BACKGROUNDER

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NCI AIDS RESEARCH

In FY 1985, the National Cancer Institute (NCI) made progress in many areas of AIDS research. These included advances in understanding the molecular biology of human T-cell lymphotropic virus-III (HTLV-III), the causative agent of AIDS; toward developing a vaccine and an animal model for the disease; in understanding the natural history and transmission of AIDS; and in identifying potential new therapies for the disease.

Molecular Biology

HTLV-III was molecularly cloned and characterized. This enabled scientists to compare closely and study the different isolates of HTLV-III and to make direct comparisons of its sequence with that of other retroviruses. This was accomplished by investigators in the Laboratory of Tumor Cell Biology (LTCB), headed by Dr. Robert Gallo, who discovered HTLV-III.

The entire genetic structure (genome) of the virus was mapped. From analyses of the genomes of HTLV-III and other retroviruses, scientists confirmed that the AIDS virus is a new virus in humans. With this map, or nucleotide sequence, researchers are now able to reproduce and analyze separately any structural part of the viral genome by molecular cloning. This knowledge has greatly facilitated direct comparison of different viral isolates.

This research is an excellent example of cooperation among government, university and industry research groups. The work was initiated by Dr. Gallo and Dr. Flossie

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Wong-Staal of LTCB and was conducted in collaboration with four other groups: Harvard Medical School and Dana-Farber Cancer Institute, Boston, in the laboratory of Dr. William Haseltine; E.I. duPont de Nemours, Wilmington, Delaware, under the direction of Dr. Mark Pearson; NCI's Laboratory of Molecular Oncology, under the direction of Dr. Takis Papas; and Centocor, Malvern, Pennsylvania, under the direction of Dr. Nancy Chang.

Scientists found the first direct evidence that HTLV-III can by itself destroy the white blood cells (helper T cells) that are the primary target of AIDS. This study proved that the virus does not necessarily need interaction with other disease "cofactors" to destroy these immune system cells. This research was conducted by Dr. Amanda Fisher and colleagues of LTCB.

Scientists also learned that the HTLV family* of retroviruses possesses a unique strategy for usurping the host cell's cellular machinery. This represents an entirely new type of regulatory mechanism for activating cells to produce more virus. The retroviruses produce a protein from a gene, called the tat gene, that turns on viral genes and possibly normal cellular genes at widely distant sites in the chromosomes of an infected cell. This phenomenon is called trans-acting transcription (tat) regulation. Identification of the tat gene of HTLV-III and its unique splicing mechanism was made by Dr. Suresh Arya and colleagues of LTCB.

In related research, NCI-funded investigator Dr. William Haseltine and colleagues at the Dana-Farber Cancer Institute and Harvard Medical School discovered that bovine leukemia virus (BLV), a retrovirus that causes leukemia in cattle, has the tat gene. They also found that HTLV-III exhibits tat activation at a very high level. This may account at

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* The family of retroviruses known as HTLV, human T-cell lymphotropic viruses, comprises HTLV-I, which is strongly associated with an adult form of leukemia-lymphoma; HTLV-II, which has not been linked to any disease; and HTLV-III, the cause of AIDS.

least in part for HTLV-III's virulent cell-killing activity, in contrast to the abnormal cell proliferation that occurs with HTLV-I and -II and BLV. However, whether tat is an integral part of HTLV-III's cell-killing activity is not yet determined.

In other research, Dr. Jay Hess and colleagues at The Johns Hopkins University School of Medicine in Baltimore found the tat gene in the ungulate lentiviruses, a family of retroviruses that infects hoofed animals and causes chronic disease. Taken together, these findings of a common tat gene and other features common to HTLV-I, -II, -III, BLV, and the lentiviruses suggest that these retroviruses may share a common ancestoral origin. This research was supported by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).

Understanding the group of retroviruses to which HTLV-III belongs and its origin is important in order for scientists to understand how HTLV-III causes disease.

The genetic structure of HTLV-III shows greater similarity to the lentivirus family than to HTLV-I and -II or BLV, which suggests that HTLV-III may be a human lentivirus. Dr. Matthew A. Gonda of Program Resources, Inc., at NCI's Frederick Cancer Research Facility, found that HTLV-III and visna virus, a pathogenic lentivirus that causes a chronic degenerative neurologic disease in sheep, are structurally and genetically very similar. In fact, these two retroviruses are more closely related than HTLV-III is to HTLV-I or -II. HTLV-III causes neurological disease in AIDS patients, as does the visna virus in sheep. This research was done in collaboration with The Johns Hopkins University School of Medicine and LTCB.

Dr. Ing-Ming Chiu and colleagues in Dr. Stuart Aaronson's Laboratory of Cellular and Molecular Biology, NCI, found, by direct nucleotide sequence comparisons, that HTLV-III also is similar in its genetic structure to the lentiviruses caprine (goat) arthritis encephalitis virus and equine infectious anemia virus. These and other similarities lend support to the belief that they share a common origin. HTLV-III and the lentiviruses both kill cells in laboratory cultures and both infect brain cells.

Some research suggests that HTLV-III may have originated in Africa. In laboratory tests by Dr. Carl Saxinger of LTCB, a virus related to, or identical to, HTLV-III was detected in serum samples collected in Uganda in 1972 and 1973. This suggests that the virus may be a predecessor of HTLV-III, or is HTLV-III, and that AIDS may have originated in Africa. The study was done in collaboration with the International Agency for Research on Cancer and the Laboratory of Epidemiology and Immunovirology of Cancer in Lyon, France, and Bispebjerg Hospital in Copenhagen.

Vaccine Development

Neutralizing antibodies, or protective antibodies, have been detected in some people who had been exposed to HTLV-III. The antibody's existence shows that our bodies are able to respond immunologically to HTLV-III in a way that may lead to new prevention and treatment approaches. These findings were reported by Dr. Marjorie Robert-Guroff and colleagues at LTCB and separately by NCI-funded investigator Dr. Martin Hirsch and colleagues at Massachusetts General Hospital in Boston.

Looking at the different isolates of HTLV-III, scientists found extensive diversity in their genetic structure, particularly in their envelope genes. The protein of the virus' outer coat produced by the envelope gene is the key protein that elicits neutralizing antibodies in some infected individuals. Consequently, variations in the envelope may mean that antibodies developed against one HTLV-III variant would not protect against a wide spectrum of other variants, perhaps making development of an effective vaccine for AIDS difficult. This research was conducted by Dr. Wong-Staal and colleagues at LTCB, Bionetics Research, Inc., in Kensington, Maryland, and Walter Reed Army Institute of Research in Washington, D.C. The lentiviruses, including the visna virus, also have variations in their envelope proteins and manage to escape their host's immune defense by "genetic drift" in their envelope proteins.

Several glycoproteins--compounds composed of a protein and a carbohydrate--of HTLV-III were identified that appear to be the major targets of antibodies produced by

the body as an immune response to the virus. These glycoproteins, gp160 which is processed into gp120 and gp41, are believed to be the major ones produced by the envelope gene of HTLV-III. Dr. M.G. Sarngadharan of Bionetics Research, Inc., made a monoclonal antibody to detect gp41 and gp160. Using this monoclonal antibody, Dr. Max Essex of Harvard University School of Public Health and colleagues, with NCI-funding, detected gp120 as well as gp160. Dr. Essex's research was done in collaboration with Harvard's Dana-Farber Cancer Center and the National Institute of Allergy and Infectious Diseases (NIAID).

In another study, Dr. Essex found that gp160 and 120 are the major target antigens for antibodies from patients with AIDS, ARC, and some healthy homosexual men. This suggests that detection of antibodies specifically against these glycoproteins may serve as a confirmatory test for the presence or absence of antibodies to HTLV-III in human serum samples. This NCI-funded study was done in collaboration with New England Deaconess Hospital, Boston.

Subunits of HTLV-III are being studied in various combinations, including gp120 and gp41, to see if they will stimulate a protective antibody response in animals infected with HTLV-III. Artificial membrane matrices that anchor the viral subunits have been developed. These "immune stimulating complexes," or ISCOMS, have elicited antibody responses in mice, guinea pigs, rabbits, and rhesus monkeys. Preliminary studies indicate that, in some rhesus monkeys, low levels of HTLV-III antibodies have been observed. Other variants of HTLV-III are now being mass produced in order to see if similar results can be obtained from different isolates, or if different isolates would have to be represented in a vaccine. This research is under way at NCI's Frederick Cancer Research Facility.

Dr. Raoul Benveniste of NCI's Laboratory of Viral Carcinogenesis, in collaboration with scientists at the University of Washington Regional Primate Research Center in Seattle, is studying the transmission of simian AIDS (SAIDS), an AIDS-like syndrome seen

in monkeys. They have successfully infected rhesus monkeys with a virus isolated from a tumor, called retroperitoneal fibromatosis, that occurs in SAIDS. The proteins of this virus have been purified. One of them, an envelope glycoprotein, has been shown to be neutralizing when tested in the laboratory. Studies are under way to develop vaccines with this viral protein.

Other isolates of a virus that is very similar to HTLV-III, called STLV-III (simian T-cell lymphotropic virus) have been obtained, analyzed, and successfully used to infect rhesus monkeys at Harvard's New England Regional Primate Research Center in Southborough, Massachusetts. The identification of such a virus in animals and the ability to transmit it is an important step toward development of an animal model for AIDS. These studies were done by Dr. Ronald Desrosiers and colleagues at the primate center in collaboration with Dr. Essex and colleagues at the Harvard School of Public Health, with support from NCI, NIAID, the Division of Research Resources of NIH, and the Massachusetts Department of Public Health.

NCI sponsored a workshop on vaccine approaches for retrovirus infections in December 1984 and issued a Request for Applications for research in this area for funding in FY 1986.

Epidemiology

To determine the natural history of AIDS, Dr. James Goedert of EEB and colleagues are following prospectively five populations at high risk for AIDS. In these groups, from 4 to 20 percent of individuals infected with HTLV-III and followed for 32 months were diagnosed with AIDS. When the study began in 1982, the highest infection rate for HTLV-III, 54 percent, was seen among homosexual men from New York City. By mid-1985, 20 percent of those who were infected had developed AIDS, and an additional 25 percent had developed lesser clinical manifestations of immune deficiency. In the other groups, the proportion of those infected with HTLV-III and who subsequently developed AIDS was much lower: 12 percent of homosexual men in Washington, D.C.; 4

percent of IV drug abusers in New York City; 8 percent of homosexual men in Denmark; and 7 percent of hemophilia-A patients in Pennsylvania. The scientists speculate that the higher rate of developing AIDS seen among the New York homosexual men most likely reflects the longer duration of infection in the New York group.

Another AIDS natural history study was conducted by Dr. Elaine Eyster of The Milton S. Hershey Medical Center of The Pennsylvania State University, in collaboration with Dr. Goedert. They found among a group of hemophiliacs that all those infected with HTLV-III for more than three years had lymphadenopathy (persistent, swollen lymph glands), lower helper T-cell counts (an immunologic abnormality characteristic of AIDS), or both. At least two years lapsed between HTLV-III infection and manifestation of AIDS-like symptoms.

The national network of NCI-funded population-based tumor registries was used to monitor the occurrence of cancers that may be associated with AIDS. Kaposi's sarcoma, a rare type of cancer that originates in blood vessel walls, occurs in AIDS patients, and some reports have suggested that other cancers may be associated with the disorder. Because information on homosexuality is not available from the registries, Dr. Robert Biggar of EEB and other NCI scientists analyzed data on never-married men 20 to 49 years old from the San Francisco-Oakland area registry, and found a 50-fold increase in the occurrence of Kaposi's sarcoma between 1973-1980 and 1981-1982, and a ninefold increase in Burkitt's-like lymphomas. These increases coincide with emergence of the AIDS epidemic. No increases were seen for other cancers that have been suggested as AIDS-related, but it is possible that there may be a longer delay for development of other cancers, and they may appear in the future.

Because Kaposi's sarcoma has been endemic in some areas of Africa for years, Dr. Luc Kestens of the Institute of Tropical Medicine, Belgium, in collaboration with Dr. Biggar, studied the occurrence of Kaposi's sarcoma in eastern Zaire. They concluded that in this region the classical form of the cancer is not associated with HTLV-III infection or with immunosuppression.

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Studying transmission of the virus, Dr. Goedert found that homosexual men from Washington, D.C., who had sexual contact with men from high-risk areas for AIDS had lower helper T-cell counts than Washington homosexuals who did not. A strong, consistent association was also seen between the number of sexual contacts with men from high-risk areas and the extent to which helper T cells were reduced. This suggests that frequent male homosexual contact with residents of areas where AIDS is common may be an important means of spread of the causal agent. Washington, D.C., was a relatively low-risk area for AIDS, but the risk has subsequently increased. The laboratory tests were performed under the direction of Dr. Dean Mann, Chief, Biochemical Epidemiology Section, NCI, through a contract with the Uniformed Services University of Health Sciences, Bethesda.

Studies of health care workers and of household contacts of persons with HTLV-III infection demonstrated that this virus is seldom transmitted through non-sexual contact, even when someone is stuck with a contaminated needle. In a study of 361 health care workers from medical institutions that care for AIDS patients, only three cases of HTLV-III infection were reported, all of whom were accidentally stuck with sharp instruments that were clearly or probably contaminated with the blood of AIDS patients. This research was conducted by Dr. Stanley Weiss of EEB and other NCI scientists in collaboration with the National Institute on Drug Abuse; Downstate Medical Center, Brooklyn; St. Luke's-Roosevelt Hospital Center, New York; and New England Deaconess Hospital.

Dr. Martin Hirsch of the Massachusetts General Hospital and colleagues studied 85 employees who had been stuck by infected needles or had repeated exposures to specimens from AIDS patients over a 3-year period. None of the hospital workers showed evidence of HTLV-III infection, according to preliminary findings. The Hirsch and Weiss

studies suggest that isolation procedures currently recommended for AIDS are adequate and reaffirm the importance of following the Public Health Service's guidelines on general hospital and laboratory precautions for AIDS.

In two studies of members of households of hemophiliacs, Dr. Goedert, Dr. Mads Melbye of the Institute of Cancer Research and Blood Bank, Aarhus, Denmark, and colleagues found no evidence of infection among household members other than sexual partners of hemophiliacs who had been infected with HTLV-III.

In other research, Dr. Michael Marmor of New York University looked at cytomegalovirus (CMV), a type of herpesvirus, as a possible co-factor for development of Kaposi's sarcoma associated with AIDS. For unknown reasons, Kaposi's sarcoma associated with AIDS has been largely confined to homosexual men. CMV infection is prevalent among homosexual men and, therefore, is difficult to study in this group. Dr. Marmor found an association between frequency of CMV infection and Kaposi's sarcoma among three AIDS risk groups: frequency of CMV infection and Kaposi's sarcoma was lowest among male drug abusers, intermediate in female drug abusers, and highest among homosexual men. Further study will be needed to clarify this association.

Treatment

Major emphasis was placed on identifying and testing antiviral agents that may be effective against HTLV-III. NCI's Clinical Oncology Program (COP), led by Dr. Samuel Broder, conducted a small feasibility study with AIDS patients to test the efficacy of suramin as a therapy. All retroviruses require a unique viral enzyme called reverse transcriptase to replicate, and Dr. Broder had found that suramin blocked HTLV-III replication in vitro.

After the NCI study found that suramin could be administered safely to AIDS patients and that it had clinical activity against HTLV-III, NCI- and NIAID-funded clinical trials were begun at six institutions: Beth Israel Hospital in New York City; New England Deaconess Hospital, Boston; M.D. Anderson Hospital and Tumor Institute,

Houston; University of California at Los Angeles; University of California at San Francisco; and the University of Southern California at Los Angeles.

The primary focus of new drug development within COP (the intramural program) is to identify agents that can be absorbed orally--because long-term administration of antiviral therapy is considered to be likely if a successful treatment for HTLV-III infection is discovered--and that also can penetrate across the blood-brain barrier. Many AIDS patients have unexplained dementia or degenerative brain disease. Dr. George Shaw of LTCB showed that this is caused by HTLV-III when he discovered evidence of HTLV-III infection in the central nervous system of AIDS patients. He has subsequently been able to visualize the viral genes in brain cells. These findings have major implications for treatment of AIDS because they indicate that the brain is a sanctuary for HTLV-III and that effective therapies must pass the blood-brain barrier. This study was done with scientists from the National Institute of Neurological and Communicative Disorders and Stroke, Cornell University Medical College in New York City, New England Deaconess Hospital, and the University of Medicine and Dentistry of New Jersey, Newark.

Preliminary feasiblity studies at the NIH Clinical Center using azidothymidine, or Compound S, have proven that this agent can be absorbed orally and that it does cross the blood-brain barrier. In addition, several (but not all) patients who are now receiving an intermediate dose of the drug are showing evidence of improvement in the numbers and function of the T cells that appear damaged in AIDS. In some instances, there also is an improvement of clinical status as measured by such factors as weight gain, lessening of fever and night sweats, and extent of mouth ulcers. It is too early to draw any conclusions about safety and efficacy of this agent. However, the results to date suggest that it might be reasonable to initiate wide-scale experimental therapy with the agent at several medical centers around the country to define what role, if any, the agent might have in the therapy of AIDS and its related conditions.

Azidothymidine was developed and provided to COP by Burroughs Wellcome in Research Triangle Park, North Carolina. Dr. Broder's laboratory tested it and found it active against HTLV-III in February 1985. The Food and Drug Administration approved it for use in patients in June 1985, and small pilot studies were then begun at the NIH Clinical Center and at Duke University. This demonstrates the cooperation that is taking place to find effective therapies for AIDS. The assays used to screen drugs for HTLV-III inhibition in vitro were developed by LTCB and COP.

Other therapies are also being tested at the NIH Clinical Center under COP and at medical institutions around the country. These include anticancer drugs and immunorestorative agents such as interferon--a natural antiviral agent--and interleukin-2 (IL-2), a protein that plays a key role in immune response. However, no therapy has been discovered yet that completely corrects the underlying immune defect that characterizes AIDS.

To better define the extent of immunologic dysfunction seen in AIDS patients, NCI-funded investigator Dr. Charles Kirkpatrick of the National Jewish Hospital and Research Center, Denver, found that AIDS patients with opportunistic infections and those with both opportunistic infections and Kaposi's sarcoma failed to produce IL-2. However, AIDS patients who had only Kaposi's sarcoma produced IL-2. Because IL-2 mediates production of interferon, inability to produce IL-2 could be an additional defect that predisposes AIDS patients to opportunistic infections. Although preliminary results of therapy for AIDS with IL-2 have not been encouraging, Dr. Kirkpatrick suggests that measurement of IL-2 may be important for selecting patient subgroups for treatment with it.

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