

received a biopsy from another young gay male patient (MOI), who was infected with both HTLV and the new lymphadenopathy-associated virus. If MOI had been our first patient, we would have been very confused.

A few months later, I received a blood sample from a young hemophiliac (LOI) with full-blown AIDS, and blood and lymph node samples from a young gay man (LAI) with advanced Kaposi's sarcoma. The LAI virus could be isolated from the patient's blood cells and grew very quickly in the patient's cultured T lymphocytes, killing them as well as killing T lymphocytes from blood donors. In September, we isolated a similar virus from the blood of a Zairian woman, ELI, who died of AIDS a week later. All of the isolated viruses showed cross-reactivity between their gag proteins (p25 and p18) (5). The viruses isolated from full-blown AIDS patients were more aggressive than the BRU virus, and so I called them immune deficiency-associated viruses (IDAV). The viruses like BRU that were isolated from patients who only suffered from lymphadenopathy were termed lymphadenopathy-associated viruses, or LAV. This classification corresponded to the later terminology of syncytium and nonsyncytium-inducing strains.

The retrovirus was new, as was the disease. My collaborator, the electron microscopist Charles Dauguet, showed me pictures of the viral particles whose dark, cone-shaped centers suggested that this virus was not the same as HTLV. Fellow virologist Edwald Edlinger suggested that I compare the new virus with animal lentiviruses, and, indeed, the pictures of viral particles we obtained in June 1983 looked identical! As I told Robert Gallo, I was convinced that we were dealing with a virus quite different from the HTLV family.

To better characterize the new virus, we tried (unsuccessfully) to grow the BRU isolate in different T cell lines. If we had tried the LAI isolate instead, we would have been able to grow the virus without any trouble. In October 1983, we were finally able to grow the BRU isolate in Epstein-Barr virus-transformed B cell lines, although we discovered later that the LAI virus had contaminated our BRU culture (6). At least six laboratories received the LAI sample (under the name BRU) from our group and experienced the same contamination. We think that the LAI virus readily contaminated the BRU culture because it associates with a mycoplasma species, *Mycoplasma pirum*, usually present in T cell lines. This physical association makes a fraction of the LAI virus highly infectious, and, in fact, this fraction can be neutralized with antibodies against *M. pirum*. As mycoplasmas are

common contaminants of cultured cells, an infectious pseudotype virus (LAI associated with *M. pirum*) may have caused several contaminations between 1983 and 1984 in different laboratories.

New evidence that this strange retrovirus was the cause of AIDS came from our team in the fall of 1983 and the winter of 1984 (7). We observed a high frequency of antibodies against the virus in lymphadenopathy patients, and noted the favored tropism of this virus for CD4⁺ T lymphocytes. Our results were still controversial, however, and we had difficulty in obtaining the funding needed to better characterize the virus and to develop a blood test. The tide only turned in France when Robert Gallo and his group in the United States made a similar discovery. In the spring of 1984, Gallo published more convincing evidence that HIV causes AIDS (8) (see the Viewpoint by Gallo on page 1728), a finding that was confirmed by Jay Levy's group (9). In 1985 came the cloning and sequencing of the HIV genome with identification of new open reading frames specific for lentiviruses (10). This was followed by identification of the HIV large surface glycoprotein (11) and of T cell CD4 as the receptor for HIV (12, 13). In 1986, HIV-2 was isolated from West African patients (14).

Over the past 20 years, the scientific and legal controversies between our team and Gallo's group have faded. We are left with the salient fact that HIV was identified and shown to be the cause of AIDS less than 2 1/2 years after this disease was first identified. It took only another 2 years for blood tests to become commercially available, reducing almost to zero the transmission of AIDS through blood transfusion in devel-

oped countries. In 1987, the first anti-HIV drug, AZT, which blocks HIV RT activity, was introduced. With the arrival of the HIV protease inhibitors and triple drug therapy in 1995, many patients are alive today who would otherwise have died.

But we must not be complacent—the task ahead is immense. We still do not understand the origin of the AIDS epidemic; the slow destruction of the immune system by factors in addition to HIV infection of CD4⁺ T cells; the importance of cofactors in AIDS progression and virus transmission; and the nature of the HIV reservoir that resists triple drug therapy. The next wave of advances in the fight against this worldwide scourge will require the contribution and energy of us all.

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VIEWPOINT: HISTORICAL ESSAY

The Early Years of HIV/AIDS

Robert C. Gallo

Animal retroviruses were among the earliest viruses discovered, and by the 1960s some were shown to cause cancer. These findings prompted the formation of the U.S. Virus Cancer Program, which aimed to identify human tumor viruses, especially human retroviruses. By the late 1970s, however, a mistaken consensus emerged that viruses did not cause human cancer and that human retroviruses did not exist, leading to termination of the program. Even more perplexing was the assertion that serious epidemic diseases were limited to the “Third

World,” culminating in the closure of certain U.S. medical school microbiology departments and a disturbing lack of support for the U.S. Centers for Disease Control and Prevention (CDC). In the midst of this complacency, my co-workers and I made human retroviruses one of our primary research objectives. We were interested in leukemia and began to characterize the DNA polymerases in blood cells (1, 2). Howard Temin had proposed that retroviruses replicate through an integrated DNA intermediate, a notion supported by his discovery with David Baltimore of a retroviral reverse transcriptase (RT).

This discovery provided me with an entry point into the field because RT is a DNA polymerase. We developed sensitive assays to de-

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fect RT in order to search for retroviruses at low levels in cell supernatants, membrane preparations, and long-term cell cultures. In 1972, we reported purification of an RT from human lymphocytic leukemia cells. We realized that to detect the human retrovirus producing the RT, we would need to culture human blood cells for long periods and in greater numbers than possible with the available system of colony growth on agar. So, we began to analyze conditioned medium from various cell types, including activated T cells, for the presence of growth factors. We found a growth factor that promoted myeloid cell growth, enabling establishment of HL-60, the first human granulocytic cell line. Next, with Doris Morgan, our group discovered interleukin-2 (IL-2), which we called T cell growth (mitogenic) factor (3–5). This enabled my colleagues and I together with my postdoctoral fellow Bernard Poiesz to isolate the first human retrovirus, human T cell leukemia virus-1 (HTLV-1), in 1979 from a patient with a T cell malignancy (6–8). Independent work the following year from several Japanese groups further documented that HTLV-1 caused a very specific adult T cell leukemia endemic to Japan. HTLV-1 targets CD4⁺ T cells; it is transmitted from mother to child, and through blood and sexual contact (9–11). This human retrovirus is prevalent in parts of Africa, is closely related to Old World primate leukemia viruses, and causes minor immune impairment. In 1982, we reported the isolation of the second human retrovirus, HTLV-2, from a case of hairy cell leukemia of the T cell type (12). The characteristics of HTLV-1 and HTLV-2 foreshadowed the discovery of an even more sinister human retrovirus.

I first heard about AIDS in 1981 from newspaper reports but more informatively from lectures given by Jim Curran of the CDC, who challenged the audience, asking “where are the virologists?” Theories of the cause of AIDS abounded, but Curran was already thinking of an infectious etiology, most likely a new virus (13). Max Essex reminded us that the feline leukemia retrovirus not only causes leukemia, but that its variants could also cause immune disorders. We knew that the risks for HTLV-1 infection included blood exposure, sexual contact, and birth to a mother with the disease, and also that HTLV-1 targeted CD4⁺ T cells. The same risk factors were described for AIDS, and combined with clinical evidence that CD4⁺ T cells were abnormal in AIDS patients and epidemiological hints that AIDS may have originated in equatorial Africa, this led us to propose that AIDS might be caused by a new retrovirus of the HTLV family. In May 1982, using protocols similar to those for isolating HTLV-1, we tested blood cells from AIDS patients for cross-reactivity with HTLV proteins and for HTLV-like DNA

sequences. But by early 1983, we had found HTLV-related DNA sequences in only 2 of 33 AIDS patients and had obtained virus isolates with equal infrequency.

It was in 1973 that I first met Luc Montagnier of the Pasteur Institute (14). In January 1983, Montagnier and his colleague Jean-Claude Chermann were beginning to study blood cell cultures from patients with suspected AIDS. They told me of their first positive result: the culturing of a virus from the peripheral blood cells of a patient with lymphadenopathy. They were able to identify the virus as a new human retrovirus, but were unable to characterize it in detail. Essex and I suggested to Montagnier and Chermann that we submit our findings jointly, and three reports from the two groups were published in May 1983. Montagnier and Chermann had not named the virus, but later called it LAV (lymphadenopathy-associated virus, isolated from patient BRU).

There was still another “curve ball” to come. In February 1983, a clinician (Jacques Leibowitch) arrived from Paris with cell samples from AIDS patients. One of these samples came from a man (CC) who had received blood transfusions in Haiti. My co-worker Mika Popovic succeeded in growing CD4⁺ T cells from the sample. These T cells were highly positive for RT, and electron microscopy revealed that they contained two viral forms, which we called “mature” and “aberrant,” believing that they were from the same virus. The virus from these T cells cross-reacted with antibodies to HTLV core proteins, yet unlike HTLV, it killed target T cells. Using more sophisticated methods, we quickly discovered that these T cells contained two distinct retroviruses: HTLV-1 and the aberrant form, later defined as HIV. We had assumed that we could only find HTLV-like viruses in 5 to 10% of our AIDS patients because our assays were not sensitive enough, and had not considered the possibility that our HTLV-positive cells were in fact infected with two separate retroviruses.

In our May 1983 paper, we had not separated and adequately cultured a retrovirus that was free of HTLV. Thus, the paper by the Montagnier/Chermann group is unequivocally the first reported true isolation of HIV from a patient with lymphadenopathy. However, the cause of AIDS was still unknown. By the summer of 1983, our group had obtained evidence for a retrovirus related to HTLV in many patients with AIDS and pre-AIDS. With a more detailed molecular analysis of the virus from patient CC, we concluded that the HTLV-positive results in samples from 5 to 10% of AIDS patients were due to a double infection with HTLV and a new human retrovirus. Moreover, the early 1983 experience with sample CC proved that the new retrovirus could be

grown in continuous culture (something that Montagnier and Chermann believed impossible because, even to this day, their LAV/BRU virus cannot be cultured).

In late 1983, Popovic and my technician Betsy Reed-Connole had a second breakthrough: They grew several viral isolates in CD4⁺ T cells in continuous culture. Several of these viral isolates—RF (1983), IIIB (1983), and MN (early 1984)—became standard tools for AIDS researchers and crucially enabled development of a blood test. In March 1984, we submitted four papers to *Science* (15) and shortly thereafter one to *Lancet* (16). In these papers, we described isolates of the new retrovirus, methods for its continuous production, analyses of its proteins, and evidence that it was the cause of AIDS. The rapid development of a blood test not only safeguarded the blood supply, but also allowed public health officials to follow the course of the disease in infected individuals before they developed full-blown AIDS. The blood test also yielded a grim vision of the future—although sera from hemophiliacs in Japan all tested negative in early 1984, by the end of that year, 20% of the sera were positive for HIV because the hemophiliacs had been treated with HIV-tainted blood products from the United States.

We visited Montagnier in Paris and gave him our cell line continuously producing HIV (called HTLV-IIIB/H9), so that he could compare it with his LAV/BRU isolate. We agreed to a joint press conference if our IIIB retrovirus turned out to be the same as their LAV/BRU isolate. However, a leak from a freelance journalist prompted Margaret Heckler, secretary of the U.S. Department of Health and Human Services (DHSS), to call an urgent press conference to which I was summoned home to attend, and from which, very regrettably, the French group was excluded.

The scientific achievements were overshadowed by a dispute between the United States and France over the patent rights to the blood test, and a temporary disagreement among the scientists. Although patents were not common at the National Institutes of Health back then, we were instructed by DHSS officials to patent the blood test so that pharmaceutical companies would be able to rapidly deploy the test worldwide. Because it grew so well in T cell lines, we selected the IIIB isolate to develop the blood test. The dispute over the origin of this isolate became sensationalized. In fact, our IIIB isolate was accidentally contaminated with a sample sent to us by Montagnier. This HIV strain (IIIB/LAI) later contaminated the cultures of several other laboratories (17).

Years later, we learnt that the same HIV strain had earlier contaminated viral isolates

of the French group. Montagnier and Chermann did not realize that virus from a patient called LAI had contaminated their LAV/BRU isolate. Although Montagnier believed he was sending LAV/BRU to us—and so did we—one culture consisted predominantly of LAI. The properties of LAI are very different from those of LAV/BRU, which does not grow in cell lines. Compounding the complexity, although IIB was clearly derived from LAI, it is not identical with LAI, but rather is a variant that grows vigorously because of mutations in some of its regulatory genes. All of this was acknowledged by our group and the French group in 1991 (18) (see the Viewpoint by Montagnier, on page 1727).

The period after the May 1984 publication of our papers was marked by rapid advances (15, 19). The HIV-1 genome was sequenced, HIV antigenic variation was discovered, the virus was found in the brain of AIDS patients, genomic sequence variation was found in viral populations from the same patient, macrophages were found to be targets for HIV, various modes of HIV transmission were elucidated, all of HIV's genes

and most of its proteins were defined, and the blood supply in most developed nations was rendered safe as a result of screening for HIV. Next, came identification of the HIV receptor (CD4), the discovery of SIV in chimps, and the development of the first anti-HIV drug, AZT.

The late Jonathan Mann heralded the years 1982 to 1985 as a period of intense discovery, noting that the pace of research was the fastest in medical history. For some scientists, these were also years of disquiet and frustration; years in which we would encounter in an unprecedented manner the negative face of politics, the media, patient activists, and legal issues. For myself and others trained in science and disciplined by the rigor and analysis that are the essence of scientific endeavor, the rough and tumble of the outside world provided harsh and bitter lessons. In retrospect, it is clear that these lessons needed to be learnt, and I can say we are better for the experience. But our job is far from over, and it is up to the scientists to ensure eradication of the AIDS epidemic that continues to rage in many regions of the world.

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VIEWPOINT: HISTORICAL ESSAY

Prospects for the Future

Robert C. Gallo and Luc Montagnier

With close to 70 million people already infected with HIV and more than 20 million dead, AIDS is one of the greatest pandemics in medical history. Not only is this a human tragedy of

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unimaginable dimensions, it is also a threat to world security because of the potential for political destabilization. The AIDS epidemic must be halted soon. We need a policy of prevention that can be adapted to the sociological and cultural conditions of the most devastated countries in Africa and Asia, and that encompasses sustained international political will. New developments in AIDS research will contribute decisively to the decline of the epidemic and eventually its eradication. From the beginning of the epidemic, science has produced the most important practical advances: from discovering the cause of AIDS to developing a blood test and anti-HIV drugs. Now, sci-

ence must develop new therapies that are practical alternatives for the developing world, as well as new microbicides that block sexual transmission, until an efficacious vaccine arrives.

In developed nations, the judicious use of combination anti-HIV drug therapy has substantially benefited HIV-infected people and has ended the pediatric epidemic. The Global Fund to Fight AIDS, Malaria and Tuberculosis—launched by Kofi Annan, the secretary-general of the United Nations—is spearheading efforts to translate these advances worldwide. However, the challenge is huge and has been complicated by many factors, including the emergence of multidrug-resistant HIV mutants. New antivirals that target not only dividing cells but also “resting” cells, and strategies that augment intracellular levels of active drug through modulation of metabolic pathways, may improve the suitability of existing drugs (1, 2). New classes of drugs, particularly HIV entry inhibitors, show promise. They have the advantage of stopping HIV before it establishes new infections in host cells. Preliminary studies show impressive results with inhibitors that block each stage of HIV entry: attachment and binding to CD4⁺ T

cells, coreceptor binding, and fusion of the viral and cellular membranes (3, 4).

What can be done to bring anti-HIV therapy to developing countries with limited infrastructure? Administering these therapies is complex, and patient compliance is a major challenge. If compliance and careful follow-up of patients is not achieved, we will see a dramatic increase in multidrug-resistant HIV mutants whose further spread will only exacerbate the epidemic. With our 20 years of experience, we propose the following priorities for eliminating AIDS worldwide.

Access to Antiretroviral Treatments

One of the main objectives of the Global Fund to Fight AIDS is to make anti-HIV drugs accessible to all of the developing world. The problem of cost can be partly solved by reducing drug prices (through lower pricing acceptable to pharmaceutical companies, use of generic drugs, and financial help from the Global Fund). But the infrastructure necessary for performing follow-up of patients during treatment will be costly and difficult, and the duration of such treatments will make them ultimately unaffordable for patients in poor countries. This is an unprecedented situation. The decrease in plasma viral load achieved with triple drug therapy does not stabilize after treatment interruption, which results in a rapid increase in circulating virus. Moreover, there are severe limitations to antiretroviral therapy, including toxic side effects (lipid

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