HIV/AIDS

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25 Years after HIV Discovery: Prospects for Cure and Vaccine (Nobel Lecture)**

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AIDS · HIV · Nobel Lecture · virology

Biography

I was born on August 18, 1932 in Chabris, France, a "bourg", larger than a village but smaller than a town, located in Berry, south of the Loire Valley. This was—and still is—a region of agriculture with some renowned products such as welsh rabbit, goat cheeses, and white asparagus. It was the place where my mother had grown up but, in fact, I never lived there. On my father's side, his parents came from Auvergne, a province of the center of France, made of rich plains and older volcanoes—the latter probably being at the origin of my family name: Montagnier, the man living in mountains.

My father had, in his youth, caught a terrible disease: streptococcus-linked arthritis, ending in irreversible lesions in aortic valvae. Therefore, he was declared unfit for military service and had to find a sedentary job: he became an accountant, and excelled in this profession which involved, at that time, mainly hand-written work. He started working in the Poitiers area, and then moved a little further north to Chatellerault, a small city between Tours and Poitiers.

As a single child, I was cherished by my mother, a housewife, but two events dominated this "prewar" period, of which I keep a vivid memory: I was badly hit by a high-speed car when crossing a main road: I suffered multiple wounds from which I still have some visible scars, spent two days in a coma, but I emerged from it as if I was born again, at the age of five (Figure 1).

Two years later came the declaration of war in 1939 while all the family was harvesting grapes in the vineyards of my mother's brother. I still remember the images in a newspaper of Warsaw ruins after bombing by German planes.

Figure 1. Luc Montagnier at the age of 5.

And then, in 1940, came the "real" war: the German invasion, my parents and I leaving their house (close to a risky railway station), fleeing on the roads in a little car, and being more exposed to German bombing during this "exodus" than if we had stayed home.

The first year of German occupation was terrible in that we had no food reserves and most of the time we were starving. I was a rather puny boy and during the four years of the war, did not gain a gram! The "ersatz" did not stimulate my appetite, when I was dreaming of chocolate and oranges! My father had chronic enterocolitis and, worse, my grandfather (his father) was diagnosed with rectal cancer. He died in 1947 after terrible suffering, and each time I visited him I could see the inexorable progression of the disease. This touched me so much that it is probably one reason why I decided later to study medicine and to start research on cancer.

In June 1944, our house (very close to the railway) was partly destroyed, this time by an allied bombing. I keep mixed feelings of this year regarding the liberation of France. It was a great relief, but I could not forget also the vision of so many killed people, civilians and soldiers, and the images of skinny deportees released from concentration camps. I have hated wars and their atrocities for the rest of my life.

At high school, I was doing well, being usually ahead of my class mates. This is the time when I became curious of scientific knowledge, having left behind my religious catholic beliefs. Following the example of my father, who was tinkering on his leisure days with electric batteries, I set up a chemistry laboratory in the cellar of the new house which was requisitioned to accommodate us. There, I enthusiastically produced hydrogen gas, sweet-smelling aldehydes, and nitro compounds (not nitroglycerine!) that had the unfortunate habit of blowing up in my face.

I was delighted to read—in popularized books—the impressive progress of physics—especially atomic physics. Being good in physics and chemistry—but not as good in maths—I decided not to prepare for the "contests" for the

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"Grandes Ecoles", but instead registered both at the School of Medicine and the Faculty of Sciences of Poitiers. My goal was in fact to start a research carrier in human biology but there was no such specialty in Poitiers, neither in Medicine, nor in Sciences. Since both Faculty and School were within walking distance, I could spend the morning at the hospital and the afternoon attending courses in botany, zoology, and geology, which were the main disciplines of the License of Sciences.

Fortunately, the new Professor of Botany, Pierre Gavaudan, was a very atypical professor in that his scientific interests went far beyond the classification of plants. In fact, I owe him for having opened up a large window on what was the beginning of a new biology, the DNA double helix, the in vitro synthesis of proteins, and the structure of viruses.

At the same time, I was installing at home a device consisting of a time-lapse movie camera and a microscope, thanks to a gift from my father. This allowed me to do my first research work. I was studying a phenomenon known since 1908 as the phototaxy of chloroplasts: the property of some algae living at the surface of ponds to orient their large unique chloroplast according to the intensity of light; if the light was too intense, the chloroplast turned inside the tubular cell to present its edge. In dark or weaker light, the chloroplast (a flat plate) exposed its larger surface. The phenomenon took a few minutes, which could be analyzed by time-lapse cinematography. Using different glass filters, I could show that it was not the wavelength absorbed by the chlorophyll (red light) which could regulate the orientation of the chloroplasts, but indirectly some yellowish pigments absorbing the blue light. I was very proud, at the age of 21, to submit this work as a small thesis at the Faculty of Sciences of Poitiers. I was asked by my mentor, Pierre Gavaudan, to dissert also on a literature-based subject: it was the L forms of bacteria. This allowed me to do my first incursion—not the last—into the world of filtering bacteria. I could only find the references on this controversial subject at the library of the Pasteur Institute in Paris. This was indeed the time when I left Poitiers to go to Paris, where I would finish my medical studies, as well as explore some aspects of biology closer to human beings, particularly neurophysiology, virology, and oncology.

Having been hired as an assistant at the Sorbonne at the age of 23, I started learning old-fashion technologies derived from Alexis Carrel's work on chick embryo heart cultures, as well as that of human cell lines in monolayers. Although my research was not productive at all, I keep from this period a solid expertise of Pasteurian technologies for working in conditions that are perfectly sterile, without the use of antibiotics.

In 1957, the first description of infectious viral RNA from the tobacco mosaic virus by Fraenkel-Conrat, Gierer and Schramm determined my vocation: to become a virologist by using the modern approach of molecular biology.

I started in Kingsley Sanders laboratory at Carshalton near London with the foot and mouth virus. Kingsley was also an atypical British man, a writer of opera music in his leisure days. He gave me complete freedom to choose the approach for elucidating the replication of small RNA viruses. I was proud to identify for the first time an infectious double-stranded RNA from cells infected with the murine encephalomyocarditis virus, a small single-stranded RNA virus. This demonstrated for the first time that RNA can replicate, like DNA, by making a base-paired complementary strand.

In order to perfect my knowledge on oncogenic viruses, I moved from Carshalton to Glasgow, where a new Institute of Virology had recently been inaugurated, headed by a remarkable virologist, Michael Stocker, and where many high-ranking visitors, among them, Renato Dulbecco, were spending sabbatical years.

Working on the small oncogenic DNA virus polyoma, I could show there, with Ian Macpherson, a new property of transformed cells, that of growing in soft agar. By using this technique, it was easy to detect the transforming capacity of polyoma virus and its DNA. We showed that naked DNA alone carried all the oncogenic potential of the virus. This looks now pretty obvious, but it was not so at that time.

Back at France at the Institute Curie, I extended this finding to a number of cancer cells that were transformed or not by oncogenic RNA or DNA viruses. However, this property allowed me to distinguish some in vitro steps in the process of transformation which were correlated with some modifications of the plasma membrane and of the carbohydrate layer surrounding it.

A great mystery remained at that time: that of the replication of the oncogenic RNA viruses, now known as retroviruses. Howard Temin (Figure 2) had proposed the hypothesis of a DNA intermediate, but other possibilities could be considered. I myself tried to find a double-stranded RNA specific of the Rous sarcoma virus, a virus able to infect and transform chick embryo cells. I indeed isolated double-stranded RNA sequences, but they were of cellular origin and existed at the same level in non-infected cells! Together with Louise Harel, I showed later that this RNA was partly coming from repetitious sequences of DNA. On retrospect, it could, at least in part, represent the recently identified interfering RNAs involved in the negative control of messenger RNA translation.

In 1969–1970, the isolation of an RNA polymerase associated with the viral particles of the vesicular stomatitis virus led to the idea that perhaps a key enzyme was also

Figure 2. Receiving an award plate of the American Society of Pathology from Howard Temin's in 1985.

associated with the oncogenic RNA viruses. Indeed, Howard Temin and Mizutani, and independently D. Baltimore, discovered in 1970 a specific enzyme associated with Rous sarcoma virus (RSV), the reverse transcriptase (RT), capable of reversely transcribing the viral RNA into DNA.

At about the same time, Hill and Hillova in Villejuif, France, demonstrated that the DNA extracted from RSV-transformed cells was infectious and carry the genetic information of the viral RNA, confirming that the enzyme was working faithfully in infected cells. I, myself, with P. Vigier, confirmed and extended this discovery by showing that the infectious DNA was associated with the chromosomal DNA of the cells, showing integration of the proviral DNA, as earlier postulated by Temin.

The work on the chicken RSV was extended to similar viruses of mammals, so that many researchers at that time believed that the RT activity was a new highly sensitive tool for detecting similar viruses in human leukaemia and cancer. This was stimulated by the highly funded virus-cancer program launched by the American NIH. Unfortunately, the hunt for human retroviruses was basically unsuccessful, but led to important basic work on the molecular biology of animal retroviruses and the discovery of oncogenes—the "cancer genes".

In 1972, I was proposed by Jacques Monod, then heading the Pasteur Institute, to create a research unit in the newly created Department of Virology of the Institute. I accepted it, and this new laboratory allowed me to develop within the general theme of viral oncology new avenues of research, with the ultimate goal remaining the detection of virus involved in human cancers.

Thus, I became interested in the mechanism of action of interferon and its role in its expression of retroviruses. I came into this field after having demonstrated the biological activity of interferon messenger RNA in collaboration with two world-known experts in the field, Edward and Jacqueline De Maeyer. From 1973 onwards, Ara Hovanessian and his co-workers joined my unit and added a new dimension to the subject: the complex biochemical mechanism sustaining the antiviral activity of this remarkable group of cellular proteins.

In 1975, two other researchers joined my unit and brought their expertise on murine retroviruses: these were Jean-Claude Chermann and his collaborator, Françoise Barré-Sinoussi (Figure 3). The latter mastered particularly the detection of retroviruses by their RT activity. I convinced them to participate in a joint study inside the unit to look again for retroviruses in human cancers. We started in 1977 with blood samples coming from different Parisian hospitals and biopsy specimens.

Two advances made in other laboratories boosted this search:

In Villejuif, France, Ion Gresser had prepared a potent antiserum neutralizing any molecule of endogenous alpha-interferon produced by individual cells. This interferon, we realized, was produced by mouse cells induced to express some of their endogenous retroviruses. Its blockade by the antiserum increased by up to 50 times the production of endogenous retroviruses in the culture medium. We could conclude that, despite the fact that endogenous retroviruses have been integrated in the genome of vertebrates for millions of years, their expression is still controlled by the interferon system, like that of exogenous viruses!

Figure 3. HIV discoverers in the park of the Pasteur Institute Annex in Garches, near Paris, during a break of a "100 guards meeting" in 1987. From left to right: Jonas Salk, Jean-Claude Gluckman, Jean-Claude Chermann, Luc Montagnier, Robert Gallo, Françoise Barré-Sinoussi, Willy Rozenbaum, and Charles Mérieux.

At about the same period, the discovery by Doris Morgan and Frank Ruscetti in Dr. Gallo's laboratory of a growth factor allowing the in vitro multiplication of human T lymphocytes (TCGF, then named interleukin 2, Il2) made it possible to propagate T lymphocytes in sustained cultures. We knew at that time that some retroviruses involved in mouse mammary tumor formation (MMTV) could not only be expressed in the tumor cells but also in the circulating lymphocytes.

Taking advantage of these two advances, we started a search for retroviruses in human cancers, using anti-interferon serum and Il_2, with particular focus on the T-lymphocyte cultures from breast cancer patients.

Indeed, in 1980, we were able to detect a DNA sequence close to the MMTV not only in the cells of an inflammatory breast cancer (from a North African woman), but also in her cultured T lymphocytes. A second patient showed similar results. Unfortunately, the molecular tools we had at that time could not tell us whether we were dealing with endogenous retroviral sequences or with an exogenous virus. Nowadays, having to hand more powerful technologies, I am planning to reinitiate these studies.

But in 1983, the same approach, the use of anti-interferon serum, and the use of long-term cultures of T lymphocytes greatly facilitated the isolation of HIV.

My involvement in AIDS began in 1982, when the information circulated that a transmissible agent—possibly a virus—could be at the origin of this new mysterious disease. There were at that time only a few cases in France, but they attracted the interest of a group of young clinicians and immunologists. They were looking for virologists, especially retrovirologists, as a likely hypothesis was that HTLV—the only human retrovirus known so far, recently described by R. C. Gallo—could be involved. Retroviruses that cause leukaemia in rodents often also cause a wasting syndrome which could be the result of secondary immune depression.

This was also the case of patients suffering from leukaemia induced by HTLV.

A member of the working group, Françoise Brun-Vézinet, was a former student of the virology course I was then directing. She called me up to organize the search for the putative retrovirus from a patient presenting with an early sign of the disease lymphodenopathy. The patient was a young gay man who had been traveling in the USA, who was consulting Dr. Willy Rozenbaum—one of the leaders of the working group—for a swollen lymph node in the neck. The gland was not painful; it was just for the patient a question of aesthetics.

The reasoning was that if we were to find a virus at this early stage of the disease, it could be more a cause than a consequence of the immune depression. Another incentive to start this research was a request from the producers of the hepatitis B virus vaccine in the industrial subsidiary of the Pasteur Institute. They were using plasma from American blood donors and were concerned by the risk of transmission of the AIDS agent through their procedure of viral antigen purification.

The lymph node biopsy arrived on January 3, 1983, a date which I remember well because it was also the first day of the virology course at the Pasteur Institute which I had to introduce. I could only dissect the small hard piece at the end of the day. I dissociated the lymphocytes with a Dounce glass homogenizer and started their stimulation in culture with a bacterial mitogen, protein A, known to be an activator of B and T lymphocytes, since I did not know which fraction of the lymphocytes could produce the putative virus. Three days later, I added the T-cell growth factor I had obtained from a colleague working in the laboratory of Jean Dausset.

The T cells grew well. As previously established in a protocol during the search of retrovirus in human cancers, it was decided with my associates, Françoise Barré-Sinoussi and Jean-Claude Chermann, to measure the RT activity in the culture medium every 3 days. On day 15, Françoise showed me a hint of positivity (incorporation of radioactive thymidine in polymeric DNA), which was confirmed the following week.

We had evidence of a retrovirus, but this was just the beginning of a series of questions: 1) Was it close to HTLV or not? 2) Was it a passenger virus or, on the contrary, the real cause of the disease?

In order to answer these basic questions we had to characterize the virus biochemically and immunologicaly, and for that, we need to propagate it in sufficient amounts. Fortunately, the virus could be easily propagated on activated T lymphocytes from adult blood donors. No cytopathic effect was observed with this first isolate, but unlike HTLV-infected cultures, no transformed immortalized cell lines could emerge from the cultures, they always died after 3–4 weeks, as do normal lymphocytes.

In contrast, subsequent isolates I made from cultures of lymphocytes of sick patients with AIDS were cytopathic for T-lymphocytes culture and—we discovered later—could be grown in larger amounts in tumor cell lines derived from leukaemia.

Shortly after the virus isolation, my co-workers and I could show that it was not immunologicaly related to HTLV, and by electron microscopy it was very different from HTLV viral particles. In fact, as early as June 1983, I noticed the quasi identity of our virus with the published electron microscopy pictures of the Visna virus of sheep, the infectious anemia virus of horse, the bovine lymphocytic virus: it was a retrolentivirus, a subfamily of viruses causing in animals long-lasting disease without immunodeficiency.

This indicated clearly that we were dealing with a virus very different from HTLV, and my task was now to organize a team of researchers to accumulate proof that this new virus was indeed the cause of AIDS.It was an exciting period since every saturday morning, when we had a meeting in my office, new data were brought by my associates favoring the causative role of the virus. The viral isolates were called LAV, for lymphadenopathy associated virus, when they were isolated from patients displaying swollen lymph nodes, a frequent sign of the early phase of infection. The isolates made from patients with full-blown AIDS were called immunodeficiency associated viruses (IDAV). The latter generally grew better in T-lymphocyte culture and induced the formation of large syncitia, resulting from the fusion between several infected cells. Some of them—we found out later—could also multiply in continuous cell lines of B- or T-cell origin. The latter property greatly facilitated the massive production of the virus for commercial use.

By September 1983, I could make a presentation of all our data favoring a causal link between the virus and the disease at a meeting on the HTLV organized by L. Gross and R. Gallo at Cold Spring Harbor. This presentation was received with scepticism by a scarce audience (it was a late-night session) and the HTLV theory was still prevailing. Mentally, most attendants were not prepared to accept the idea of a second family of retroviruses (lentiretroviruses) existing in humans and causing immune deficiency, and having no counterpart in animals!

This situation is not infrequent in science, as new discoveries often raise controversy. The only problem is that it was a matter of life and death for blood-transfused people and haemophiliacs, since a serologic blood test using our virus antigens was already working at the laboratory scale, but awaited industrial and commercial development.

This came in 1985, after two other teams of researchers—first that of Dr. Gallo at NIH in early 1984 and that of Jay Levy in San Francisco—confirmed and extended our findings. In particular, Dr Gallo and his associates gave more strength to the correlation between the virus and the disease, improved the detection of the antibody response, and could grow several viral strains, including ours, in T cell lines of cancer origin. Meanwhile, my co-workers showed the tropism of the virus for $CD4^+$ cells, and identified the CD4 surface molecule as the main receptor for the virus.

The rest of the story is described in the next section. I would just like to illustrate how I discovered what I believe are two important phenomenons for explaining the destruction of the immune system induced by HIV.

During the latent phase of the infection, no virus is found in the blood, it is mostly localized in lymphocytes of lymphatic tissues and yet, we found that most of the lymphocytes present in the blood are sick! In 1987, a young visitor from

Sweden, Jan Alberts, came to my lab. He wanted to cultivate human lymphocytes in serum-free synthetic medium and to learn some technologies concerning HIV culture. The surprise came when we compared in his medium the viability of lymphocytes from healthy donors and those from HIV-infected patients, even in their early asymptomatic-stage of infection. While the former could survive several days without dying, the majority (more than 50%) of the latter died very quickly. Addition of interleukin 2 partially prevented their death.

When we used normal culture medium supplemented with fetal calf serum, the same difference was observed, although the survival time of the lymphocytes from infected patients was longer.

It did not take very long before three of my collaborators found the reason for such deaths: apoptosis. This is an active process by which the cell "decides" to die in a clean way, without releasing too much toxic compound into the medium.

It is a physiological way to prevent abnormal proliferation of activated lymphocyte clones, but here the phenomenon was enormous and affected not only the main cellular targets of HIV infection, $CD4^+$ T-lymphocytes, but also cells which were not infectable by the virus, such as $CD8^+$ T lymphocytes, B lymphocytes, monocytes, and natural killer cells (NKs). Clearly, it was a general phenomenon: the culture simply revealing a predisposition to apoptosis of the majority of circulating blood cells, although most of them were neither infected nor infectable. Indeed, my collaborator Marie-Lise Gougeon found a very good relationship between in vitro apoptosis and the observed in vivo drop of CD4 T cells in patients.

We have spent a lot of time trying to find the origin of this massive apoptosis, without finding a completely satisfactory explanation: the most likely is the intensive oxidative stress existing in patients since the beginning of their infection. This is also a finding I am very proud of: although oxidative stress has been—and still is—completely overlooked by AIDS researchers, it is likely to aggravate the wrong activation of the immune system at the origin of its decline, and also to trigger inflammation through the production of cytokines.

Of course, the next question arises: What are the factors causing oxidative stress? Viral proteins, fragments of viral DNA, co-infection with mycoplasmas? Even after 25 years, we still do not know the complete answer. But the phenomenon does exist and needs to be treated, while most AIDS clinicians do not care about it at all!

The treatment by combined antiretroviral therapy has, without doubt, changed the prognosis of this lethal disease, from death condemnation to an almost "normal" life. However, the virus is still there, ready to multiply when the treatment is interrupted, and not all HIV-infected patients of the developing world have access to it! And the epidemic still kills 2–3 million people each year. There is, therefore, an absolute necessity to resolve these problems, and basic research, as well as clinical research, has to be continued.

In addition, I realized in the 1990s that research should not only be localized in the wealthy laboratories of the developed countries, but also in southern countries in which a lot of patients were suffering from AIDS and many other diseases like tuberculosis and malaria.

Too many examples showed that collaboration between northern and southern research laboratories is unequal, the south providing serum samples to be analyzed in the north. This "safari" concept is wrong. There are now many young researchers trained in northern laboratories who would like to go back to their own countries, but are prevented from doing so because laboratories and adequate structures are missing. Moreover, one has to be in the regions where disease proliferates to realize how complex is the reality.

This is why I initiated with the former Director General of UNESCO, Federico Mayor, a Foundation aimed at creating in African countries centers for research and prevention. Although the task was difficult, this concept met with enthusiastic colleagues and medical doctors, and also found the support of local governments, particularly in the Ivory Coast and Cameroun. I wish that from these pilot experiments, a whole network of similar centers could cover all the countries of the developing world where the populations are badly hit by the epidemics.

Another lesson I learnt from my AIDS experience was the weakening effect of oxidative stress on the immune system and its pro-inflammatory role in many chronic diseases, such as Parkinson, Alzheimer, and rheumatoid arthritis; a likely consequence of chronic infections? Or both consequence and cause? Questions which can be resolved only by hard work and innovative thinking. I hope to be able to continue both.

Nobel Lecture

The impressive advances in our scientific knowledge over the last century allow us to have a much better vision of our origin on Earth and our situation in the universe than our ancestors. Life probably started on Earth around three and a half billion of years ago, and a genetic memory early emerged, based on an extraordinary stable molecule, the DNA double helix, bearing a genetic code identical for all living organisms—from bacteria to men. We are thus the heirs of myriads of molecular inventions which have accumulated over millions—sometime billions—of years. The environmental pressure has of course both maintained these inventions and also modulated them over the generations, through death of the individuals and sexual reproduction.

For the last 30000 years, our biological constitution has not changed: a hypertrophic cortical brain, a larynx to speak, and a hand to manipulate. However, for the last 10000 years another memory has emerged, which makes our species quite different from the others: this is the cultural memory which transmits knowledge and societal organization from one generation to the next generation, through the use of language, writing, and more recently virtual communication means.

This revolution occurred at several sites on the Earth almost simultaneously through sedentarization of human populations by agriculture, leading to several civilizations. Each human being thus receives two packages, the genetic memory at birth and cultural memory during all his life, and he will become a real human only if he benefits from both. For the last three centuries, particularly in the 20th century, our

scientific knowledge has increased exponentially and has diffused all over the world.

We have a tendency to consider ourselves as pure spirits, but the hard reality still reminds us of our biological nature: each of us is programmed to die and, during his life, is exposed to diseases. At the dawn of this new century, we are still facing two major health problems:

- new epidemics related to infectious agents (mostly bacteria and viruses);
- chronic diseases (mostly cancers, cardiovascular, neurodegenerative, arthritic, autoimmune diseases, diabetes) linked to the increase in life expectancy and environmental changes related to human activities.

This presentation will obviously be focused on one new epidemic, AIDS, but we should not forget that there are other persisting and life-endangering epidemics, such as malaria and tuberculosis, especially in tropical countries.

Moreover, other new epidemics should not be excluded as human activities generate more favoring factors:

- lack or loss of hygiene habits;
- lack of water;
- globalization and acceleration of exchanges, travel;
- atmospheric and chemical pollution leading to oxidative stress and immune depression;
- malnutrition, drug abuse, aging, also leading to immune depression;
- global warming leading to new ecological niches for insect vectors;
- changes in sexual behaviors.

This last factor and immune depression caused by malnutrition, drug abuse, and increased co-infections, are probably the causes of the emergence of AIDS as a global epidemic, affecting most if not all the continents, including recently polynesian islands.

The causative agent existed in Africa before the emergence of the epidemics in Central Africa and North America in the 1970s. As there exist related viruses apparently well tolerated in nonhuman primates, it is tempting to consider AIDS as a zoonosis, resulting in the transmission to humans of related viruses infecting primate species without causing disease.

But let us first recall the circumstances of HIV discovery in my laboratory at the Pasteur Institute (Figure 4).

AIDS as a pathologically distinct entity was first identified in June 1981 by members of the Center for Disease Control (CDC; particularly James Curran) after reports received from two medical doctors, Michael Gottlieb in Los Angeles and Alvin Friedman-Kien in New York, of clusters of opportunistic infections and Kaposi sarcoma occurring in young gay men who had related sexual intercourse. Following publication of this report in the CDC Bulletin, similar cases were described in Western European countries and particularly in France by a group of young clinicians and immunologists led by Jacques Leibovitch and Willy Rozenbaum.

It was soon recognized that a similar disease, characterized at the biological level by a profound depression of cellular immunity and clinically by infections previously described in chemically or genetically immunodepressed patients, also existed in haemophiliacs and blood-transfused patients.

Figure 4. The Pasteur team involved in the discovery of HIV in 1983–1984.

The case of haemophiliacs was giving a clue as to the nature of the transmissible agent: these AIDS patients had received purified concentrates of factor 8 or 9 made from pools of blood donors which had been filtered through bacteriological filters. This purification process should have eliminated any soluble toxic compound and the filtration should have retained bacterial or fungal agents: only viruses could be present in the preparations given to the patients. This is why I become interested in a search for viruses; but what kind of viruses? Many viruses have immunodepressing activity, in order to persist in their hosts. This is particularly the case of herpes viruses (cytomegalovirus) and retroviruses. A putative candidate was the human T leukemia virus (HTLV) described by R. C. Gallo and Japanese researchers.

Having more expertise on retroviruses (see biographical section), we embarked on the search for an HTLV-like virus at the suggestion of the French working group and also incited by the Institute Pasteur Production, an industrial subsidiary of the Institute, producing an hepatitis B vaccine from a pool of plasma from blood donors.

Knowing that retroviruses are usually expressed in activated cells, I set up classical conditions to grow, in culture, activated lymphocytes, using at first a bacterial activator of both T and B lymphocytes protein A, since I ignored in which subset of the cells the virus was hiding out.

The reasoning at that stage was that we should look first in lymphocytes from swollen lymph nodes, supposedly the site where viruses accumulate in the early phase of infection. I received on January 3 a biopsy from a patient with cervical adenopathy, a symptom already recognized as an early sign of AIDS. After dissection of the sample into small pieces and their dissociation into single cells, the lymphocytes were cultured in nutrient medium in the presence of protein A and anti-interferon serum.

In fact, after addition of interleukin 2, only T lymphocytes were multiplying well and produced a small amount of virus, as detected by its reverse transcriptase activity, measured by my associate Françoise Barré-Sinoussi. Only some 9 months later could I show also growth of the virus in B lymphocytes transformed by Epstein–Barr virus.[4]

The viral growth ceased as the cellular growth started declining, but we could propagate the virus in cultures of lymphocytes from adult blood donors as well as in lymphocytes from cord blood. This allowed characterization of the virus, and showed for the first time that it was different from HTLVs. A p24-25 protein could be immunoprecipitated by the serum of the patient and not by antibodies specific for the p24 gag protein of HTLV1, kindly provided by Dr. R. C. Gallo.

Electron microscopy of sections of the original lymph node biopsy, as well as those from infected cultured lymphocytes, showed rare viral particles with a dense conical core, similar to the retrolentiviruses of animals (infectious anaemia virus of horse, Visna virus of sheep, etc.), but different from HTLV. Unlike the case of HTLV, we could never see emergence of permanent transformed lines from the infected lymphocyte cultures (Figure 5).

These results were published in a *Science* paper in May 1983,[1] together with two papers by the Gallo and Essex groups in favor of HTLV being the cause of AIDS. During the following months, more data accumulated in my laboratory showing that this new virus was not a passenger virus, but was really the best candidate to be the cause of AIDS.

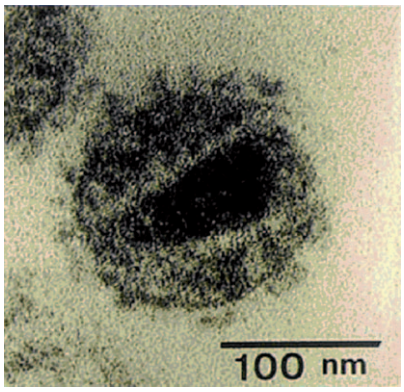

Figure 5. An HIV particle under an electron microscope.

1) The same type of virus was isolated from patients of different origins: gay men with multiple partners, haemophiliacs, drug abusers, Africans (Table 1).

Table 1: First viral isolates of the viral oncology unit.

Patient initials	Origin	Clinical conditions	Cytopathic effect
Bru, ♂	gay man, caucasian	pre-AIDS	–
Loi, ♂	haemophiliac, caucasian	AIDS	+
Lai, ♂	gay man, caucasian	AIDS (Ks)	++
Eli, ♀	Zaire, african	AIDS	+

2) Besides immunoprecipitation of viral proteins (p25, P18), serums from patients with lymphadenopathy syndrome and a fraction of the serums from patients with advanced AIDS, were positive in an ELISA test using proteins from partially purified virus.[2]
3) In vitro, the virus was shown to infect only $CD4^+$ T lymphocytes and not the $CD8^+$ subset.[3]
4) A cytopathic effect was observed with isolates made from patients with late symptoms of AIDS. Particularly the third isolate made from a young gay man with Kaposi Sarcoma (Lai) caused the formation of large syncitia, presumably due to the fusion of several infected cells (Figure 6). Attempts to grow the first isolate Bru in T cell lines isolated from patients with leukaemia or lymphoma were unsuccessful. However, we discovered later[5] that the Bru isolate was contaminated with the Lai isolate, which in contrast could be grown in T cell lines (CEM, HUT78) in laboratories which received our Bru isolate on their request.

In fact, a few laboratory isolates were shown to grow in mass quantities in T cell lines, facilitating analysis of the virus and its use for detection of antibodies by commercial blood tests.

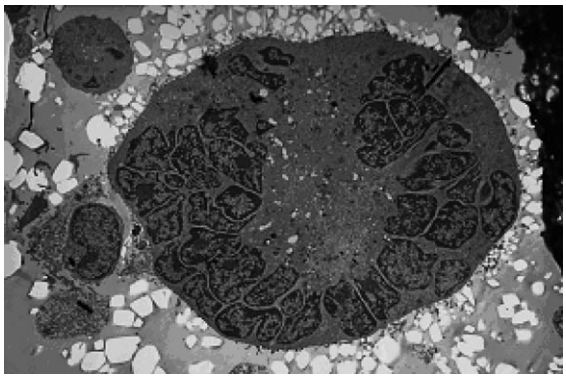

Figure 6. Electron micrography picture of a giant cell (syncitium) resulting in the fusion of many lymphocytes expressing the HIV fusion protein (from Ref. [6]).

Our data, which I presented in September 1983 at a meeting on HTLV in Cold Spring Harbor,[6] were met with scepticism, and only in the Spring of 1984 did the description of a quasi identical virus under the name of HTLV III by the group of R. C. Gallo[7] convince the scientific community that this new retrolentivirus was the cause of AIDS. The group of Jay Levy in San Francisco also isolated the same kind of virus,[8] followed by many other laboratories.

However, a few opponents led by P. Duesberg argued, and are still arguing, that there is no real demonstration that the virus does exist and is the cause of AIDS according to Koch's postulates.

In fact, the proviral DNA of the virus, renamed HIV (human immunodeficiency virus) by an international nomenclature committee, was cloned and sequenced,[9–11] showing the classical gene structure of animal retroviruses which Dr. Duesberg himself helped to uncover earlier. But in addition, new genes (*tat*, *nef*), important in the regulation of the expression of the viral genetic information, were recognized from the DNA sequencing, making the viral genome probably the most complex known in the retrovirus family (Figure 7). HIV and its primate cousins are therefore well characterized entities only composed of DNA sequences, none existing in the human genome.

A posteriori, two facts should have provided to the few remaining sceptics final conviction that HIV is the culprit in AIDS:

1) Transmission of AIDS by blood transfusion has practically disappeared in countries where the detection of HIV antibodies in blood donors has been implemented.
2) The inhibition of virus multiplication by a combination of specific inhibitors of the viral enzymes (reverse transcriptase, protease) has greatly improved the clinical conditions of the patients. Mutations in the genome of HIV-inducing resistance to these inhibitors has led to relapses and aggravation of the patients' condition.

In 1986, thanks to a collaboration with Portuguese colleagues, we isolated a second virus (which I named HIV2) from West African patients hospitalized in a Lisbon hospital.[12] They all had the signs of AIDS but had no antibodies against our first virus. In fact, they had only antibodies to the most variable protein of HIV, the surface glycoprotein. The patients had lost antibodies against the well-conserved internal proteins of HIV2 which show common epitopes with their counterparts of HIV1, unlike the glycoprotein (Figure 8).

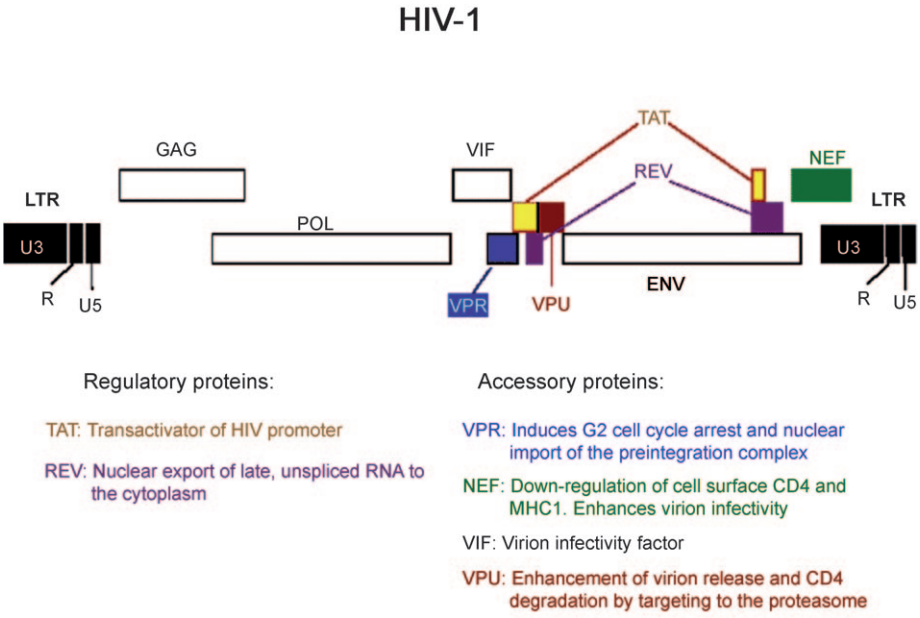

Figure 7. Genome struture of HIV1: *gag*, *pol*, and *env* are the genes coding for the structural proteins.

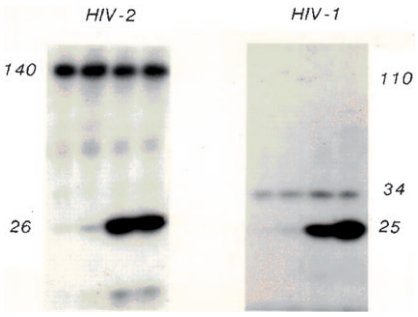

Figure 8. Immunoprecipitation of radioactively labeled proteins of HIV1 and HIV2 by a serum of an AIDS patient infected with HIV2; note in the HIV2 panel, the precipitation of the envelope protein.

The isolation of HIV1[6] and HIV2[12] viruses from AIDS patients in Africa made us realize that we were dealing with a large epidemic of heterosexually transmitted viruses.

Evidence that HIV was not transmitted by casual contacts came from our study in a French boarding school, where HIV-infected haemophilic children were in close contact, day and night, with HIV negative nonhaemophilic children: none of the latter were found to be HIV positive.[13]

The isolation of the virus causing AIDS allowed to implement rational prevention measures and also to start a search for efficient viral inhibitors. The first candidate, azidothymidine (AZT), was an efficient inhibitor of HIV reverse transcriptase in in vitro experiments (Mitsuya and Broder). However, its use in AIDS patients was soon recognized as disappointing.[14] In fact, the treatment readily induced mutants of the virus resistant to AZT and did not extend the life span of the patients. The main obstacle of treatment with a single or two inhibitors was the capacity of the virus to mutate, which also impedes the design of an efficient vaccine and also explains the complexity of the pathophysiology of AIDS.

Only a combination of three inhibitors proved to be efficient on the clinical outcome. Since 1996, clinicians are using HAART (highly active antiretroviral therapy) to treat patients with high virus load and low $CD4^+$ T-cell numbers, preventing them most of the time from falling into lethal opportunistic infections (Table 2).[15]

Table 2: Some milestones in the research of AIDS

1981	identification of the disease in the USA
1983	first isolation of HIV
1984	confirmation of HIV as the causal agent of AIDS; biological and molecular characterization
1985	first blood test to eliminate transmission of HIV by blood transfusion
1986	isolation of HIV-2
1987	first use of AZT as an antiretroviral drug
1991	apoptosis as a mechanism of cell death in AIDS
1995	decrease of HIV perinatal transmission with AZT
1995	demonstration of the high rate of HIV replication during the silent period of infection
1996	identification of HIV main co-receptors
1996–97	generalization of HAART in developed countries

HIV Variability

In fact, in order to escape to the immune reactions of their hosts, most viruses have a strategy to change their immunogenic epitopes. In the case of HIV, a combination of several factors put it to an unprecedented level.

I have listed below the factors which seem to be most responsible for this variability:

1) errors of reverse transcription;
2) genetic recombination;
3) incomplete neutralization by Vif of the activity of the APOBEC3G cellular gene;
4) oxidative stress.

The first is that the replicative enzyme, reverse transcriptase (RT), has no editing compensation, so that the transcription errors may reach $1/10^5$ nucleotides, far from the $1/10^9$ of the cellular DNA polymerases.

However, some other retroviruses, such as HTLV, do not show this variation rate, since once integrated, the proviral DNA remains replicated by the cellular DNA replicative machinery. The difference could be explained by the fact that the HIV-infected cells die, so that the virus can maintain itself only by many cycles of new infections involving, each time, reverse transcription of its RNA into DNA. However, in in vitro infection of cell lines, also involving a cytopathic effect and many cycles of re-infection, the virus seems to be stable in the absence of immunoselective pressure.

Another factor of variation is genetic recombination. The immune responses (humoral and cellular) against the virus are unable to prevent a second virus infection of the host (because of virus variability induced by the previous factor and other causes), so that some cells could be co-infected by two viruses: this will also allow genetic recombination between the two viral RNAs existing each in two copies. The result is a "mosaic" virus in which many sequences from the two original viruses are entangled, starting from "hot spots" of recombination. This is particularly visible in Africa, probably because of repeated exposure to infection by many patients. The mosaic viruses, because of their selective advantage, then disseminate in the infected population. The original subtypes called A, B, C, D, E, G, etc defined by the sequence of their envelope gene are thus replaced by A/G, B/C, etc depending on the geographic location.

Moreover, two other factors have been more recently identified: In the lymphocytes are expressed a family of genes coding for enzymes able to convert guanosine into adenosine in the viral DNA, fouling the viral genetic code (APOBEC3G). However, the virus has evolved a gene, *vif*, which can more or less counteract this effect, rendering viable the viral DNA without completely avoiding mutations.[16]

A last factor of variability, whose importance has probably been overlooked, is oxidative stress (see below), a cause of RNA and DNA mutations (before integration of the proviral DNA): highly reactive molecules derived from oxygen can oxidize the bases, particularly guanine or deoxyguanine, thus modifying their coding capacity or inducing a wrong replacement in repair.

A combination of these factors could explain both the intrinsic variability of the virus in the host during the long evolution of infection, and also the increasing variability of the circulating strains as the epidemic spreads in various populations. We can at least act on this variability by decreasing the viral multiplication rate inside the host by antiretroviral treatment and also by neutralizing the oxidative stress. However, some problems remain.

How HIV Infection Results in the Destruction of the Immune System.

In the early years following the virus discovery, it was generally thought that the drop in $CD4^+$ T cells was due to their direct infection by a cytopathic virus. In fact, the viral isolates (like Bru) made in the early stage of the disease are

not cytopathic, they use, after binding to the $CD4^+$ receptor of activated lymphocytes, a co-receptor (CCR5) which is the receptor for a chimiokine. Only viruses isolated from patients at the late stage of the disease are cytopathic (like Lai) and their direct infection of the remaining T lymphocytes (by using another chemokine co-receptor CXC4) could account for the final drop of these cells.

In fact, the number of activated $CD4^+$ T lymphocytes (the ones which only allow full replication of the virus) is probably a limiting factor of the initial infection, after the first contact with dendritic cells and monocytes of genital or rectal mucosa. It is obvious that inflammation and co-infections (bacterial, viral) could increase the number of activated T lymphocytes and therefore could increase the risk of HIV infection. Recently, the virus has been found associated with the Peyer patches existing around the small intestine, which constitutes a major source of activated T lymphocytes.

At the onset of infection, virus replication is high in all the lymphatic tissues, taking advantage of the delay in the reaction of the immune system (in time order: interferon, NK cells, CD8T cells, antibody response) and then decreases while persisting in some lymph nodes (Figure 9).

This is the beginning of the chronic phase which is generally asymptomatic, although the lymphadenopathy is often present. It has been shown that the virus replication continues in the lymph nodes, despite the immune response. This starts declining, although there is a continuous renewal of T lymphocytes, both $CD4^+$ and $CD8^+$, which could last for years.

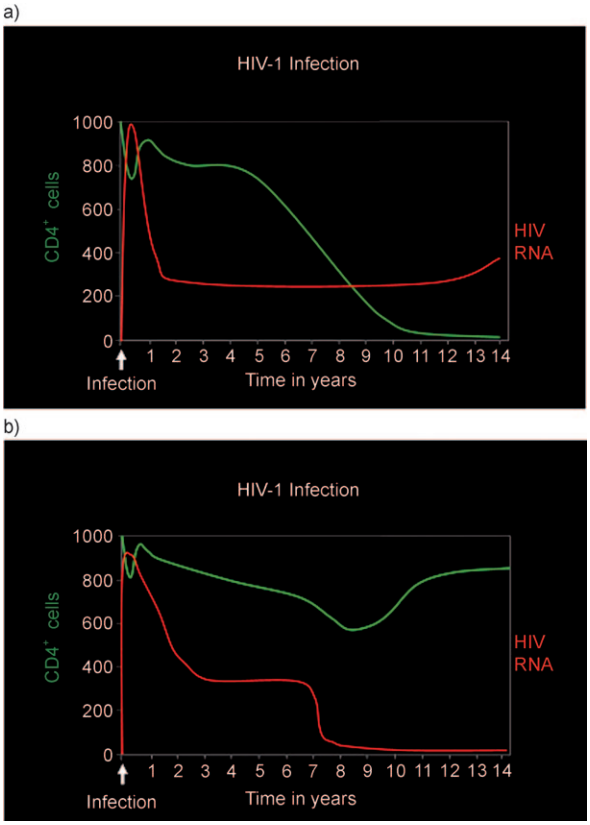

Figure 9. Evolution of HIV-1 infection in AIDS: a) Untreated patients; b) patients treated by antiretroviral therapy at year 6.

During this period, we have found two phenomenons which could help to explain the indirect destruction of the immune system: one biological, namely apoptosis, and one biochemical, namely oxidative stress

Apoptosis: my laboratory was the first to describe this programmed cell death in white blood cells cultured in medium deprived of interleukin 2.[17] All the subsets, not only the $CD4^+$ T cells, were affected when taken from the blood of asymptomatic HIV patients, as well as in patients presenting with full blown AIDS: $CD8^+$ T cells, NK cells, B lymphocytes, monocytes. However, we found a good correlation between the drop in $CD4^+$ T cells in patients and this in vitro phenomenon[18] We surmised that in the in vivo situation, cells were still alive, but in pre-apoptosis. Indeed, we could detect in infected patients a general phenomenon of immune activation,[19] which has now been well recognized as a major factor of AIDS pathogeny.

At the biochemical level, we also showed that the lymphocyte population of asymptomatic patients ($CD4^+$, $CD8^+$, NK) displayed the biochemical signs of oxidative stress (excess of free radicals derived from oxygen): namely fast degradation of oxidized protein and carbonylation of some of their amino acids.[20] In the patients' blood, we could detect similarly a hyperoxidation of plasma lipids[21] and oxidization of guanine.

What could be the origin of this strong oxidative stress? At least one HIV protein may contribute to it. It was shown by C. Flores, McCord, and their collaborators that the Tat protein, among many functions, inhibits the expression in lymphocytes of the Mn-dependent superoxyde dismutase gene.[22] This enzyme is key to transforming the anion superoxide, highly oxidant, into hydrogen peroxide. Tat has been shown to circulate in nanogram amounts in the blood of infected patients and to penetrate inside cytoplasm.

In addition, bacterial and viral co-infectors can also induce oxidative stress. We have been studying the possibility that a "cold" persisting bacterial infection could co-exist in HIV-infected patients. These studies were initiated because we observed that in vitro co-infection of lymphocytes with some mycoplasma species (*M. pirum*, *M. penetrans*, *M. fermentans*) and HIV could greatly reinforce the cytopathic effect of the latter. Moreover, these small bacteria lack catalase, an enzyme able to convert hydrogen peroxyde into water. Therefore, they also generate oxidative stress and, furthermore, are activators of lymphocytes.[23]

In summary, the pathophysiology of AIDS is complex. HIV is the main cause, but could also be helped by accomplices, and also have some indirect effects by wrongly activating the immune system through oxidative stress.

Prospects for the Future—No cure, No vaccine, but Maybe a Cure by a Vaccine

The advent of HAART has transformed AIDS into a tolerable infection, but whatever the length of the treatment the inhibitors used have not reached the level of a cure! As soon as this treatment is interrupted, virus multiplication

resumes within a few weeks and the immune system declines again.

This observation led researchers to think that there is a reservoir of virus, to which the drugs have no access,[24] probably because the virus stays in a latent form in some tissues.

Our project is to design quantitative tests to evaluate the size of this reservoir and to prevent it from giving rise to actively multiplying virus, by boosting the immune system against the most conserved parts of viral proteins.

A possible protocol for this therapeutic immunization, aimed at achieving a functional eradication of HIV,[25] could be the following:

1) First, antiretroviral therapy (HAART) for 3–6 months to reduce viral load in the plasma to undetectable levels and maintain it until the protocol has been terminated.
2) Then, treat with antioxidants and immunostimulants, such as an orally absorbable form of glutathione, to reduce the oxidative stress induced by viral proteins and by HAART. Reduced glutathione is known to induce a shift from Th2 to TH1 responses, therefore reinforcing cell-mediated immunity. Its effect can be enhanced by some synthetic immunostimulants, which are now close to approval for clinical use by regulatory authorities.
3) After two week treatment with the former products, start specific immunization against HIV proteins with a therapeutic vaccine. Trials with vaccine preparations made for therapeutic use have already been carried out, with mixed results—probably because the immune system of the patients was not sufficiently restored, and/or also due to the inadequacy of the immunogens. Our data from genetic engineering indicate that the native HIV glycoprotein must be modified in order to make immunogenic the most conserved parts of the protein, including the pocket involved in HIV binding. This will result in a neutralization capacity broad enough to cover potential escaping mutants. I advise also to add in the vaccine preparation two other proteins involved in immunosuppression, Tat and Nef, also modified to become nonfunctional while remaining immunogenic.
4) After this vaccination, interrupt HAART. If the protocol has been successful, there will be no virus rebound, as evidenced by a low viral load and an increase of the $CD4^+$ T-cell component. Regular monitoring of these two parameters will assess the durability of the immunization. A strong cell-mediated immunity, in addition to the induction of neutralizing antibodies, will permit interruption of a cycle of new cell infections by newly formed viral particles. This control already exists spontaneously in a small number of HIV-infected patients, which show no immune depression, even after many years.

This protocol is complex, but will be less expensive and for the patient much more tolerable than a life-long antiretroviral therapy. The protocol can also be applied to patients in the early stages of HIV infection, perhaps with a better chance of success, as their immune system will have a better ability to respond.

If, in this optimistic scenario, HIV infection becomes a curable disease, the impact on the epidemic itself will be considerable: In developing countries, HIV infection represents a stigma for the familial and professional life. Many infected individuals do not want to be tested and to learn their status, and as a consequence, they keep transmitting the virus to new partners. The perspective of being treated for a cure immediately after the diagnosis of HIV infection will ease early testing and the emergence of responsible behavior.

Moreover, the success of a therapeutic vaccine will facilitate the design of an efficient preventive vaccine based on the same viral components.

Meanwhile, it will be essential to make accessible the use of antiretroviral drugs to all patients who are eligible for them. This implies not only an international effort to lower the price of the drugs, which already has been partly achieved, but also a comparable effort to create adequate medical structures with trained doctors and research centers in developing countries. Our Foundation has chosen the mission to contribute to achieving these tasks in Africa. The World Foundation for AIDS Research and Prevention, in association with UNESCO and local governments, has created two Centers for AIDS Research and Prevention: The "Centre Intégré de Recherches Biocliniques d'Abidjan" (CIRBA; Figure 10) in Abidjan (Ivory Coast) and the International

Figure 10. CIRBA in Abidjan (founded in 1996).

"Chantal Biya" Reference and Research Centre for HIV-AIDS Prevention and Care-Taking (CIRBC) in Yaoundé (Cameroun).

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