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The early years of HIV research: integrating clinical and basic research

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Between the first analysis of patient samples in early 1983 and the determination of the sequence of HIV-1 in 1985, a vast amount of data was accumulated on HIV through the integrated efforts of clinicians, virologists, immunologists, molecular biologists and epidemiologists. These early years of HIV research quickly led to strategies for the diagnosis, monitoring and treatment of HIV/AIDs

In 1981, Gottlieb *et al*. reported five young men in Los Angeles affected by *Pneumocystis carinii* pneumonia combined with a severe immune depletion of T cells[1]. Clinicians in France soon started observing individuals with similar symptoms. Together with their research colleagues from different hospitals in Paris, including the Pitié-Salpétrière and the Bichat–Claude Bernard hospitals, French clinicians Willy Rozenbaum, Françoise Brun-Vézinet, Christine Rouzioux and others established a working group to study the emerging disease.

Clinical and epidemiological investigations had provided persuasive evidence that the disease was caused by an infectious agent, probably a virus, transmitted by sexual routes and in blood derivatives. But all initial attempts to establish a link between the epidemiological and clinical features of this disease and a known virus failed. The French working group became convinced that the cause was probably an as yet unidentified virus.

The search for a new virus

At that time, the first human retrovirus discovered, human T lymphotrophic virus 1 (HTLV-1), had just been reported to infect T cells. In addition, the feline leukemia retrovirus (FeLV) had been shown to induce severe immunodeficiency in cats. It therefore seemed possible that an unknown human retrovirus was causing the newly recognized disease. To test this hypothesis, Rozenbaum and Brun-Vézinet contacted Luc Montagnier and Jean-Claude Chermann of the Viral Oncology Unit of the Institut Pasteur. I was then a research associate on Chermann's team, which was working on murine retroviruses and was thus familiar with the techniques used to identify such viruses. We were indeed very interested in the possibility that an unknown retrovirus was causing immunodeficiency in humans.

This is how, in late 1982, the Institut Pasteur embarked on the adventure that led to the discovery of the virus that causes AIDS. It began with a meeting at the Institut Pasteur among Rozenbaum, Brun-Vézinet, Rouzioux, Montagnier, Chermann and myself to discuss the first patient sample to be studied. On the basis of the profound $CD4^+$ T cell depletion observed in individuals affected by opportunistic infections characteristic of the new disease, we postulated that the $CD4^+$ lymphocytes were the virus' target cells and were being destroyed as a consequence of their infection. We therefore decided that our first step should be to examine a sample from a patient in the early stages of disease, who would still have $CD4^+$ T cells from which to isolate the virus. We also reasoned that the generalized lymphadenopathy that often precedes AIDS might represent a strong immune reaction to the infection and, therefore, that the causative virus might be easily detected in lymph node tissues.

Our first sample, which arrived in January 1983, was from a lymph node biopsy from an individual suffering from generalized lymphadenopathy. Montagnier isolated lymph node cells and cultured them in the presence of the cytokine interleukin-2 (IL-2), which had just been reported to allow T cell proliferation *in vitro*. I regularly checked the culture supernatant for the presence of retroviruses—in particular, for the presence of reverse transcriptase activity. Because we were convinced that we were dealing with a new retrovirus with unknown characteristics, we did not follow any preset protocol. Instead, we decided to check for reverse transcriptase activity every 3–4 days. Each time, we were anxious to see a positive result.

The strategy paid off. After 3 weeks we detected reverse transcriptase activity, but it was soon followed by cell death. Thinking that we were losing the viral culture, we rushed to the Pasteur Blood Transfusion Center to obtain peripheral blood mononuclear cells from blood donors and added them to the culture. After a couple of days, we once again detected reverse transcriptase activity followed by cell death. This was the first observation of the cytopathogenicity of the virus (**Fig. 1**). And we now knew that we could maintain the viral culture by adding fresh blood cells.

Charlie Dauguet, who was in charge of electron microscopy in our group, screened the reverse transcriptase–positive samples to try to detect viral particles. Showing great patience in the face of our constant

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I have it!

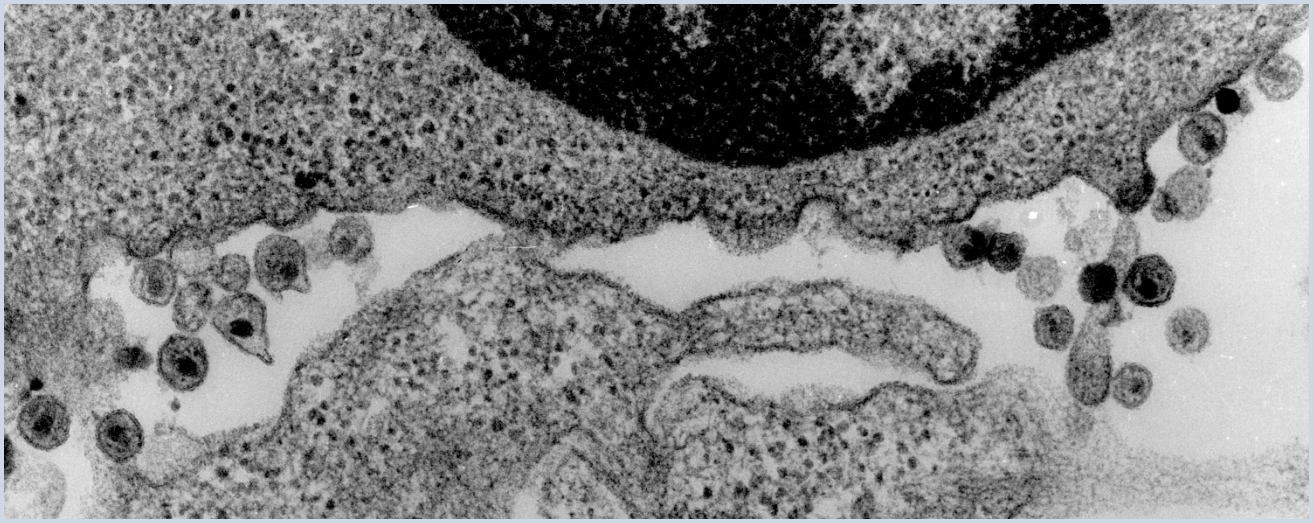

Photo courtesy of Institut Pasteur (magnification 100,000)

It happened on Friday, 4 February 1983, at 5:45 p.m. A week before, Monsieur Montagnier had come to see me in my laboratory one evening to ask me whether I'd agree to work on an infectious human sample. I agreed straight away. He recommended that I take all possible precautions; at that time we only had a simple laminar flow hood.

Before turning off the electron microscope, I had to let it cool down. That late Friday afternoon, just as I was about to turn off the cooling water, I saw a virus under the screen. I ran out of the lab, screaming, "I have it, I have it!" Someone walking in the corridor at that moment might have doubted my mental capacities. I immediately took several photos. Time flew by. Later that evening, I arrived at the railway station to take the train to Trouville, where I was planning to spend the weekend, only to see the red taillights of the departing train.

I didn't get to the Normandy coast, but that weekend was probably the most intense experience in my 39 years doing electron microscopy at the Pasteur Institute.

After that, I hardly left the electron microscope. It was often tiring, particularly on the eyes—tiring but productive. Examining viral cultures, I saw the virus stick to the cell surface, sometimes disappearing inside, which was a sign of infection. The budding process, a sign of productive infection, was readily visible. And of course, I also saw cells with altered morphology and others killed by the virus.

I was surprised by the appearance of the virus. Viruses with a similar morphology had been described in goats and horses, but nothing like this had ever been described for a human virus. Because I was looking at cells of human origin, I was convinced that we had the first human virus of that family. I remember saying to myself that it would be possible to make a vaccine. I was a bit naive, or at least optimistic, at that moment.

Charlie Dauguet, as told to Hugues Fleury and Simon Wain-Hobson

questions, Dauguet detected retroviral-like particles within a few days. His images showed characteristic immature particles budding at the plasma membrane. We began to accumulate data indicating that we were dealing with a retrovirus, such as the association of reverse transcriptase and RNA in particles at a density of 1.16 in a sucrose gradient, a characteristic of most retroviruses. By immunofluorescence analysis, we identified antibodies against the virus in the serum of both the first patient and a second patient. The virus was not recognized by type-specific antisera to HTLV-1 (provided by R. Gallo), indicating that we were dealing with an unknown virus.

It was a very exciting period for our group, and we completely forgot how many hours we were spending in the laboratory each day. All of our data were corresponding precisely with what we expected. Although we began to realize the importance of our discovery, our aim was to continue working and to generate data that would convince the scientific community.

In May 1983, our early data led to the publication in *Science* of the initial description of the AIDS-causing virus, named lymphadenopathy-associated virus, which was later designated human immunodeficiency virus-1 (HIV-1)[2]. From 1983 to 1985, a vast amount of data was accumulated through further collaborations between scientists and clinicians and contributions from Robert Gallo's laboratory, which strongly reinforced our initial findings. The detection of antibodies against the virus in individuals at risk for AIDS and in those with AIDS or AIDS-related conditions, but not in individuals suffering from other diseases, confirmed that HIV was responsible for the disease[3]. The virus-selective tropism for $CD4^+$ T cells[4], leading to T cell death, was demonstrated early in 1983 in collaboration with Klatzmann and Gluckman. The viral reverse transcriptase was characterized, providing further evidence that we were dealing with a retrovirus[5]. The cloning of the virus in 1984 (ref. 6) and the sequencing of its genome[7]

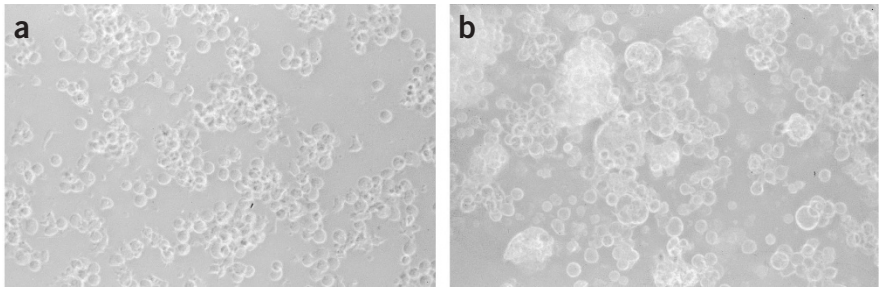

Figure 1 Early observation of HIV cytopathogenicity. This figure shows one of the first images taken of HIV-induced cytopathic effects on T cells. (**a**) Uninfected T cell cultures. (**b**) T cell cultures derived from an individual infected by HIV-1 LAI, a virus isolated in 1983 at the Institut Pasteur. Characteristic syncytium formation was observed before cell death under the microscope.

proved definitively that the virus belonged to the retrovirus family and, more precisely, was a lentivirus. The sequence revealed a complex genetic structure that was shown soon after to be highly variable.

How did we manage to get all of these results so quickly? Probably because an extremely efficient synergy occurred between clinicians, virologists, immunologists, molecular biologists and epidemiologists—a perfect example of integrated research.

Twenty years on

Despite all the progress in HIV and AIDS science, the epidemic unfortunately continues to progress in the developing world, where over 95% of people infected with HIV live. The Institut Pasteur in Paris, a close partner of the National Agency for AIDS Research in France, is still deeply involved in HIV and AIDS research, together with the Institut Pasteur International Network. About 15 teams in Paris and 14 teams in the International Network are conducting research in three main areas: virus-host interactions and their impact on HIV pathogenesis, and strategies for treatment and for vaccine development. Most research programs within the International Network are developed based on the priorities of local health authorities, and are designed to help reinforce local structures and aid in training and technology transfer, particularly in Africa and southeast Asia.

Twenty years after we first recognized the AIDS-causing virus, many questions remain to be answered, especially in the field of HIV pathogenesis. Much more attention should be paid to the very early signals of immune activation in response to HIV infection. Indeed, in the early 1980s, soon after the discovery of HIV-1, our group postulated that immune activation might be a key element to consider in AIDS pathogenesis because of its potential impact on HIV dissemination and on the control of HIV replication *in vivo*[8]. Since then, a good deal has been learned about signaling pathways that lead to initial T cell responses, but there is still a long way to go in understanding the key determinants of HIV transmission and protection against HIV infection. Resolving these issues will certainly generate new concepts and will probably result in further insights into HIV immunopathogenesis and, consequently, into vaccine and therapeutic interventions in the near future.

ACKNOWLEDGMENTS

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