25 YEARS AFTER HIV DISCOVERY: PROSPECTS FOR CURE AND VACCINE

by

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The impressive advances in our scientific knowledge during 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 emerged early, based on an extraordinarily 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. Environmental pressure has of course both maintained these inventions and also modulated them over the generations, through the deaths of individuals and sexual reproduction. For the last 30,000 years, our biological constitution has not changed: a hypertrophic cortical brain, a larynx to speak and a hand to manipulate. But for the last 10,000 years, another memory has emerged, which make our species quite different from the others: this is the cultural memory which transmits knowledge and societal organisation from generation to generation, through the use of language, writing and more recently virtual means of communication.

This revolution occurred in various sites on the earth almost simultaneously through sedentarisation of human populations by agriculture, leading to several civilisations. Each human being thus receives two pieces of luggage: genetic memory at birth and cultural memory during all his life, and he will become a real human only if he is benefiting 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, auto-immune diseases, diabetes) linked to the increase in life expectancy and environmental changes related to human activities.

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

Moreover, other new epidemics should not be ruled out as human activities generate more favourable factors:

- Lack or loss of hygiene habits
- lack of water
- globalisation and acceleration of exchange and travel
- atmospheric and chemical pollution leading to oxidative stress and immune depression
- malnutrition, drug abuse and ageing, also leading to immune depression
- global warming leading to new ecological niches for insect vectors
- changes in sexual behaviours.

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 continents, recently including the 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 non-human primates, it is tempting to consider AIDS as a zoonosis, resulting from the transmission to humans of related viruses infecting primate species without causing disease.

But let us first recall the circumstances of the discovery of HIV in my laboratory at the Institut Pasteur (Figure 1).

Figure 1. The Pasteur team involved in the discovery of HIV in 1983–1984.

AIDS as a pathologically distinct entity was first identified in June 1981 by members of the CDC (particularly Harold Jaffe and 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 and related to sexual intercourse.

Following the 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 recognised that a similar disease, characterised 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.

The case of haemophiliacs gave 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 donated blood which had been filtrated by 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 patients. This is why I became interested in a search for viruses; but what kind of viruses? Many viruses have immunodepressive 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 biography Chapter I), we embarked on the search for an HTLV-like virus, at the suggestion of the French

working group and also encouraged by Institut Pasteur Production, an industrial subsidiary of the Institute producing a hepatitis B vaccine from pool of plasmas from blood donors.

Knowing that retroviruses are usually expressed in activated cells, I set up classical conditions to culture activated lymphocytes, using first a bacterial activator of both T and B lymphocytes, Protein A, since I did not know in what subset of cells the virus was hiding out.

The reasoning at that stage was that we should look first at lymphocytes from swollen lymph nodes, supposedly the site where viruses accumulate in the early phase of infection.

On January 3, 1983, I received a biopsy of a patient with cervical adenopathy, a symptom already recognised 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 detected by its reverse transcriptase activity, measured by my associate Françoise Barré-Sinoussi. Only some 9 months later could I also show 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 characterisation of the virus, and showed for the first time that it was different from HTLVs. A p24-25 protein could be immuno-precipitated by the serum of the patient and not by antibodies specific to 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 in horses, visna virus in sheep, etc.), but different from HTLV. Unlike the case of HTLV, we never saw the emergence of permanent transformed lines from the infected lymphocyte cultures (Figure 2).
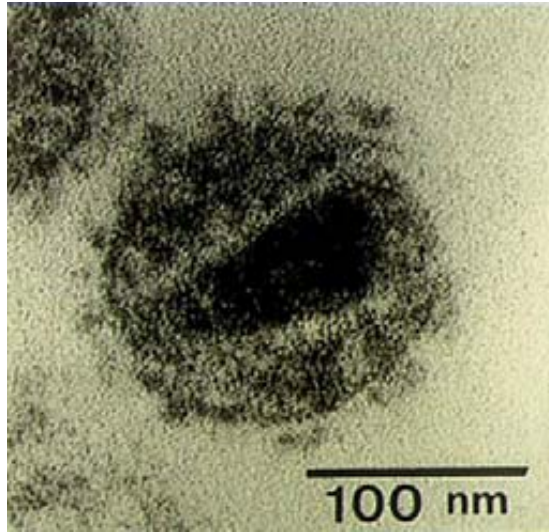
Figure 2. HIV through the electron microscope.

These results were published in a *Science* paper in May 1983 (1), together with two papers by the Gallo and Essex groups in favour 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.

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

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	♀	Zaïre, african	AIDS	+

Table 1.

2) Besides immune-precipitation 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 3). 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 by contrast could be grown in T cell lines (CEM, HUT78) in laboratories which received our Bru isolate at their request.
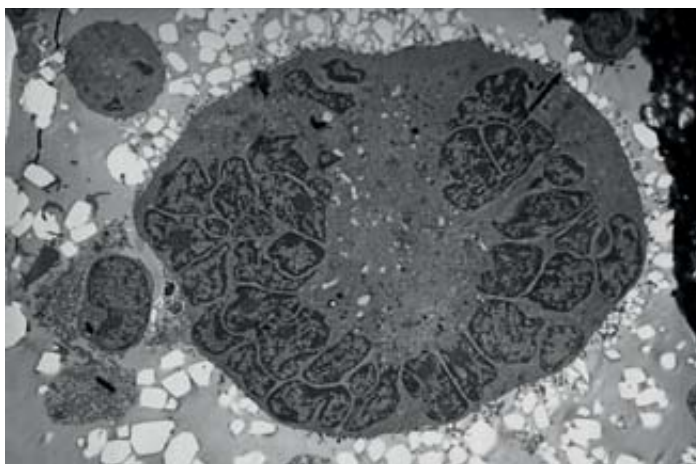

Figure 3. Electron micrography picture of a giant cell (syncitium) resulting of the fusion of many lymphocytes expressing the HIV fusion protein (6).

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.

Our data, which I presented in September 1983 at a meeting on HTLV in Cold Spring Harbor (6), were met with scepticism. Only in the spring of 1984 did the description of a quasi identical virus under the name of HTLV III by the R. C. Gallo group (7) convince the scientific community that this new retrolentivirus was the cause of AIDS. The Jay Levy group 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 helped himself to uncover earlier.

But in addition, new genes (Tat, Nef), important in regulation of the expression of the viral genetic information, were recognised from the DNA sequencing, making the viral genome probably the most complex known in the retrovirus family (Figure 4). HIV and its primate cousins are therefore a well-characterised entity only composed of DNA sequences, none existing in the human genome.
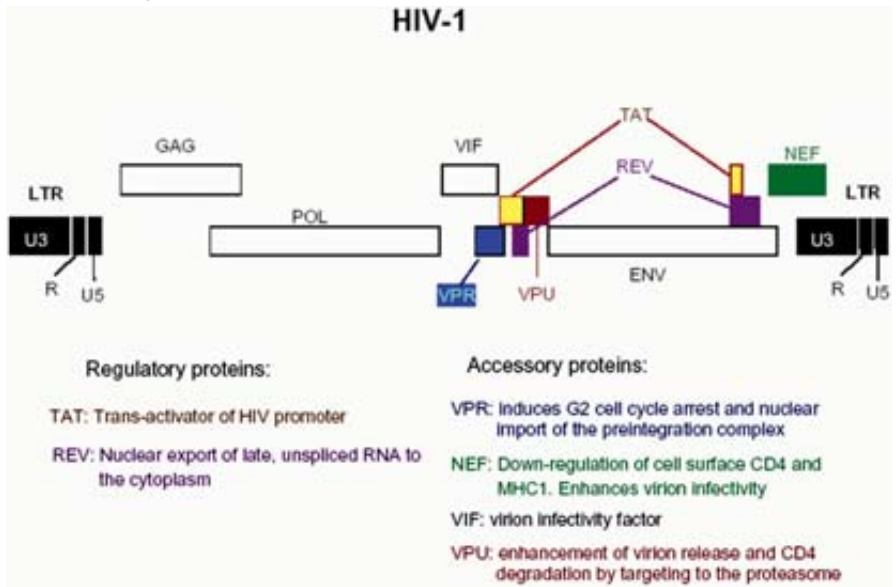

Figure 4. Genome structure of HIV1: gag, pol, env are the gene codes for the structural proteins.

A posteriori, two facts should have provided to the few remaining sceptics final proof 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 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 our collaboration with Portuguese colleagues, we isolated a second virus (which I named HIV2), from West African patients hospitalised 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 5).
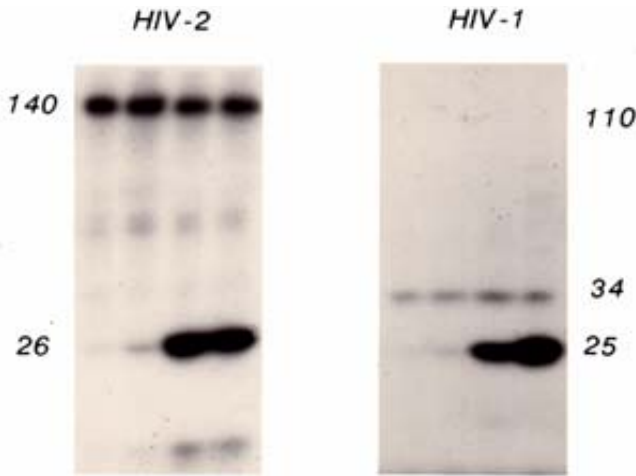
Figure 5. Immunoprecipitation of radioactively labelled proteins of HIV1 and HIV2 by a serum of an AIDS patient infected by HIV2; note in the HIV2 panel the precipitation of the envelope protein.

The isolation of HIV1 (6) and HIV2 (12) viruses from AIDS patients in Africa made us realise 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 non-haemophilic children: none of the latter was found HIV positive (13).

The isolation of the virus causing AIDS allowed the implementation of rational prevention measures and also the beginning of a search for efficient viral inhibitors.

The first candidate, azidothymidine, was an efficient inhibitor of HIV reverse transcriptase in *in vitro* experiments (Broder, Mitsuya *et al.*); and however, its use in AIDS patients, first looking promising, was later recognised 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 to 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 explains the complexity of the pathophysiology of AIDS.

Only a combination of three inhibitors proved to be efficient in 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 number, preventing them most of the time from contracting lethal opportunistic infections (15).

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
1986	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 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

Table 2.

HIV VARIABILITY

In fact, in order to escape the immune reactions of their hosts, most viruses have a strategy of changing their immunogenic epitopes. In the case of HIV, a conjunction of several factors put this at 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 neutralisation 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 $1/10^9$ 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 variation factor 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 in 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... 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 variability factor, 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 oxidise 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 is spreading 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 neutralising the oxidative stress.

THE REMAINING PROBLEMS:

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; after binding to the CD4+ receptor of activated lymphocytes, they use a co-receptor (CCR5) which is the receptor for a chimiokine.

Only viruses isolated from patients at late stages 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 in 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 in 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, the 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 6).
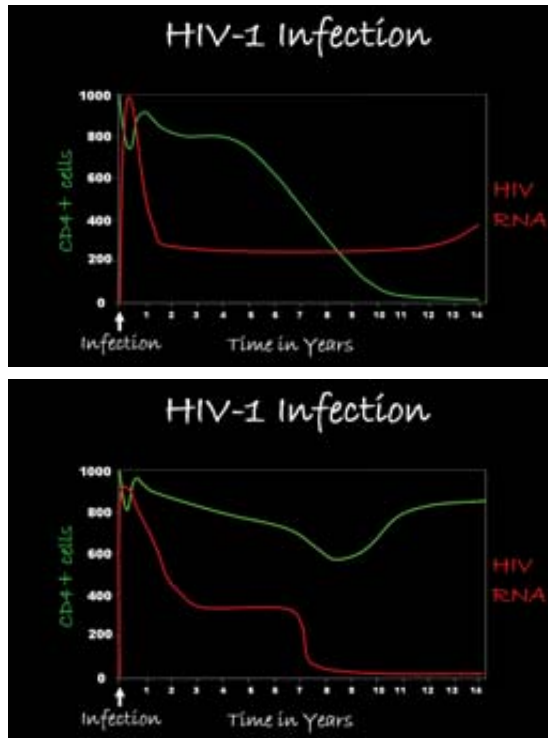

Figure 6. Evolution of HIV-1 infection in AIDS. Left: Untreated patients; Right: Patients treated by antiretroviral therapy at year 6.

This is the beginning of the chronic phase, which is generally asymptomatic, although 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.

During this period, we have found two phenomena which could help explain the indirect destruction of the immune system:

One biological: apoptosis

One biochemical: oxidative stress

Apoptosis: my laboratory was the first to describe this programmed cell death in white blood cells cultured in a 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 from 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 *in vitro* phenomenon (18) We surmised that in the *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 become well recognised 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 oxidised proteins, carbonylation of some of their amino acids (20). In the patients' blood, we could detect a similar hyper-oxidation of plasma lipids (21) and oxidisation 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 superoxide dismutase gene (22). This enzyme is key to the transformation of 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 studied 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 peroxide 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 schematic protocol of this therapeutic immunisation, 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, treatment by 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 Th_2 to TH_1 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 a two-week treatment by the former products, start specific immunisation against HIV proteins by a therapeutic vaccine. Trials with vaccine preparation made for a therapeutic use have already been carried out, with mixed results probably because the immune system of the patients was not sufficiently restored, or/and also due to the inadequacy of the immunogens. Our genetic engineering data 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 neutralisation capacity broad enough to cover potential escape mutants. I also advise adding during vaccine preparation two other proteins involved in immunosuppression, Tat and Nef, modified to become non functional 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 immunisation. A strong cell-mediated immunity, in addition to the induction of neutralising antibodies, will permit the 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 it will be less expensive and for the patient much more tolerable than 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 family and professional life. Many infected individuals do not want to be tested and to learn their status, and as consequence, they keep transmitting the virus to new partners. The prospect of being treated and cured immediately after the diagnosis of HIV infection will ease early testing and emergence of responsible behaviours.

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 these drugs, which has already been partly achieved, but also a comparable effort to create adequate medical structures with trained doctors and research centres in developing countries. Our Foundation has chosen the mission to contribute to fulfilling these tasks in Africa.*

Figure 7. Centre Intégré de Recherche Bioclinique d'Abidjan (CIRBA), created in 1996.

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* The World Foundation for AIDS Research and Prevention, in association with UNESCO and local governments, has created two Centres for AIDS Research and Prevention; The "Centre Intégré de Recherches Biocliniques d'Abidjan" – CIRBA (Figure 7) in Abidjan (Côte d'Ivoire) and the International "Chantal Biya" Reference and Research Centre for HIV-AIDS Prevention and Care-taking" (CIRBC) in Yaoundé (Cameroon).

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