you may be able to reverse the immune deficiency."

But there is another problem. An individual who displays no or only mild AIDS symptoms is still capable of harboring the agent and may transmit it to others. Since sexual intercourse both of the heterosexual and homosexual varieties is a major pathway of transmission, an individual who learns that he or she shows signs of infection will face a major dilemma, to say the least.

There may be an enormous demand for the test. Virtually every blood bank will have to examine all donated blood. The high-risk groups in this country include some 15,000 hemophiliacs, an unknown number of users of illicit intravenous drugs, and about half a million Haitians. In addition, there are perhaps 20 million homosexual males, although this figure is only an estimate at best. However, only an unknown percentage of these are highly promiscuous and therefore at high risk.

Identification of the AIDS agent also

opens the way to a vaccine to protect high-risk persons against the disease. "The data in these four reports make a very good case that this virus is associated with the disease," notes Vincent DeVita, director of NCI. "Attempts to develop a vaccine would be the next step." One thing that will have to be learned is whether antibodies to HTLV-III can protect against the development of AIDS. Vaccine testing may begin in 2 to 3 years, according to officials of the Department of Health and Human Services.

HTLV first turned up a few years ago when Gallo's group and that of Yorio Hinuma of Kyoto University in Japan independently isolated a virus from leukemia cells of patients with malignancies affecting the T lymphocytes, which play a major role in immune responses. This virus, now designated HTLV-I, is currently the best candidate for a human cancer virus. The Gallo group also identified a second, much more rare HTLV variant, designated HTLV-II, which is also associated with T-cell malignancies.

The first reports linking HTLV's to AIDS began to appear about a year ago (*Science*, 20 May 1983, p. 806). The Gallo group found either infectious HTLV particles or DNA related to the HTLV genome in T cells from a few AIDS patients. At the same time investigators from the Pasteur Institute in Paris also reported the isolation of an HTLV-like virus from an individual with signs of early AIDS. The Paris isolate, which has not yet been fully characterized, appeared different from HTLV-I and -II. In addition, Max Essex of the Harvard University School of Public Health found antibodies against HTLV-I in about 30 percent of patients with AIDS or early AIDS.

Although these findings were considered intriguing and "a good lead," they did not constitute definitive proof of HTLV involvement in AIDS. For one thing, most of the patients did not show evidence of HTLV infection. For another, a hallmark of AIDS is high susceptibility to infection by opportunistic pathogens that can take advantage of the patient's compromised immune system. The HTLV infections might also be opportunistic—that is, consequences rather than causes of AIDS.

Nevertheless, all during this research Gallo and his colleagues were tantalized by indications of a viral presence in cells from many additional AIDS patients. The indications included electron micrographs showing what appeared to be viral particles, the presence of reverse transcriptase (an enzyme that copies RNA into DNA and is a component of viruses such as HTLV that have RNA as their genetic material), and the ability to infect fresh human lymphocytes by culturing them with the AIDS cells. Infected cells always died, however. "We couldn't characterize these isolates because, unlike the leukemic cells, we couldn't get T cells from AIDS patients to grow," Gallo explains. The investigators were able to tell that the isolates were neither HTLV-I nor -II because they were not detected by monoclonal antibodies against those strains.

The turning point came about 10 months ago. Mikulas Popovic of the Gallo laboratory identified cloned cell lines, derived from human lymphoid leukemia cells, that would continue to grow after infection with the uncharacterized virus. "We were able to mass-produce the virus for the first time," Gallo notes. They at last had enough material to determine the properties of the virus and compare it with HTLV-I and -II. Moreover, they could develop analytic reagents for characterizing all their isolates, which now total 51.

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The newly identified virus has RNA for its genetic material, like the other HTLV's, and also resembles them in size and shape. Its reverse transcriptase has a molecular weight of about 100,000 and requires magnesium ions for its activity, additional points of resemblance to HTLV-I and -II. There are significant differences, however. Jörg Schüpbach of the NCI laboratory has found that the proteins of the newly isolated virus, now called HTLV-III, are similar to those of the other HTLV's, especially HTLV-II, but they are not identical. In addition, Suresh Arya and Flossie Wong-Staal have found that the viral nucleic acids, although showing homology throughout the genome, are different.

The most significant difference between HTLV-III and the other two variants, at least with respect to their potential pathogenicity, involves their effects on cells. According to Gallo, when cultured cells are infected with HTLV-I or -II, a few cells undergo cancerous transformation. This is consistent with the association of these two viruses with the T-cell malignancies. In contrast, HTLV-III does not appear to transform cells in that way, although it does have cell-killing effects.

All three HTLV's specifically infect T cells of the class that includes the helper cells, which, as their name suggests, help other immune cells to carry out their functions. The immune deficiencies of AIDS patients are largely, if not entirely, caused by a severe depletion of helper T cells—such as might be produced by a virus that specifically kills them.

In any event, the Gallo group has now isolated HTLV-III from roughly 85 percent of 21 individuals with early AIDS symptoms and from about 35 percent of patients with the full-blown disease (26 of 72). The latter figure may be lower than it is in actuality. When the investigators calculated the percentage, they used the total of all the AIDS samples sent to them, even though some had deteriorated to the point where they were of questionable value for analysis. In addition, it may be more difficult to detect virus-infected cells in patients with advanced AIDS because the susceptible cell population is so severely depleted in them.

The Gallo group did not detect the virus in blood samples from 115 heterosexual donors. Only 1 of 22 homosexual males who appeared healthy when their blood samples were taken carried the virus. That individual subsequently developed AIDS.

In addition, as described in their *Science* report, M. G. Sarngadharan of Litton Bionetics, Inc., in Kensington, Maryland; Gallo; and their colleagues found antibodies to HTLV-III in nearly 90 percent of AIDS patients (43 of 49) and in about 80 percent of 14 homosexual men with the milder, early symptoms. They also detected the antibodies in 7 of 17 homosexual men with no clinical AIDS symptoms. At least two of these were long-term sex partners of AIDS victims, and all the men were clinic patients, which means that they may not be representative of the total homosexual population.

Only 1 of 186 other controls was positive for the antibodies. The controls included a number of patients with conditions in which the function of the immune system is disrupted. This makes it unlikely that the antibodies are associated with nonspecific immune defects instead of HTLV-III infection.

In another study that was just completed, with the use of an improved method for detecting the HTLV-III antibodies, 100 percent of the AIDS patients were positive. Groopman, who supplied samples for this study, says, "The assay

picked out 100 percent of the AIDS and lymphadenopathy syndrome [pre-AIDS] patients. There were no false positives and no false negatives. I can tell you it was remarkable." The study was conducted under double-blind conditions. The investigators did not know which samples were which until the antibody analyses were completed and the code was broken on 10 April.

The antibodies detected in the blood of the AIDS patients are directed primarily against the major protein forming the outer envelope of the HTLV-III particle. These antibodies are apparently capable of cross-reacting to some extent with the envelope protein of HTLV-I, which probably explains why roughly one-third of AIDS patients were positive in the earlier study by the Essex group. The antigen actually detected by the Essex assay is a membrane protein, which has a molecular weight of roughly 60,000 and is found on the surfaces of cells infected with HTLV-I. Recent work by the Essex and Gallo groups has shown that this is the precursor of the 46,000-dalton envelope protein of HTLV-1.

Meanwhile, the Pasteur workers have now made nearly a dozen isolates of their virus, which they call lymphadenopathy-associated virus (LAV), from AIDS and pre-AIDS patients. Using samples supplied by the CDC, they have also detected antibodies to LAV in blood from about 90 percent of U.S. AIDS and pre-AIDS patients.

LAV, like the HTLV's, has RNA as its genetic material. It appears to infect the same subpopulation of T cells as the HTLV's. The presumption is that LAV will turn out to be the same as HTLV-III. Gallo plans to collaborate with the Pasteur group to determine whether that is the case. Gallo notes, incidentally, that HTLV-III does not appear to be closely related to the virus that has recently been identified as the cause of an AIDS-like disease of monkeys.

According to Gallo, the nucleic acid studies of HTLV-III suggest that the virus may not be new, as has been speculated. AIDS was just identified in 1981. The nucleic acid data show that the HTLV-III RNA is similar throughout the genome to the RNA's of HTLV-I and -II. "It looks as though there is some kind of common ancestor," Gallo says. "It has probably existed for a long time." The possibility remains, however, that HTLV-III underwent some recent subtle change.

Gallo speculates that the HTLV's originated in Africa. HTLV-I has been detected in Old World monkeys, but not in New World monkeys. AIDS may have emerged only recently as a result of population shifts from rural areas of Africa to the cities where there would be greater chances of contact with foreign visitors who could have carried the agent to new locales, such as the United States or Haiti, which also has a relatively high incidence of the disease. Alternatively, the virus might have been exported directly by an emigrating African. Many of the AIDS cases identified in Europe have links to Central Africa and the condition has been found there as well.

Traditionally, final proof that a particular agent causes a disease usually involves showing that Koch's postulates can be met. One of the postulates requires that the host be injected with the agent to see whether the disease develops. With an illness as deadly as AIDS this will never be possible with human subjects. But showing that HTLV-III can be used to produce an effective vaccine would go a long way to removing whatever doubts might remain about whether it is the AIDS agent.

—JEAN L. MARX

Ancestors Worshiped

Paleoanthropologists have been discussing their agreements and disagreements in the presence of most of the world's hominid fossils

The culmination of 4 years of ambitious planning, wildly fluctuating enthusiasm and, it must be said, occasional dread, the American Museum of Natural History, New York, has finally staged its Ancestors exhibit. It is without doubt an extraordinary event: unprecedented and probably unrepeatable too. Some 40 fossils—original fossils note, *not* casts—representing a great proportion of the most significant evidence of human evolution are to be seen through bullet-proof display panels in the museum's number one gallery.

"There has not been a more significant event in the museum's past 30 years," said its director, Thomas Nicolson, a few days before the exhibit finally opened to the public. Confronting the anticipated creationist reaction to the exhibit, Nicolson added: "This is a statement about science, an active statement about evolution. We don't seek controversy but we often find ourselves in the midst of it. We insist on the right to learn and on the primacy of the material evidence."

Some 10 days before the exhibit opened, and while final adjustments were being made to the support structures in the display cabinets with high-quality casts serving as temporary surrogates for the real things, the material evidence itself was spread out over five or six tables in an upper room and subjected to treatment that would make even the most hardened fossil jock wince. During a series of study sessions paleoanthropologists from around the world pored over these fragile and priceless relics, comparing, probing, contrasting, disputing—frequently disputing—and sometimes, believe it or not, agreeing.

Although the initial sessions were marked by a palpable tension and unexpressed awe—"It was," says Michael Day of St. Thomas' Hospital Medical School, London, "like discussing theology in a cathedral"—the atmosphere grew more and more relaxed as time went on. The original strict rules of handling and movement about the room, which had been instituted to minimize risk to the fossils, began to break down. Neanderthals made first aquaintance with australopithecines, Miocene apes with *Homo erectus*, and so on: taxonomic, temporal and geographical boundaries were shattered. And the paleoanthropologists occasionally broke ranks too.

Curators held their collective breaths at first as their fragile charges were variously held aloft, angled to the light, and passed from hand to hand, not always with the relative safety of a padded tabletop below. But nothing—repeat, nothing—was broken. "That was a near miracle," observed Milford Wolpoff of the University of Michigan, a man whose frequent global expeditions has brought him into contact with more original fos-