

L'UNE DES TOUTES PREMIÈRES PHOTOGRAPHIES DU VIRUS VIH-1 RÉALISÉE LE 4 FÉVRIER 1983. © INSTITUT PASTEUR/CHARLES DAUGUET

# 40 YEARS AFTER THE DISCOVERY OF **HIV**

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PRESS KIT

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## EDITORIAL

**CHRISTOPHE D'ENFERT**  
SENIOR EXECUTIVE SCIENTIFIC VICE-PRESIDENT  
OF THE INSTITUT PASTEUR

Research on the HIV/AIDS epidemic – which continues to be a major public health issue, with 1.5 million new infections worldwide in 2020<sup>1</sup> – is now a 40-year-long tradition at the Institut Pasteur. The story began when the HIV retrovirus was identified in 1983, and continued with the award of the 2008 Nobel Prize in Physiology or Medicine to Françoise Barré-Sinoussi and Luc Montagnier for this discovery. Another milestone was the sequencing of HIV-1 in 1985, then HIV-2 a few years later, which would pave the way for different therapeutic approaches. The sequencing of reverse transcriptase, a crucial enzyme for viral replication, led to the first antiretroviral drug, AZT, in 1987.

Today, 40 years after the identification of HIV, a wealth of knowledge has been accumulated on its mechanism of action, the way in which it attacks immune cells and effectively spreads from one cell to the next, and also the ability of some people living with HIV to mount an effective defense. An international scientific conference set to take place at the Institut Pasteur from November 29 to December 1, 2023, will take stock of the latest advances in research.

But two scientific problems remain unanswered: how can we develop an effective vaccine against HIV, and how can we eliminate the persistent viral reservoir in people living with the virus? Several research teams at the Institut Pasteur and in the international Pasteur Network are working on the latter issue, and clinical trials will begin in 2023 to test novel broadly neutralizing antibodies and promising natural killer cells.

In a context dominated by the recent COVID-19 pandemic, and more recently by the monkeypox epidemic, it is more important than ever that we pool our research efforts. The knowledge accumulated on HIV, especially diagnostic tools and techniques for analyzing and producing broadly neutralizing antibodies, proved useful in tackling SARS-CoV-2 and monkeypox. We now have to hope that the knowledge gained about these novel viruses can help us solve the mystery of viral reservoirs so that we can offer lasting remission or even a cure for HIV infection.

<sup>1</sup> - UNAIDS.



Françoise Barré-Sinoussi in 1987.

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# FRANÇOISE BARRÉ-SINOUSSI'S SOUVENIR ALBUM INTERVIEW

«**H**IV changed everything for me...» confirms Françoise Barré-Sinoussi, a retrovirologist at the Institut Pasteur, laureate of the Nobel Prize in Physiology or Medicine and President of the French HIV/AIDS charity Sidaction, as she looks at a selection of photos charting the 40 years that followed the discovery of HIV at the Institut Pasteur. «I was a fairly conventional scientist, very focused on basic laboratory research, working on the links between retroviruses and cancer, but I had no direct contact with patients or with clinicians and healthcare workers. The discovery of HIV and my research on the virus brought me closer to these people and helped me align my work more closely to their needs,» she explains.

## FROM BASIC RESEARCH TO PATIENTS

«We had detected the presence of retroviral enzyme activity in the culture supernatant, but we needed to visualize the virus under a microscope,» continues the scientist. This tricky task fell to Charles Dauguet, a microscopist in the laboratory. «We gave him some indications: he needed to look for viral particles with a diameter of approximately 100 nanometers, with an envelope, and see budding at the surface of the lymphocytes in culture,» remembers Françoise Barré-Sinoussi. «Charlie spent hours and hours looking for the virus, and eventually he called us saying «I think I've got it, come and see!».» The first photo of the virus was taken on February 4, 1983, and on May 20, 1983 the first paper on the novel human retrovirus was published in the journal Science.

## THE HUNT FOR A NOVEL RETROVIRUS

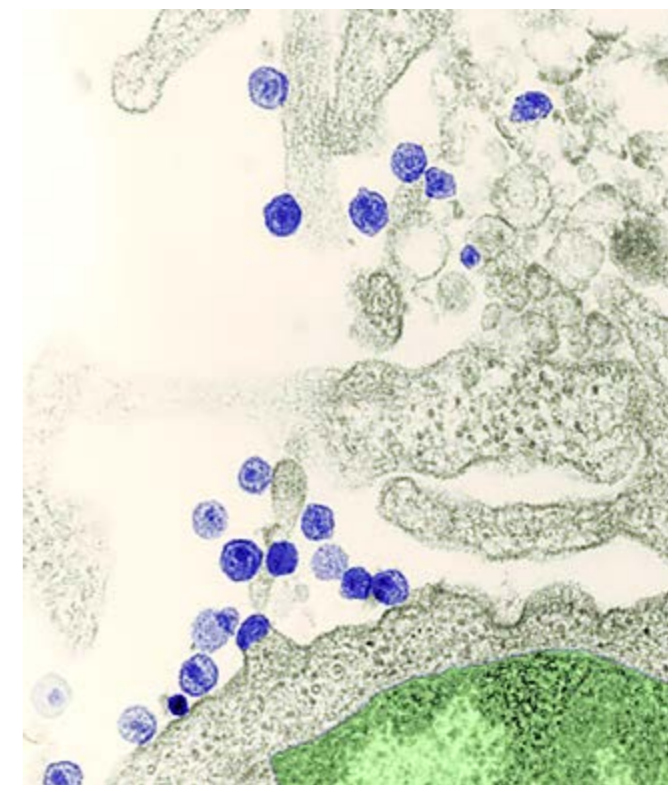
It all began in late 1982. The scientist was working in Jean-Claude Chermann's laboratory, in the Viral Oncology Unit led by Luc Montagnier. In December 1982, Chermann and Montagnier were contacted by a group of clinicians and virologists at Bichat hospital, including Willy Rozenbaum and Françoise Brun-Vezinet, who were in charge of the first patients with HIV in France. The physicians hoped that the Institut Pasteur scientists could identify the infectious agent behind the new disease, which they thought might be a retrovirus similar or related to HTLV.<sup>1</sup> In January 1983, a first lymph node biopsy from an HIV patient who had not yet developed AIDS was sent to the laboratory. The investigation could begin. Marie-Thérèse Nugeyre, Françoise Rey, Jean-Claude Chermann and Françoise Barré-Sinoussi worked in collaboration with Luc Montagnier's team in the hunt for a retrovirus.

In the cell culture supernatants, Françoise Barré-Sinoussi was trying to detect activity of a specific retroviral enzyme known as reverse transcriptase. Retroviruses rely on reverse transcription to replicate in order to convert their RNA into DNA for integration into the genome of the infected cell. «We tested different reverse transcriptase activities with different reagents,» remembers Françoise Barré-Sinoussi, flicking through the pages of her old lab notebook. «And it happened incredibly quickly: we performed the first tests on January 12, 1983, and we had already found evidence of significant reverse transcriptase activity by January 27.» In the space of just a few days, a novel human retrovirus, initially named «lymphadenopathy-associated virus» (LAV), had been detected.



Jean-Claude Chermann, Françoise Barré-Sinoussi and Luc Montagnier at the Institut Pasteur in 1986.

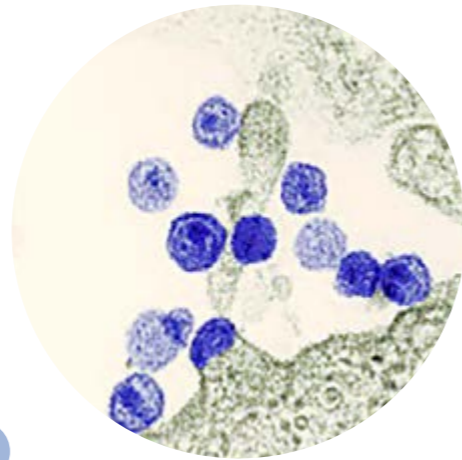
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One of the very first photographs of the HIV-1 virus (colored blue) taken on February 4, 1983.

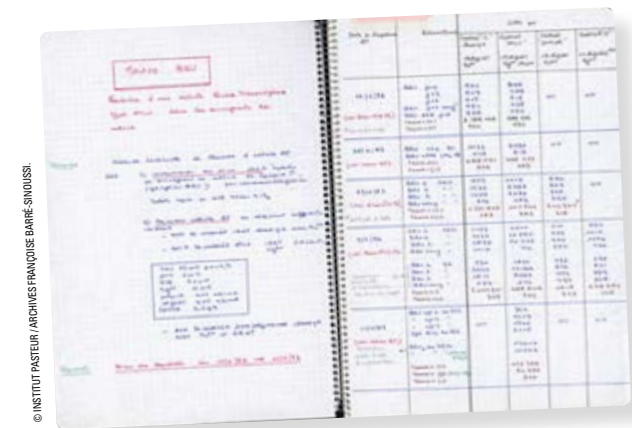
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«As soon as the virus was identified in 1983, and once we had gathered enough evidence – that same year – confirming that the virus was responsible for AIDS, we quickly started to develop diagnostic tests, characterize the virus, consider tools for treatment and think about vaccine candidates. There was a huge amount to do,» admits Françoise Barré-Sinoussi. To tackle this vast undertaking, the small team led by Jean-Claude Chermann and the team headed by Luc Montagnier began working with immunologists, molecular biologists, clinicians and hospital virologists. A partnership was formed with Françoise Brun-Vezinet and Christine Rouzioux, virologists at Bichat hospital, to develop diagnostic blood tests for infected patients, which were brought to market in 1985. Isolation of the virus, renamed human immunodeficiency virus (HIV) in 1986, was the starting point for intensive international research efforts to respond to this new emerging infection.



For the time, it was remarkable to have images like that, showing the virus budding at the lymphocyte surface.

FRANCESCA DI NUNZIO, HEAD OF THE INSTITUT PASTEUR'S ADVANCED MOLECULAR VIROLOGY LABORATORY



Françoise Barré-Sinoussi laboratory notebook.

### CHARLES DAUGUET, THE FIRST SCIENTIST TO VISUALIZE THE VIRUS, TELLS HIS STORY



It happened on Friday February 4, 1983, at 5.45pm. Late in the afternoon, as I was letting the electron microscope cool down before turning it off, I saw a virus under the screen. I ran out of the lab, shouting «I've got it, I've got it!». If anyone had seen me in the corridor at that point they might have wondered if I was in my right mind. I immediately took several photos.

After that I barely took my eyes away from the electron microscope. It was often tiring, my eyes got very tired, but it was productive. When I examined the viral cultures, I saw the virus binding to the cell surface, sometimes disappearing inside, which was a sign of infection. The budding process, a sign of productive infection, was easily visible. And of course, I also saw cells with altered morphology and others that had been killed by the virus.

EXTRACT OF THE ACCOUNT BY CHARLES DAUGUET, AS TOLD TO HUGUES FLEURY AND SIMON WAIN-HOBSON



Odile Croissant and Charles Dauguet with the electron microscope used to take the first pictures of HIV.

### YEARS OF RESEARCH RECOGNIZED WITH THE NOBEL PRIZE

In 1988, Françoise Barré-Sinoussi took over as head of the Institut Pasteur's retrovirus biology laboratory. She worked in particular on the genetic variability of HIV in collaboration with Institut Pasteur institutes in Africa, then South-East Asia. With Marc Girard, Head of the Institut Pasteur's Molecular Virology Unit, she was involved in research on development of a vaccine candidate. But as the years passed, the retrovirologist became convinced that finding an effective vaccine would only be possible with a thorough understanding of the virus' pathophysiology. It was at this point that the laboratory changed its orientation. It began to focus on the mechanisms of pathogenesis, (2) with Michaela Müller-Trutwin studying African monkey models that did not develop AIDS and Asier Sáez-Cirión investigating cohorts of patients that were able to control HIV. In 2008, 13 years after the discovery of HIV, the work of Françoise Barré-Sinoussi and Luc Montagnier was recognized when they were awarded the Nobel Prize in Physiology or Medicine. Françoise was at the Institut Pasteur du Cambodge when she heard the news. «After the announcement, the news spread through the streets of Phnom Penh. A number of people arrived at the Institut Pasteur, including some living with HIV who I knew. They hugged me, brought me flowers,» remembers Françoise Barré-Sinoussi. «It was an incredibly moving moment, and that was when I realized that it was not my Nobel Prize but our Nobel Prize, that it belonged to the whole community that had been fighting the virus for years.»

- 1 - the first oncogenic human retrovirus to be discovered
- 2 - the study of mechanisms resulting in disease.



I remember my first day at the laboratory: my interest was focused on the molecular biology of HIV, but I was convinced that I would only truly understand the virus by getting closer to patients. When I shared my thoughts with Françoise, she replied, «You're in exactly the right place – here we're interested in people.» And it's something that she showed throughout her career.

ASIER SÁEZ-CIRIÓN, HEAD OF THE INSTITUT PASTEUR'S VIRAL RESERVOIRS AND IMMUNE CONTROL UNIT



I started my pharmaceutical studies when HIV was discovered at the Institut Pasteur by Françoise Barré-Sinoussi and her colleagues. That great discovery made me want to work on viruses, and it went on to shape my entire career.

OLIVIER SCHWARTZ, HEAD OF THE INSTITUT PASTEUR'S VIRUS AND IMMUNITY UNIT



Françoise Barré-Sinoussi and members of the Regulation of Retroviral Infections Unit in October 2008, three days after the Nobel Prize was announced.



Photo taken in Cambodia in 2008 when the Nobel Prize was announced.



Photo taken during the interview at the Institut Pasteur in December 2022.

# HIV MILESTONES

## SCIENTIFIC BREAKTHROUGHS

- 1981**
  - First cases of AIDS described in the United States
- 1985**
  - First International AIDS Conference held in the United States
- 1987**
  - First antiretroviral drug, AZT, brought to market
- 1988**
  - First World AIDS Day

## DISCOVERIES INVOLVING THE INSTITUT PASTEUR

- 1983**
  - Isolation of the virus responsible for AIDS, HIV-1
- 1984**
  - Identification of CD4 T cells as the prime target of HIV
  - Sequencing of the HIV-1 genome and identification of viral genes
- 1985**
  - Publication of the whole genome of HIV-1
  - First HIV-1 screening test, Elavia, brought to market
  - Isolation of a second virus responsible for AIDS, HIV-2
- 1987**
  - Sequencing of the HIV-2 genome
  - Sequencing of the genome of SIVmac (simian immunodeficiency virus in rhesus macaques)
  - HIV-2 screening test brought to market

- 1990**
  - Identification of predictive markers of progression to AIDS
- 1994**
  - Discovery of the first broadly neutralizing antibodies (bNAbs), capable of neutralizing dozens of HIV variants
  - Identification of the role of HIV-specific CD8 cells in partially controlling infection
- 1996**
  - First triple-drug therapies (HAART) brought to market; this becomes the standard HIV treatment and leads to a global slowdown in the epidemic
  - Identification of HIV coreceptors CCR5 and CXCR4
- 1997**
  - Identification of the persistence of viral reservoirs in patients receiving treatment
- 2000**
  - International AIDS Conference held in Africa for the first time

- 1990**
  - Sequencing of the genome of SIVcpz (simian immunodeficiency virus in chimpanzees)
- 1994**
  - AZT shown to be effective in preventing mother-to-child transmission of HIV during pregnancy
- 1998**
  - Identification of HIV-1 group N (the first HIV-1 group closely related to SIVcpz)
- 1999**
  - Discovery of the origins of HIV when SIVcpz is shown to be the animal reservoir of HIV-1

## 1985 > Sequencing of HIV-1

The situation was rather worrying, like at the beginning of the COVID crisis, because a new disease had emerged, caused by an unknown pathogen, and people were dying from it. At the time, I was leading a team in the Genetic Engineering Group at the Institut Pasteur. The sequencing that we performed revealed all the HIV-1 genes and helped speed up the development of tools for diagnostic and vaccine strategies. Back in 1983, there was no PCR – sequencing, together with much of the analysis that is now performed entirely by computers, was done manually! It was a stimulating time, even quite exhilarating – the team was very driven and worked non-stop to rise to the challenge. Of course it should be a source of great pride for us – Institut Pasteur scientists played a major, decisive role from the beginning of the pandemic. The diagnostic tools had multiple applications and helped save lives.

STEWART COLE, PRESIDENT OF THE INSTITUT PASTEUR

## 2000 > International AIDS Conference in Africa for the first time

I remember the International AIDS Conference in Africa (Durban) in 2000. The conference was attended by thousands of scientists, as well as activists, artists, people living with HIV, representatives of pharmaceutical companies, decision-makers, frontline healthcare workers, political leaders, members of the clergy, judges and ordinary citizens. The conference had a major impact on access to antiretroviral therapy worldwide. It was quite remarkable to see that such a political impact could be achieved by everyone gathering together at a scientific conference.

MICHAËLA MÜLLER-TRUTWIN, HEAD OF THE HIV, INFLAMMATION AND PERSISTENCE UNIT AT THE INSTITUT PASTEUR.

# HIV MILESTONES

## SCIENTIFIC BREAKTHROUGHS

2001

- Authorization to manufacture **generic drugs** for developing countries

2002

- Identification of the first **viral restriction factors**

2009

- Description of the **first case of probable cure** of HIV infection (Berlin patient)
- **Improvement of single-dose** combination therapy
- Identification of **cytokine storm** during primary infection

2011

- Confirmation that there is **no risk of transmission** of the virus in individuals taking antiretrovirals who maintain an undetectable viral load (*Treatment as Prevention or TasP*)
- **Identification and characterization of second-generation bNAbs** that are more potent and offer broader coverage

2012

- **First effective antiretroviral cocktail** to prevent transmission (pre-exposure prophylaxis or PrEP)

## DISCOVERIES INVOLVING THE INSTITUT PASTEUR

2004

- Demonstration of the major influence of **genetic factors** on HIV control in «**natural controller**» patients in the absence of treatment

2005

- Confirmation of the role of **chronic inflammation** in progression to AIDS

2007

- **Demonstration** of the role of **CD8 T cells** in the ability of «**natural controllers**» to control infection in the absence of treatment

2008

- **Nobel Prize in Physiology or Medicine** awarded to Françoise Barré-Sinoussi and Luc Montagnier for the discovery of HIV

2010

- **Demonstration** of the benefits of **controlling inflammation** in the first weeks after infection

2015

- HIV home **blood test kit** available in pharmacies

2019

- **Second case** of probable cure of **HIV infection** (London patient)
- **Monoclonal antibody** targeting the HIV CD4 receptor that inhibits target cell infection (to treat multidrug-resistant HIV) brought to market

2020

- First probable case of **natural cure** in a «**natural controller**» in whom no trace of infectious HIV is found in the absence of treatment

2021

- First **injectable antiretroviral** therapy (in the United States, PrEP also becomes available in injectable form)
- **HIV home saliva test** available in pharmacies in France

2013

- Description of the **VISCONTI cohort**, a group of «**post-treatment controllers**» in HIV remission

2015

- **First case of extended remission** (12 years) in a child infected with HIV

2016

- Demonstration that some «**natural controllers**» have **CD4 T cells** that are better able to recognize certain HIV fragments

2017

- Demonstration of the role of **NK cells** in controlling the **viral reservoir** in lymph nodes

2019

- **Demonstration** of the role of **cell metabolism** in establishing infection

2022

- **Demonstration** that mutations on the **CCR5 coreceptor** influence the ability of CD4 T cells to control the virus in some «**natural controllers**»

2023

- **Third case of probable cure** after a bone marrow transplant (Düsseldorf patient)

# WORDS ASSOCIATED WITH HIV/AIDS



## AIDS AND HIV

«AIDS is a late stage of HIV infection,» clarifies Asier Sáez-Cirión, Head of the Institut Pasteur's Viral Reservoirs and Immune Control Unit. «We really need to break the direct link between HIV and AIDS because it is an obstacle to eradicating HIV infection. This is our daily battle.»

In other words, all people with AIDS have HIV, but those with HIV do not necessarily have AIDS. And nowadays, antiretroviral therapy means that people living with HIV can lead a relatively normal life without ever developing AIDS.

On June 5, 1981

the US Centers for Disease Control (CDC) reported a rare form of pneumonia in young homosexual men in California. This was the first alert about AIDS.

1981

«gay plague»  
«gay syndrome»

The disease was originally described as GRID (gay-related immune deficiency), or the «gay plague» or «gay syndrome,» as it only seemed to be affecting homosexual men.

1981

«rare cancer seen in 41 homosexuals»

On July 3, 1981, the New *York Times* printed its first article on a «rare cancer seen in 41 homosexuals.»

1982

«mysterious cancer»

On January 6, 1982, the newspaper *Libération* published an article about a «mysterious cancer» seen in homosexual men in America.

1982

«gay syndrome»

On July 24, 1982, *Le Figaro* reported on AIDS, referred to at the time as the «gay syndrome.»

1982

«4H disease»

AIDS then came to be known as «4H disease» as it seemed to affect homosexuals, heroin users, hemophiliacs and Haitians, until it was realized that «those populations» were not the only ones affected.

1982

«AIDS / SIDA»

In 1982, the disease was renamed AIDS (acquired immunodeficiency syndrome).

1985

«virus of panic»

In 1985, fear of the virus began to take hold: *Le Point* referred to a «virus of panic.»

1986

«HIV / VIH»

In 1986, the virus responsible for AIDS, known as LAV in France and HTLV-3 in the United States, was officially named HIV (*human immunodeficiency virus*).

1987

«AIDS will not be passed on by me»

In 1987, a first *French information campaign about AIDS* was launched. The slogan, «AIDS will not be passed on by me,» caught public attention.

1991

«red ribbon»

The red ribbon became the international symbol of awareness for HIV/AIDS in 1991.

1993

«1 franc condom»

The «1 franc condom» *campaign* was launched in 1993 by the French Agency for the Fight Against AIDS (AFLS) to encourage condom use among young people.

2013

«90-90-90»

In 2013, UNAIDS set *new targets* to bring the HIV epidemic to an end by 2020: the 90-90-90 targets (90% of people with HIV diagnosed, 90% of people diagnosed receiving antiretroviral therapy and 90% of people receiving antiretroviral therapy with a suppressed viral load).

2016

«U=U»

An information campaign with the slogan *U=U* (Undetectable = Untransmittable) was launched globally in 2016.

Today

In France, for the year 2015, approximately 85% of patients with HIV were undetectable,<sup>1</sup> meaning that the virus could not be detected in their bloodstream. And the scientific evidence today is unequivocal: a person with an undetectable viral load cannot transmit HIV.

38 million people worldwide are currently living with HIV, and as many have died of AIDS-related causes since the beginning of the epidemic. In 2021, there were 1.5 million new cases worldwide.<sup>2</sup> In France, approximately 5,000 people discovered they were HIV-positive in 2020, and 26,000 are still living with HIV without knowing it.<sup>3</sup>

2030

«zero AIDS-related deaths by 2030»

The target of «zero AIDS-related deaths by 2030» is one of the international strategies (UNAIDS, UNICEF and WHO) for reaching the «end of the AIDS epidemic by 2030,» together with «zero new HIV infections» and «zero discrimination.»

1 - VIH.org. 2 - Onusida. 3 - Santé publique France.

# HIV, A MASTER OF EVASION

The HIV virus is microscopic, measuring more than a hundred times smaller than a cell. Like all viruses, it requires a host cell to replicate, but HIV does not infect just any cells in the body. Instead, it targets cells in the immune system, such as CD4 T cells and macrophages. The virus binds to CD4 surface proteins and releases a capsid containing two identical RNA strands into the cytoplasm of the cell. Similar to all retroviruses, the RNA of HIV must undergo reverse transcription into DNA to be integrated into the host cell's genome, allowing it to replicate and coexist with the host. From that point on, each time the cell divides, the virus is inherited by the daughter cells, giving it a highly effective Trojan horse. In summary, this is how HIV replicates.

But our knowledge of HIV is constantly evolving, and we are realizing that the mechanisms that regulate and influence HIV entry and replication in cells are still poorly understood. If we are to develop effective drugs or even try to produce a vaccine, it is crucial that we understand the pathogenesis of the virus, as Françoise Barré-Sinoussi, one of the scientists who discovered HIV, soon concluded. This is why the Institut Pasteur's teams are pursuing their research, and they are constantly making new discoveries. For example, the team headed by Elisabeth Menu, leader of the Mucosal Immunity and Sexually Transmitted Infection Control (MISTIC) group, is investigating the factors that regulate local inflammation (the menstrual cycle, microbiota composition, co-infections, exposure to seminal fluid, etc.) and influence susceptibility to HIV-1 in the mucosa of the female reproductive system. The team led by Francesca Di Nunzio, Head of the Advanced Molecular Virology Unit, recently demonstrated that the HIV capsid can pass through the pores of the host cell nucleus,<sup>1</sup> and that the viral RNA genome accumulates in the nucleus.<sup>2</sup>

## INSIDE INFECTED CELLS

«We are investigating the early stages of infection as it is becoming clear that they are crucial for the establishment of viral reservoirs, and the persistence of these reservoirs is a major obstacle to HIV cure,» explains Francesca Di Nunzio. «And nowadays we are able to observe living cells containing the HIV genome, taken directly from infected humanized mice from James Di Santo's laboratory (the Innate Immunity Unit).» Using a patented fluorescence microscopy method, Francesca Di Nunzio's team is able to monitor the evolution of viruses in the different cell compartments in vivo, in different organs. The scientists have demonstrated that uncoating and reverse transcription occur in the nucleus, and not in

the cytoplasm as previously thought. «We also observed that when a cell is invaded by several viruses, the viral RNA migrates to the nucleus and aggregates into highly dynamic «membraneless organelles» (HIV MLOs),» explains Francesca Di Nunzio.

Microscopy images show small puncta containing viral genomic RNA in the host nucleus, which group together over time to form large clusters where budding of viral DNA occurs.<sup>3</sup> «This is the first demonstration that RNA travels to the nucleus, that it is concentrated inside membraneless organelles for reverse transcription into DNA before being integrated into the cell genome,» marvels Francesca Di Nunzio. And the images are astonishing as they actually show spheres measuring a few hundred nanometers encapsulating viruses from the beginning of an infection in the nucleus of living cells. Since these organelles persist in some cells, they could potentially serve as markers for identifying infected cells in the viral reservoir. Moreover, they may represent a promising target for future treatments. For HIV is indeed a master of camouflage...



I have always found this virus fascinating. It is one of the «smartest» in the way it uses human cells.

FRANCESCA DI NUNZIO, HEAD OF THE ADVANCED MOLECULAR VIROLOGY LABORATORY

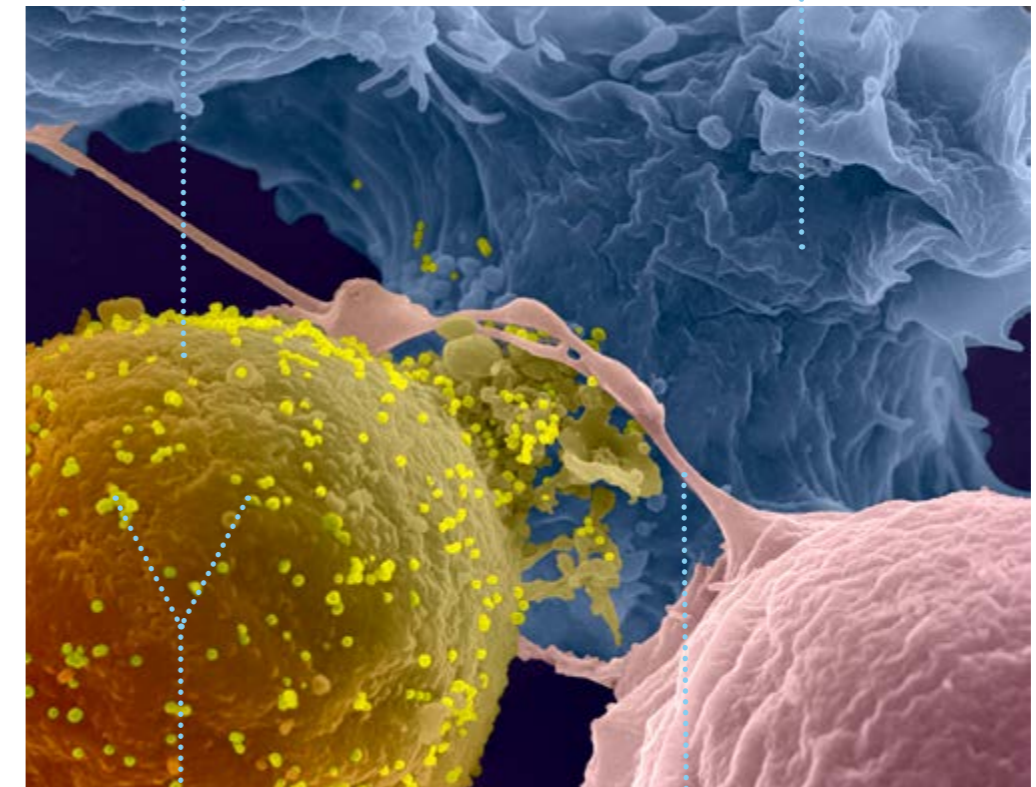


Immune cells provide the virus with a sort of Trojan horse that enables it to reach environments in which it either replicates actively or persists silently, without needing to do anything.

ASIER SÁEZ-CIRIÓN, HEAD OF THE VIRAL RESERVOIRS AND IMMUNE CONTROL UNIT

Lymphocytes are the main targets of HIV. It enters cells, hijacks the cell machinery to replicate, then buds from the cell surface before spreading and infecting other cells.

As soon as it enters the body, the virus accumulates in certain cells in the immune system, and in just two or three days it forms viral reservoirs that no drugs can destroy.



HIV viruses are a hundred times smaller than the lymphocytes they infect, measuring approximately 100nm.

Cells establish contacts with each other to communicate, a sort of synapse that viral particles can use to infect a neighboring cell.

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## TARGETING HIV RESERVOIRS

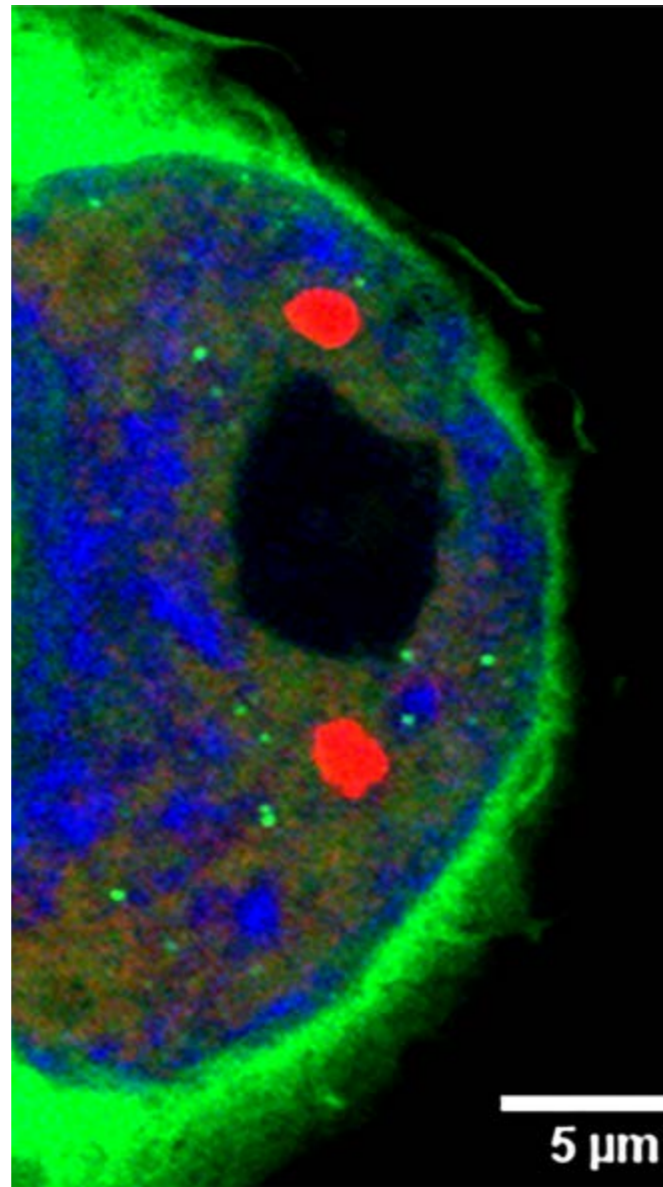
When viral DNA is «active,» it literally takes control of the infected cell and prompts it to produce thousands of copies of the virus. Viral particles produced in huge numbers – especially in the early stages of infection – bud from the surface of the hijacked cell, then invade neighboring cells. Research by the team led by Olivier Schwartz, Head of the Virus and Immunity Unit, has demonstrated that the virus essentially spreads via connections between immune cells. «The virus uses these synapses to spread effectively from one cell to another without being detected by antibodies,» explains Olivier Schwartz.

But sometimes the viral DNA remains dormant, and the latent virus stays «camouflaged» inside some immune cells, without causing any damage but unable to be eliminated either by the immune system or by antiretroviral therapy. This is a major challenge of research at the Institut Pasteur and in laboratories worldwide: how to locate HIV reservoirs, or in other words how to identify the cells in which the dormant virus is hiding so that they can be destroyed and lead to remission or even a cure for HIV infection. «We still need to understand the mechanisms by which the reservoir is formed, maintained and reactivated when treatment is stopped,» says Olivier Schwartz. «Broadly neutralizing antibodies represent a promising avenue for reducing or eliminating infected cells. We have shown that antibodies act not only by neutralizing viral particles and preventing new cells from being infected, but also by blocking the release of viruses produced by infected cells and facilitating the destruction of these cells.»<sup>4</sup>

## EXTREME DIVERSITY

Another characteristic of interest to researchers is the remarkable genetic diversity of HIV, for which there are two main types: HIV-1 and HIV-2. In a single infected individual receiving no treatment, HIV can generate more diversity than during a worldwide influenza epidemic. This is because HIV makes multiple mistakes while copying RNA into DNA, giving rise to thousands of variants with slight differences.<sup>5</sup> So the virus varies not just from one individual to the next, but also from one cell to the next.

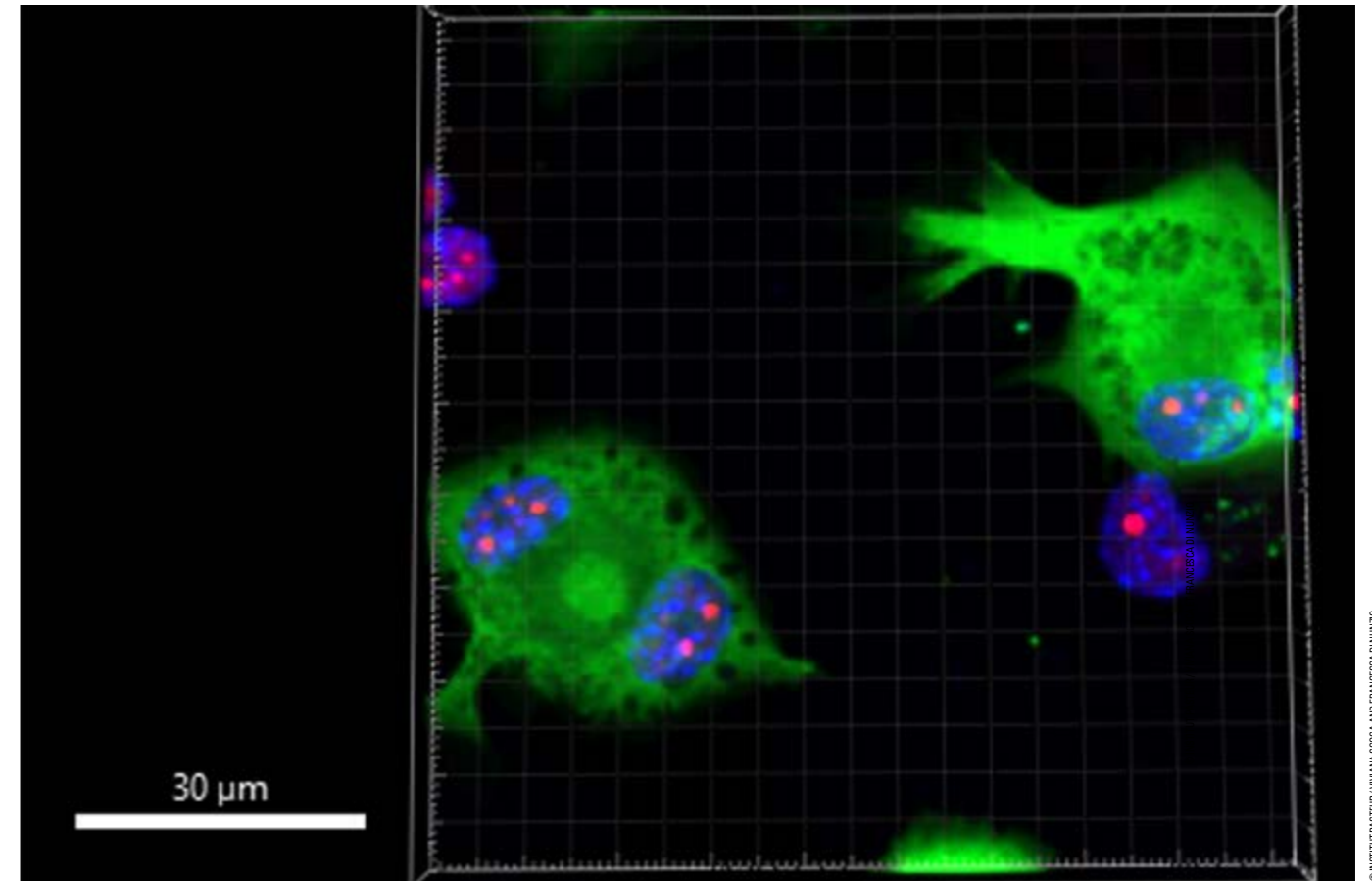
This means that a person with HIV needs to defend themselves from a huge number of different viruses, even if they were initially only infected by a single virus. And if the infection is not rapidly brought under control with treatment, the task soon becomes impossible. The virus evolves so quickly that the anti-HIV cells or antibodies produced are ultimately of no use. The immune system ends up running out of steam. This extreme variability, and the need to bring the virus under control quickly before it develops resistance to drugs, led to the development of triple therapies. The diversity of HIV is also what makes it so complicated to develop a vaccine – even the influenza virus, which is also skilled in the art of metamorphosis, requires a new vaccine each year.



In the nucleus of this infected cell, we can see membraneless organelles (in red) where viral RNA is concentrated. Newly reverse transcribed viral genomes are represented in green.



- 1 - <https://journals.asm.org/doi/10.1128/JVI.00135-20>
- 2 - <https://www.embopress.org/doi/full/10.15252/emj.2020105247>
- 3 - <https://academic.oup.com/jmcb/advance-article/doi/10.1093/jmcb/mjac060/6781009?login=false>
- 4 - <https://rupress.org/jem/article/210/13/2813/41501/Broadly-neutralizing-antibodies-that-inhibit-HIV-1>  
<https://www.nature.com/articles/s41467-022-28307-7>
- 5 - <https://science.sciencemag.org/content/320/5877/760>



HIV triggers the formation of protein condensates in the macrophage nucleus known as «HIV membraneless organelles.» Similar membraneless organelles with a different composition have also been identified in the cytoplasm of cells infected with SARS-CoV-2. They could help preserve the viral genome from cell degradation and also concentrate a number of enzymes in a given location to facilitate enzyme interactions.



## THE IMMUNE SYSTEM IN ACTION

When dealing with an infectious agent, the body has two types of defense mechanism: innate immunity, which is immediate, and adaptive immunity, which kicks in later but lasts longer. Innate immunity involves several cell types (macrophages, dendritic cells, NK cells, etc.) and protein types (cytokines, interferons, etc.). Following the interaction between infectious agents and innate immunity, adaptive immunity kicks into action in the lymphoid organs. The infectious agent is captured by specialized cells which stimulate B and CD8 T cells, with the help of CD4 T cells. B cells then produce specific antibodies against the infectious agent, while CD8 cells become capable of recognizing and destroying infected cells.

# INSPIRING PATIENTS

The Berlin, London and Düsseldorf patients, «natural controllers» and «post-treatment controllers» all have one thing in common: they are all capable, in one way or another, of dealing with HIV without antiretroviral therapy. They all represent unique models to help scientists understand how the immune system is able to combat the virus, curb its progression or prevent viral reservoirs from being reactivated once treatment has been discontinued. They are an irreplaceable source of inspiration for improving future treatments, and they are now also serving as a springboard for promising new clinical trials, after four decades of observation and research at the Institut Pasteur.

## STRONG RESISTANCE CONFERRED BY MUTATIONS

«My very first research at the Institut Pasteur, 20 years ago now, involved people who were frequently exposed to HIV (sex workers or drug users with high-risk behaviors) but who had

never been infected,» recalls Asier Sáez-Cirión, Head of the Institut Pasteur's Viral Reservoirs and Immune Control Unit. «This led us to identify some specific mutations that made immune cells resistant to infection.» Some individuals are naturally resistant to HIV because of mutations, the most well known being delta 32, which alters the surface coreceptor CCR5 in CD4 cells and macrophages and prevents the virus from entering these cells (the mutation is found in around 1% of the population<sup>1</sup>).

This mutation has also led to some individuals with HIV who developed leukemia being «cured of HIV» after receiving a bone marrow transplant. This is because the cells from the bone marrow donor that replaced those of the person receiving the transplant carried the CCR5-delta 32 mutation, enabling the recipient to develop resistance to the virus. There have so far been five persons who have gone into HIV remission after receiving bone marrow transplants: the Berlin patient (who had been in HIV remission for more than ten years when he died following a leukemia relapse), the London patient, the Düsseldorf patient, the New York patient and the City of Hope patient. They are inspiring examples, but it is important to remember that they are very special cases. «Bone marrow transplants are high risk, complicated procedures and are not suitable for the 40 million people living with HIV,» concludes Asier Sáez-Cirión.

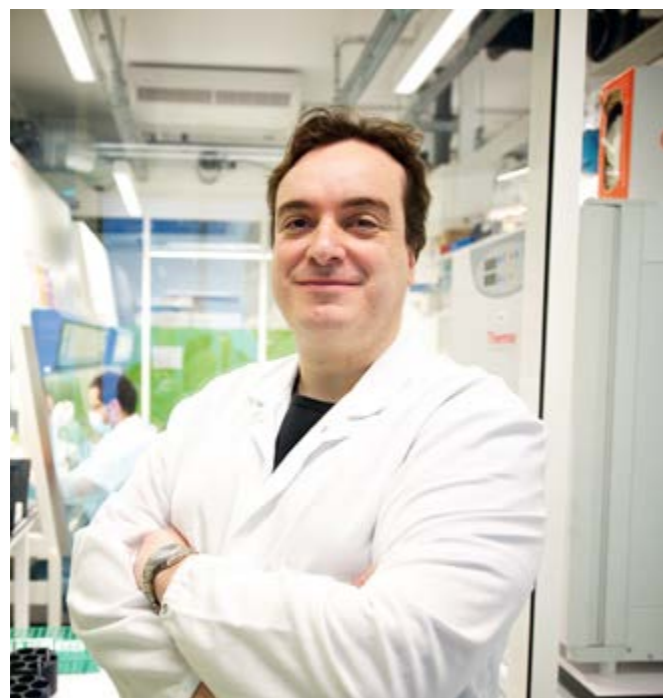
## REMARKABLE CD8 CELLS THAT ATTACK HIV

Asier Sáez-Cirión's team focuses on another group of people: «natural controllers.» These people are not resistant to the virus but, as their name suggests, they are able to spontaneously control viral replication and maintain a very low, often undetectable viral load in the absence of antiretroviral therapy. We now know that the CD4 cells of these individuals, who represent less than 0.5% of people living with HIV,<sup>2</sup> are slightly less susceptible to HIV infection. Recent research by Lisa Chakrabarti from the Institut Pasteur's Virus and Immunity Unit suggests that the ability of these rare «natural controllers» to respond effectively to infection can be explained by mutations that reduce CCR5 surface expression in CD4 T cells, limiting HIV entry. In other cases, the CD4 T cells of controllers are highly sensitive in detecting HIV antigens, resulting in an enhanced antiviral response. These extremely sensitive CD4 T cells secrete a number of chemokines that induce CCR5 receptor internalization, which again makes the cells less able to be infected with HIV.<sup>3</sup>

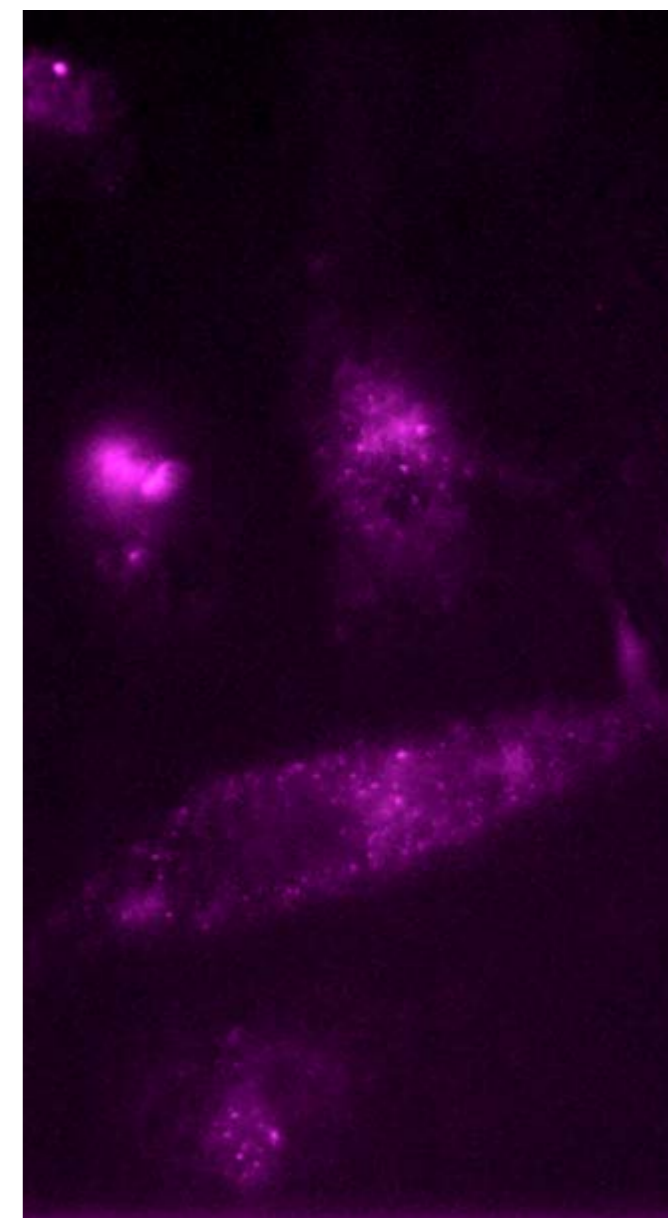
CD8 cells also seem to play a central role in controlling HIV in the absence of treatment. «The research we have conducted on the CODEX and PRIMO cohorts<sup>4</sup> has shown that the CD8 cells of «natural controllers» are more capable of recognizing and eliminating infected cells because their memory potential and cytotoxic capacity are much better than average,» explains Asier. After identifying the highly specific molecular profile of these cells, the scientists tried to reprogram the CD8 cells of non-controllers so that they would acquire the same characteristics as controllers' CD8 cells. This process, implemented successfully in vitro in 2022,<sup>5</sup> is currently being tested in an animal model for HIV/AIDS. «The long-term goal is to use this strategy as part of cell therapy to achieve remission of HIV infection. This would involve isolating non-controllers' cells, reprogramming them ex vivo, and re-injecting them before potentially discontinuing treatment,» comments Asier Sáez-Cirión. «We are also exploring the possibility of directly modifying CD8 cells in vivo using immunotherapies.»

## THE IMPORTANCE OF EARLY TREATMENT

Other avenues are also being explored in parallel in a final group of people known as «post-treatment controllers.» The VISCONTI cohort<sup>6</sup> is composed of a number of these individuals, who are able to control viral infection for years or even decades after discontinuing treatment, achieving sustained HIV remission. What they have in common is that virtually all of them began their antiretroviral therapy at a very early stage, in the first few weeks after infection. The team led by Asier Sáez-Cirión therefore decided to investigate the impact of early treatment on viral control in rhesus macaques. «We observed that early treatment has various beneficial effects, one of which is to give CD8 cells the same memory characteristics as the CD8 cells of «natural controllers,» significantly boosting their antiretroviral potential after discontinuation of treatment,» says the scientist. «This is yet further evidence that we are on the right track with our research on CD8 memory cells.»



Asier Sáez-Cirión, Head of the Viral Reservoirs and Immune Control Unit at the Institut Pasteur.



CD4 cells like macrophages can carry mutations in the CCR5 coreceptor which make them resistant to HIV. Here fluorescence imaging shows CCR5, a cellular coreceptor for HIV, expressed at the macrophage surface.

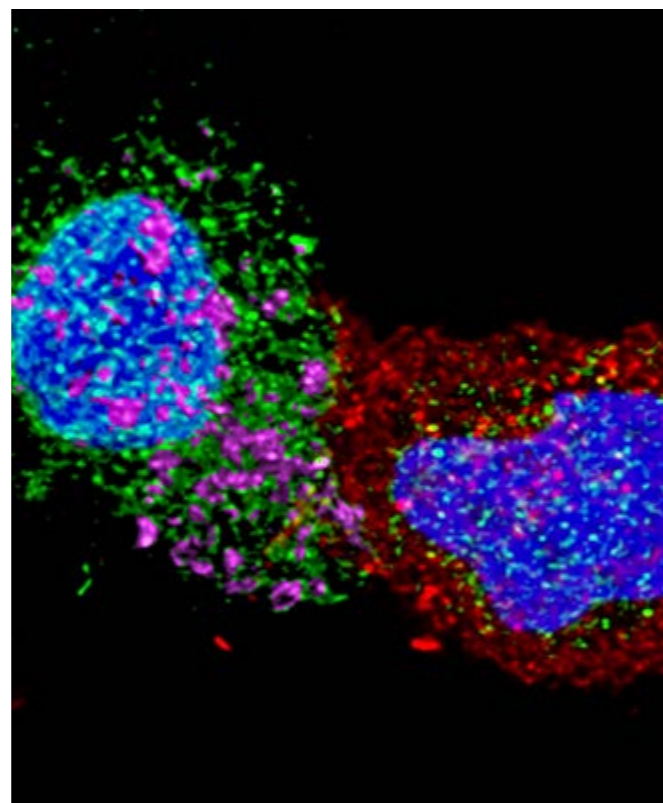
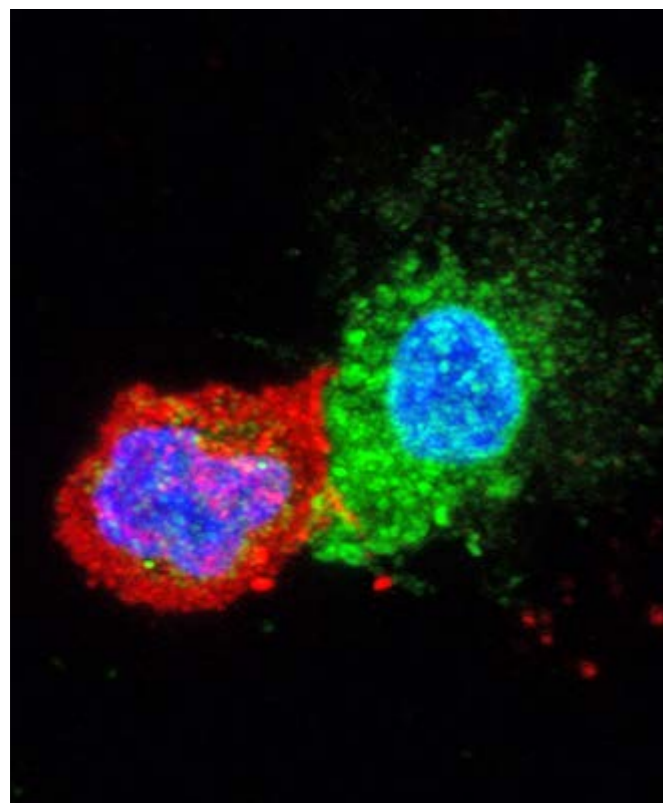
Following these encouraging results, a clinical trial (RHIVIERA02) will begin in 2023, as part of the ANRS RHIVIERA program<sup>7</sup> led with the Paris Public Hospital Network (AP-HP) in collaboration with Hugo Mouquet's team, in a group of 70 individuals with early diagnosis of HIV infection.<sup>8</sup> «We will offer them conventional antiretroviral therapy and also immunotherapy combining two broadly neutralizing antibodies (bNAbs). The bNAbs should help reduce the quantity of viral particles in circulation, help NK cells to eliminate infected cells and therefore reduce the reservoir, and finally boost their CD8 cells and their anti-HIV antibodies,» explains Asier. And after one year, the treatment will be discontinued. «We think that the participants will better control the virus after discontinuing treatment and may become «post-treatment controllers,»» says Asier Sáez-Cirión. «This raises the hope of long-term remission for people living with HIV, but in any case early treatment is always beneficial in epidemiological terms as it prevents the virus from spreading.»

And the discoveries keep coming. Institut Pasteur scientists have identified something else that several of the «post-treatment controllers» in the VISCONTI cohort have in common, namely an immunogenetic footprint linked to the presence of particular NK cells. Another clinical trial (RHIVIERA01) has started in 2023 to explore this aspect as part of the RHIVIERA program.<sup>9</sup> Around 70 people from

the PRIMO cohort, who started treatment during primary infection and carry this specific immunogenetic footprint, will discontinue their treatment to assess their ability to control viremia. «If the result is confirmed, for the first time we will have a predictive marker for the chance of remission from HIV infection,» says Asier Sáez-Cirión. «It will also confirm the important role of NK cells and guide us to new avenues for immunotherapy.»



- 1 - <https://www.sciencedirect.com/science/article/pii/S0198885917305104>
- 2 - <https://onlinelibrary.wiley.com/doi/10.1111/imr.12076>
- 3 - <https://www.nature.com/articles/s41467-022-28130-0>
- 4 - <https://www.nature.com/articles/s42255-019-0081-4>
- 5 - <https://www.pasteur.fr/fr/espace-presse/documents-presse/vih-reprogrammer-cellules-controler-infection>
- 6 - <https://www.jci.org/articles/view/157549>
- 7 - <https://www.pasteur.fr/fr/espace-presse/documents-presse/vih-conferer-aux-cellules-t-cd8-capacites-celles-controlers-naturels>
- 8 - <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003211>
- 9 - <https://rhiviera.com/>
- 8 - <https://clinicaltrials.gov/ct2/show/NCT05300035>
- 9 - <https://clinicaltrials.gov/ct2/show/NCT05482854>



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In controllers, CD8 cells play a key role in controlling infection. Here we see a CD8 cell (in red) coming into contact with a CD4 cell infected with HIV (in green) and preparing to destroy it by releasing granzyme (in pink).



## TENTH ANNIVERSARY OF THE VISCONTI COHORT

The VISCONTI cohort (Viro-Immunological Sustained Control after Treatment Interruption), described in 2013, is composed of 30 people, most of whom received early antiretroviral therapy and have been able to control their viremia when their treatment has been discontinued, some for more than 20 years. The VISCONTI study has provided proof of concept that sustained remission from infection is possible for people living with HIV-1. It is the world's largest cohort of long-term «post-treatment controllers.»



## CURE VERSUS REMISSION

The term remission is used when HIV infection is controlled in the absence of antiretroviral therapy but there may still be some infected cells that could be reactivated. The term cure is used when all the infected cells in the body have been eliminated, which is currently impossible to prove beyond doubt (we cannot analyze every single cell in the immune system!).



## THE DÜSSELDORF PATIENT

After the Berlin patient in 2009 and the London patient in 2019, the [IciStem](#) consortium, which includes Asier Sáez-Cirión's team at the Institut Pasteur, presented a new case of probable HIV cure following a bone marrow transplant from a donor with the CCR5-delta 32 genetic mutation, known to provide natural protection against HIV. The man, treated in Düsseldorf, received a stem cell transplant to treat leukemia, then stopped his antiretroviral therapy for HIV under supervision. Four years later, no trace of HIV virus can be detected in his body.

# BROADLY NEUTRALIZING ANTIBODIES, STARS OF THE LAB

Antibodies are undoubtedly among the most effective and popular players in our immune system. These Y-shaped proteins inhibit the action of pathogens by binding to some of their surface antigens, while also helping eliminate them. But some antibodies are more effective than others, especially when dealing with viruses. Some can stop a specific type of virus, while others are remarkably versatile and are capable of neutralizing several viral variants. These are known as broadly neutralizing antibodies, or bNAbs.<sup>1</sup> And at the Institut Pasteur, like in dozens of other

laboratories worldwide, they are real stars: they were the subject of more than 3,000 publications between 2013 and 2023.<sup>2</sup> «There is huge interest in broadly neutralizing antibodies because they are powerful, effective at low doses and generally multifunctional,» confirms Hugo Mouquet, Head of the Institut Pasteur's Humoral Immunology Unit. «We see them as crucial tools for developing novel therapies for chronic infectious diseases (HIV-1, hepatitis B, etc.) – as was also the case for COVID-19 – and also for helping vaccine development.»

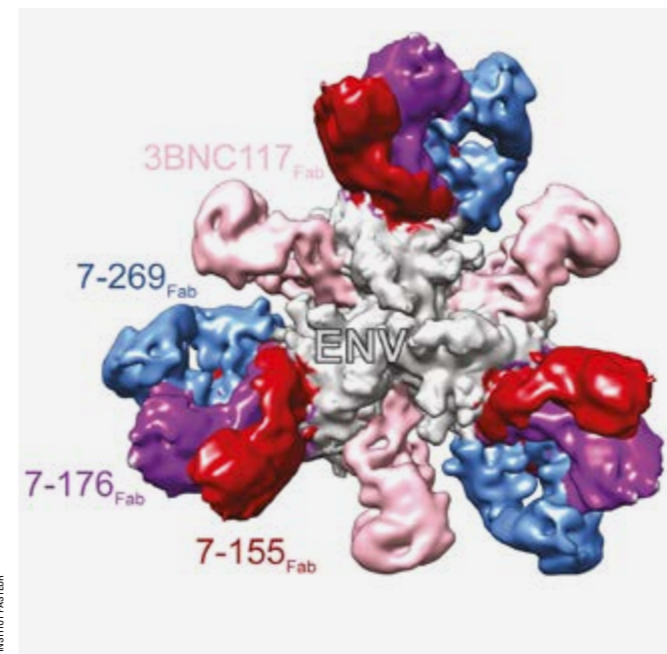
Broadly neutralizing antibodies targeting HIV were discovered in the 1990s in people living with HIV, who would later be referred to as «elite neutralizers.» The ability of these antibodies to recognize and neutralize multiple strains of HIV immediately piqued the interest of scientists, who saw them as the ultimate solution to overcome this multifaceted virus. The impact would not be felt straight away, but research confirmed the potential of these super antibodies, some of which are capable of neutralizing more than 95% of the hundreds of HIV variants tested in vitro in labs.<sup>3</sup> Not only do these antibodies have good neutralizing capabilities, with the arms of their «Y» binding to specific proteins in the viral envelope and preventing the virus from entering immune cells; they also have good effector functions – they cause various immune cells to bind to the tail of their «Y» and destroy viruses or infected cells.

## SWISS KNIVES OF THE IMMUNE SYSTEM

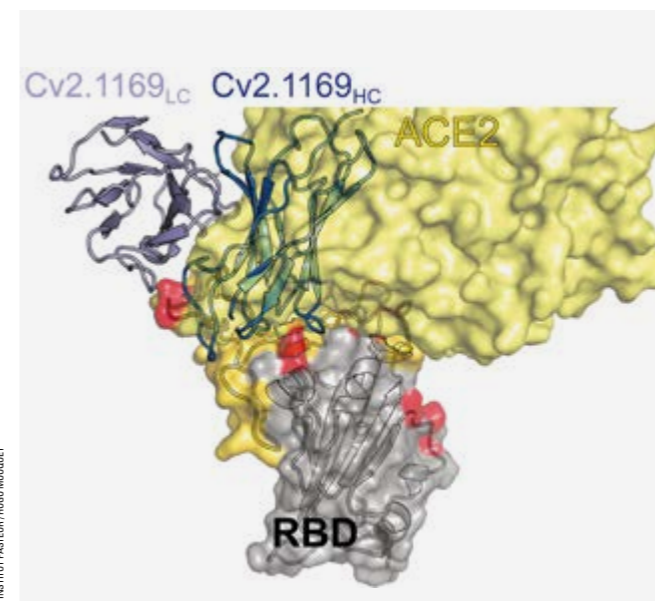
«Antibodies are the Swiss knives of the immune system,» comments Hugo Mouquet. «They are able to neutralize circulating viruses, at the point of entering or leaving target cells, they can eliminate infected cells and can stimulate immune responses by forming complexes with viruses.» To understand the mechanism of action of bNAbs and pinpoint what makes them so effective, Hugo Mouquet and his team have been identifying, reproducing and characterizing anti-HIV bNAbs in detail, one by one, for more than 15 years. This is a painstaking task, bearing in mind that thousands of neutralizing antibodies circulate in the blood of elite patients, who represent approximately 1% of infected individuals.<sup>1</sup> In other words, the scientists have to work their way through an «antibody soup» to find the most effective antibodies, those that are likely to be active against the majority of viral strains.



Hugo Mouquet, Head of the Humoral Immunology laboratory at the Institut Pasteur.



Fragments of three broadly neutralizing antibodies (bNAbs) discovered at the Institut Pasteur by Hugo Mouquet's team (blue, purple and red), here forming a complex with the HIV envelope protein (Env) (in gray).



All the knowledge and expertise acquired about HIV paved the way for considerable progress when it came to COVID-19. In just two years, 150 monoclonal antibodies like this one were produced and characterized by Hugo Mouquet's team.

But one thing is important to point out: the scientists are actually not hunting for the antibodies themselves among those circulating in the blood; what they are looking for is the B lymphocytes that produce them. Each B lymphocyte that they isolate offers access to the genetic material encoding a unique antibody. The DNA sequences coding for the antibody are then inserted into immortalized human cells, which produce a large number of exact copies of the antibody as they divide. The antibody is described as «monoclonal» as it comes from a single cell or clone. «When we have a sufficient quantity of monoclonal antibody, we characterize it and establish its profile: we perform gene sequencing and identify interaction sites with the virus, affinity for binding to the viral envelope, structure and atomic interactions with the envelope, and neutralizing activity in vitro and in vivo in animal models,» describes Hugo Mouquet. «These scientific and methodological approaches, which began with HIV research, have been adopted for other viral infections – for example, they were used for the rapid generation of hundreds of monoclonal antibodies against SARS-CoV-2 after the start of the COVID-19 epidemic.»

## TOWARDS THERAPEUTIC ANTIBODIES

In the Institut Pasteur's Virus and Immunity Unit, led by Olivier Schwartz, the antiviral properties of bNAbs are also investigated in vitro. The team was able to provide a detailed description of the mechanism of action of bNAbs in tackling the viral budding that occurs at the surface of infected immune cells. «We have been working for ten years on the mechanism of action of bNAbs and we were soon able to show how effective they are in blocking cell-to-cell viral transmission.<sup>4</sup> More recently, in 2022, Timothée Bruel, a scientist in the lab, and I described how bNAbs were able to bind to viral particles and form aggregates at the surface of CD4 cells,» says Olivier Schwartz. «These aggregates prevent the release of viral particles and the formation of synapses used by viruses to move from one cell to another.»<sup>5</sup> This neutralizing phenomenon was photographed with an astonishing level of detail using scanning electron microscopy.

The scientists in the team are also investigating the effector functions of bNAbs. Timothée Bruel used videomicroscopy to film interactions between HIV-infected T cells, neutralizing antibodies and natural killer (NK) cells. The images taken every five minutes show how the antibodies attract and trigger the destruction of cells infected by NK cells via a cell cytotoxicity process.<sup>6</sup> «When we use broadly neutralizing antibodies, destruction is more effective because NK cells



Olivier Schwartz, Head of the Virus and Immunity Unit at the Institut Pasteur.

coevolution between virus and antibodies. «This was a very important discovery,» confirms Hugo Mouquet. «After 40 years of research, no vaccine has been able to induce production of broadly neutralizing antibodies. And that is partly because we didn't have the right playbook. Now that we know how bNAbs develop, scientists are exploring the possibility of vaccines that guide bNAb production step by step, based on what happens in «elite neutralizers.»»

Another factor that explains the lack of conclusive results in the search for a vaccine is the fact that viral proteins, which play a key role in triggering antibody production in the immune system, have a highly complex structure with multiple folds that are difficult to reproduce in the lab. But in 2016, structural biologists and vaccinologists in the US successfully produced recombinant viral envelope proteins that were virtually identical to those expressed at the viral surface. «With our ability to generate recombinant native antigens in labs and the fact that we now have access to the bNAb playbook, we have a very promising strategy to develop vaccines that induce bNAb production,» says Hugo Mouquet. «For the first time, recent research has demonstrated the induction of effective broadly neutralizing antibodies in animals using this type of vaccine strategy,»<sup>8</sup> he concludes.

establish more contacts with antibodies and the infected cell,» explains Timothée Bruel. The scientists also studied how antibodies collaborate with another key player in the immune system, the complement system.

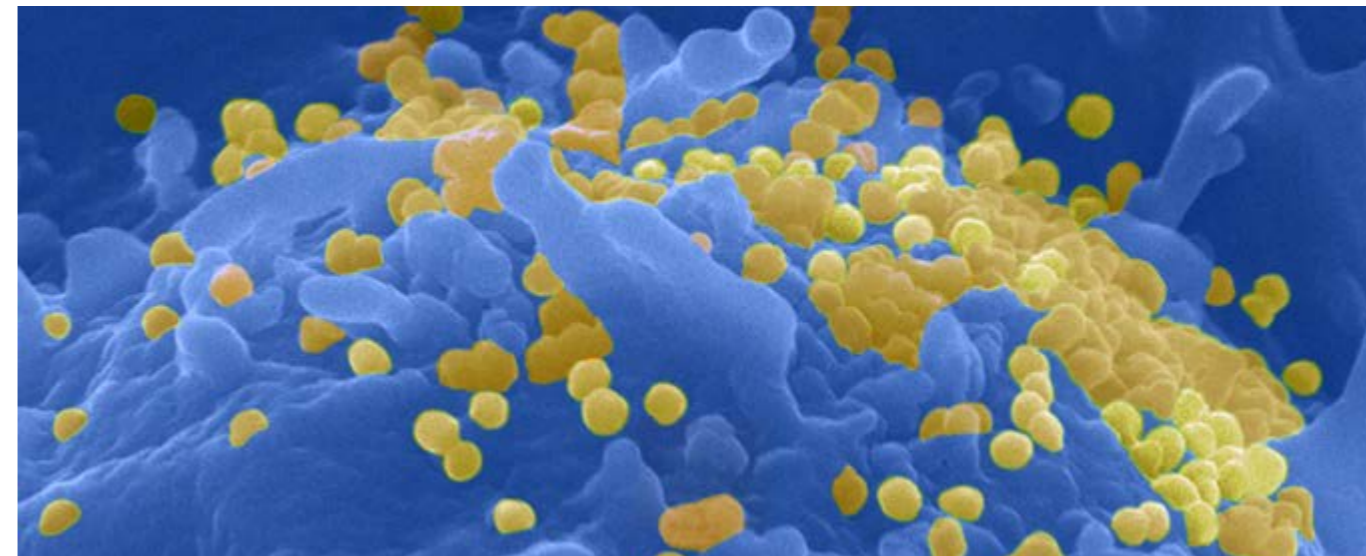
## ANTIBODIES: HOPES FOR A VACCINE?

With all this new knowledge, broadly neutralizing antibodies are being used to explore novel therapeutic approaches. In France, a clinical trial, ANRS-RHIVIERA, will begin in 2023 with the teams led by Asier Sáez-Cirión and Hugo Mouquet at the Institut Pasteur in coordination with the Paris Public Hospital Network (AP-HP), to test the joint effect of an antiretroviral triple therapy and a cocktail of long-acting bNAbs in newly diagnosed individuals during the early stage of HIV infection. «The aim is to administer these antibodies early to help reduce the reservoir and promote control of infection after discontinuation of treatment, with the hope of achieving functional remission,» explains Hugo Mouquet. «This type of treatment could also help evaluate what has been described as the vaccine effect of antibodies,» points out Olivier Schwartz. «Antibody-virus complexes could stimulate and educate the immune system and make the protective effect conferred by antibodies last longer.»

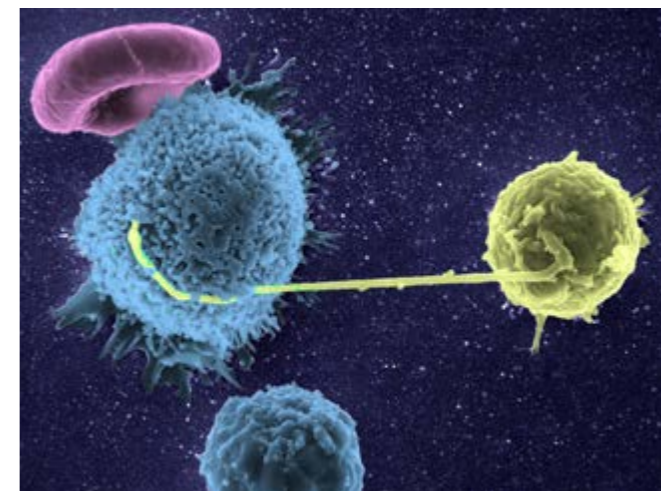
In terms of vaccines, two major advances raised new hopes around a decade ago. The first involved the way in which these super antibodies are formed. In 2013, a US team demonstrated that broadly neutralizing antibodies are sculpted over time in reaction to viral mutations and escape, and that they sometimes take two or three years to reach their final form and become fully effective<sup>7</sup>. In other words, there is a



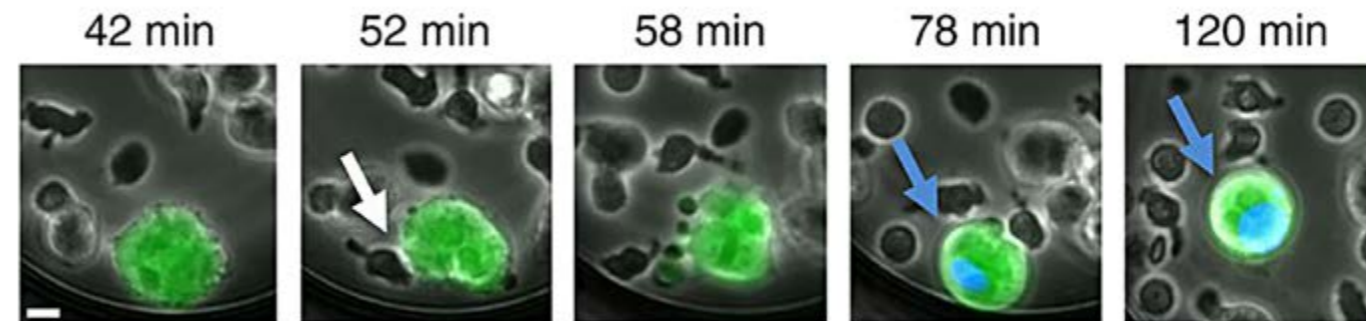
- 1 - [https://www.cell.com/trends/immunology/fulltext/S1471-4906\(14\)00149-5](https://www.cell.com/trends/immunology/fulltext/S1471-4906(14)00149-5)
- 2 - PubMed
- 3 - <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7042716/>  
<https://www.nature.com/articles/nature11544>
- 4 - <https://rupress.org/jem/article/210/13/2813/41501>
- 5 - <https://www.nature.com/articles/s41467-022-28307-7>
- 6 - <https://www.nature.com/articles/ncomms10844>
- 7 - <https://www.nature.com/articles/nature12053>
- 8 - [https://www.cell.com/cell/fulltext/S0092-8674\(19\)30733-0](https://www.cell.com/cell/fulltext/S0092-8674(19)30733-0)



By binding to budding viral particles at the surface of CD4 T cells, broadly neutralizing antibodies form clusters that prevent transmission.



A lymphocyte infected with HIV (in yellow) comes into contact with a non-infected lymphocyte (in blue) by forming a long membrane extension.



A cell infected with HIV-1 (green cell) and NK cells (smaller dark cells) are incubated with broadly neutralizing antibodies and trapped in microwells. A series of photos taken every five minutes reveals how the HIV-1-infected cell is destroyed by the NK cells (a blue dye shows the dying cell).

## CELLS UNDER THE MICROSCOPE

«We spend a lot of time looking after our cells, almost as if they were little creatures,» says Florence Guivel, an engineer in the Institut Pasteur's Virus and Immunity Unit.

«Every day, we check under the microscope that they are OK. If there are too many cells, we eliminate some so that the others can feed and replicate properly.»

For research on HIV, these cells can be healthy or infected CD4 cells, dendritic cells or other immune cells, such as killer cells (CD8+ cells or NK cells), which are particularly «fragile and tricky to keep alive.»

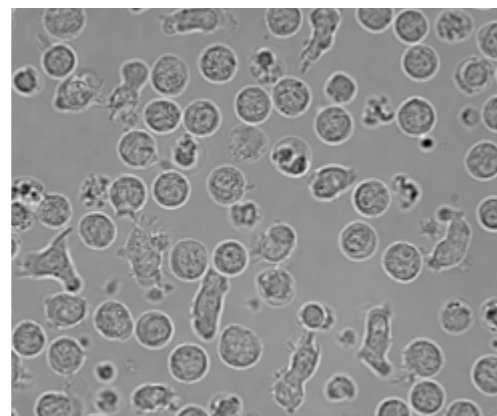
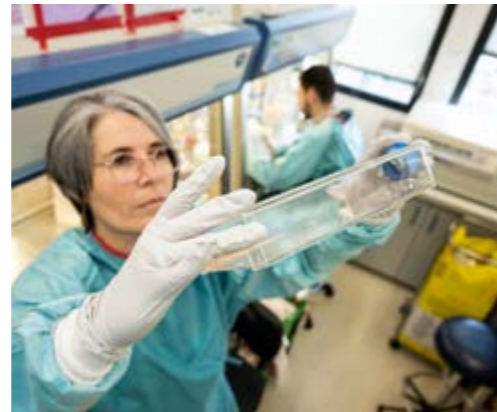
But where do these cells actually come from?

«They are extracted from patients' blood samples using tiny magnetic beads, or by laser in flow cytometry,» describes Florence Guivel. In both methods, the cell surface is labeled beforehand with magnetic or fluorescent antibodies. If the cells are infected with HIV, they can be identified with antibodies that recognize the virus. The process takes place in a BSL3 laboratory, where the use of laminar flow hoods and negative pressure containment prevent any biological agents from spreading.

Once selected, the precious cells are kept alive in a nutrient culture medium (amino acids, sugars, vitamins, etc.), in suspension or on a substrate, until they are used in experiments. Healthy and HIV-infected lymphocytes can be put in culture with killer cells and antibodies, to assess whether they can control the infection.

«When it comes to antibodies, either we buy them from suppliers or we use those produced by Hugo Mouquet. They require a lot of work and we need to take great care with them,» stresses Florence Guivel as she points to a tiny flask. «We follow the dilution instructions to make sure we get the dosage right.»

At the Institut Pasteur, more than 300 technicians and 1,600 scientists and research engineers work together on a wide range of topics.



Lymphocyte culture

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Florence Guivel, a technician in the Virus and Immunity Unit.



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From top to bottom: Augustin Martin, Lou-Léna Vrignaud, William Henry Bolland and Mariem Znaidia.

# NATURAL KILLER CELLS THAT TARGET HIV

The immune system is composed of an army of immune cells, all very different from each other. There are B cells, which produce antibodies, CD8 T cells, CD4 T cells, and then there are cells belonging to the body's first line of defense, like macrophages and NK cells. In humans, all these cells spring into action to try to control and eradicate HIV – very often in vain. But it seems that NK (natural killer) cells are naturally predisposed for this task. These innate immune cells are able to control simian immunodeficiency virus (SIV) – the virus that gave rise to human immunodeficiency virus (HIV) – in some species of monkey.



Michaela Müller-Trutwin, Head of the HIV, Inflammation and Persistence Unit at the Institut Pasteur.

## TRACKING AFRICAN GREEN MONKEYS

It was while investigating African green monkeys that virologist Michaela Müller-Trutwin, now Head of the Institut Pasteur's HIV, Inflammation and Persistence Unit, revealed the underestimated potential of NK cells. It all began in the 1990s, when the young scientist was working at the Institut Pasteur de Bangui in the Central African Republic, then with the Institut Pasteur de Dakar in Senegal and the Pasteur Center in Cameroon. «I started studying the different HIV variants circulating in Central Africa – this research was crucial for developing PCR tests for viral load measurement<sup>1</sup> and searching for a vaccine that would be effective against all variants – and I then turned my attention to the animal reservoir of HIV-1 to gain a better understanding of the origin of HIV-1» explains the scientist. «After that I focused on African green monkeys, the largest SIV reservoir in Sub-Saharan Africa.»

And for Michaela this begged the question: why are African green monkeys persistent carriers of SIV but never develop AIDS? After designing suitable immunological and genomic tools,<sup>2</sup> her research led to an important initial observation in understanding HIV infection in humans. Monkeys that never develop AIDS have no signs of persistent inflammation.<sup>3</sup> «We discovered that the virus replicates to high levels in the blood but does not cause chronic inflammation, unlike what happens in humans. The monkeys that stay healthy are those that manage to protect themselves from inflammation,» describes Michaela Müller-Trutwin. The scientists then demonstrated early on that limiting the intensity of inflammation in primary HIV infection reduces the risk of progressing toward disease.<sup>4</sup>

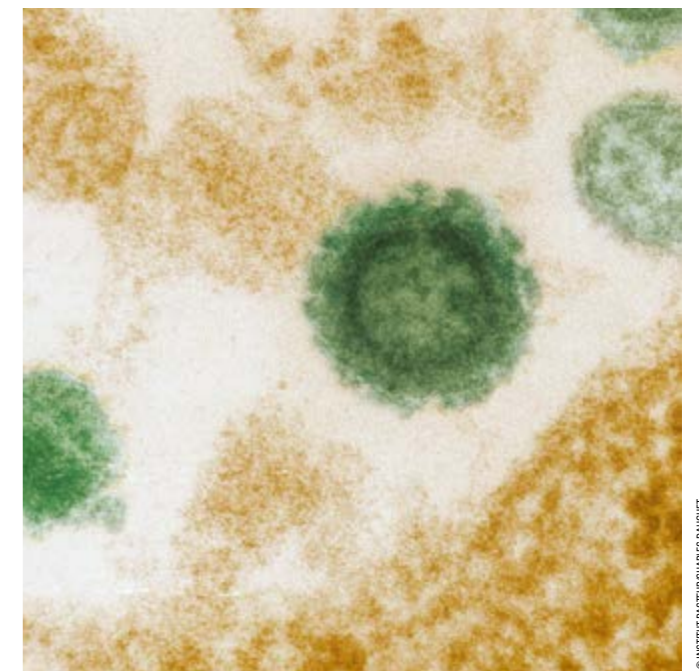
## HIGHLY PROTECTED LYMPH NODES

Another observation made in African green monkeys was that the virus is present in large quantities in the blood but generally not found in the lymph nodes. «I was struck by the idea of a tissue-specific control mechanism» marvels Michaela Müller-Trutwin. «It seemed completely paradoxical and hard to believe at first, until a strong viral control in lymph nodes despite high levels of virus in blood was confirmed in another monkey species, sooty mangabeys, where HIV-2 originated.» The scientists pursued their research further and observed that the virus entered the lymph nodes and then stopped replicating. It was as if the monkeys were allowing the virus to circulate in their blood but were able to control viral replication in the lymph nodes, a place where

HIV normally hides in humans, and is hard to control even in people receiving treatment.

But what was the explanation for this remarkable feat in green monkeys? How were they able to control SIV so effectively in the lymph nodes? The virologist set out to uncover what was behind this formidable defense system, and after 15 years of research her team put the finger on it: the key are NK cells.<sup>5</sup> «We didn't think of NK cells straight away because they are part of the innate immune system,» recalls Michaela Müller-Trutwin. «We thought they played a role at the beginning of the infection, before the adaptive responses kick in, and not as much in the chronic phase. We also knew that NK cells are normally scarce in lymph nodes.

Moreover, we didn't expect that innate responses could play such an important role by their own in primates.» But they do – NK cells are indeed capable of recognizing and killing infected cells in lymph nodes, especially in B cell follicles, the primary HIV reservoir in humans. This was a «holy grail» for scientists.



The SIV virus, which causes AIDS in monkeys, belongs to the same retrovirus family as HIV. Around 40 species of African primates are natural carriers of SIV.



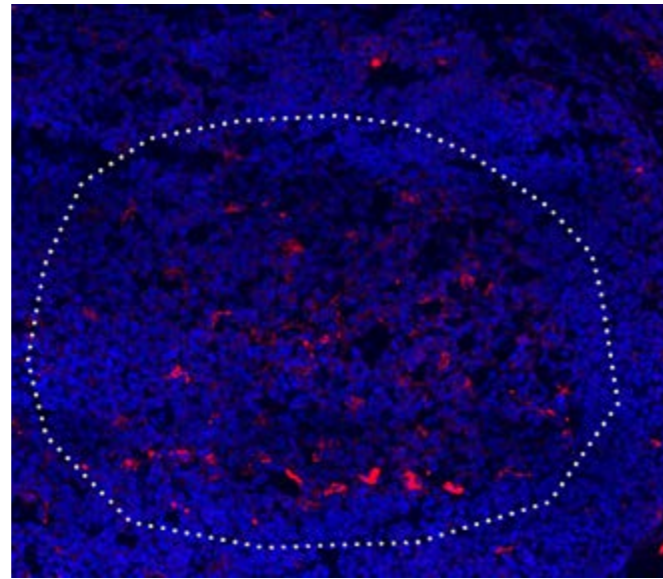
## VIRUSES THAT ORIGINATED IN MONKEYS

We know of two types of HIV: HIV-1 and HIV-2, both derived from viruses found in African monkeys (SIV or simian immunodeficiency virus). HIV-1 is the more prevalent type and is found in every world region. HIV-2 is less virulent and less transmissible than HIV-1. It is more often found in West Africa and some other countries including Angola, Mozambique, India, Brazil and Cuba.

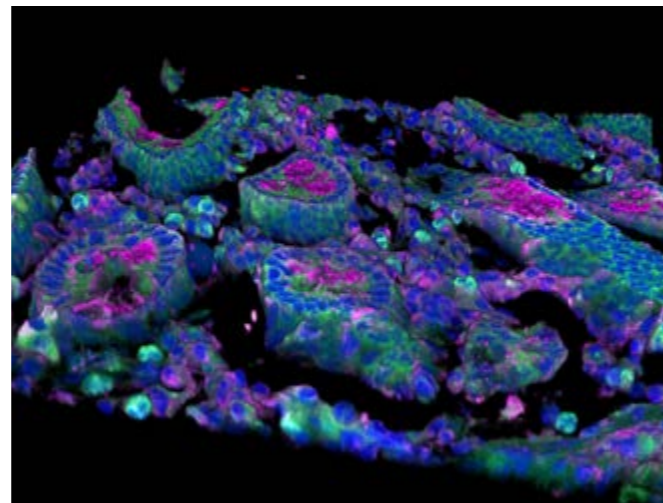
## STIMULATING NK CELLS TO TACKLE HIV

The results of this major discovery were published in 2017.<sup>5</sup> «It was an important discovery for scientists working on a cure for HIV/AIDS, as one goal consists in finding a way of reducing viral reservoirs in the lymph nodes, especially in B cell follicles,» emphasizes Michaela Müller-Trutwin. Other research by the Institut Pasteur team revealed that in pathogenic infection, NK cells are often unable to complete terminal differentiation and are therefore incapable of effectively killing infected cells, unlike in the natural simian host.<sup>6</sup> «This is a recent concept that is currently being investigated. In the natural simian host, we have shown that NK cells evolve and differentiate during infection: their capacity to recognize and kill cells infected by SIV improves with time during the infection. We think that this adaptation is also related to their stage of maturity,» explains the scientist.

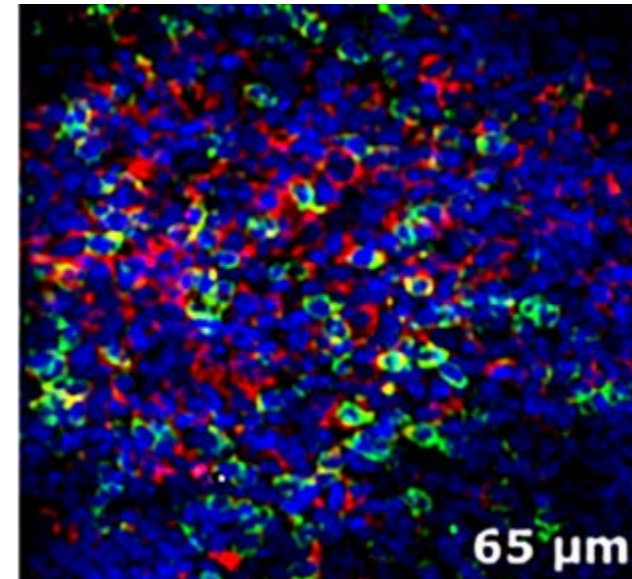
«We have long known that NK cells play a very important role in immunity, but now we have discovered that they are capable of entering B cell follicles and responsible for tissue-specific control. The fact that these capacities are adaptable opens up a whole new avenue for immunotherapy that we were unaware of before,» says Michaela Müller-Trutwin. In 2021, a preclinical trial in antiretroviral-treated rhesus macaques, animal models for HIV/AIDS, showed that stimulating NK cell differentiation using interleukin induced adaptive NK cell activity and reduced the viral reservoir in lymph nodes.<sup>7</sup> A clinical trial (RHIVIERA01) led by two clinicians and Institut Pasteur scientist Asier Sáez-Cirión will start at the Institut Pasteur in 2023 to assess the extent to which better education of NK cells can help people living with HIV who started treatment early to achieve remission.



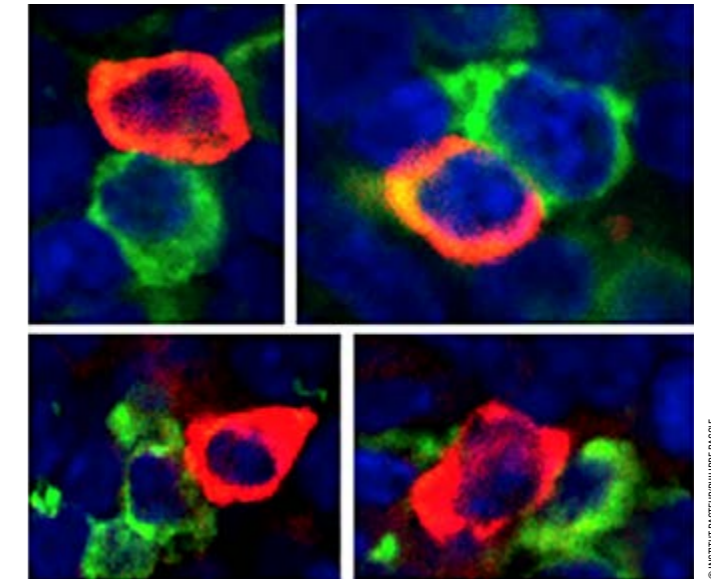
A lack of NK cells in lymph nodes induces a high rate of replication of SIV (in red) in B cell follicles (dotted white circle) in green monkeys, a natural animal reservoir for SIV.



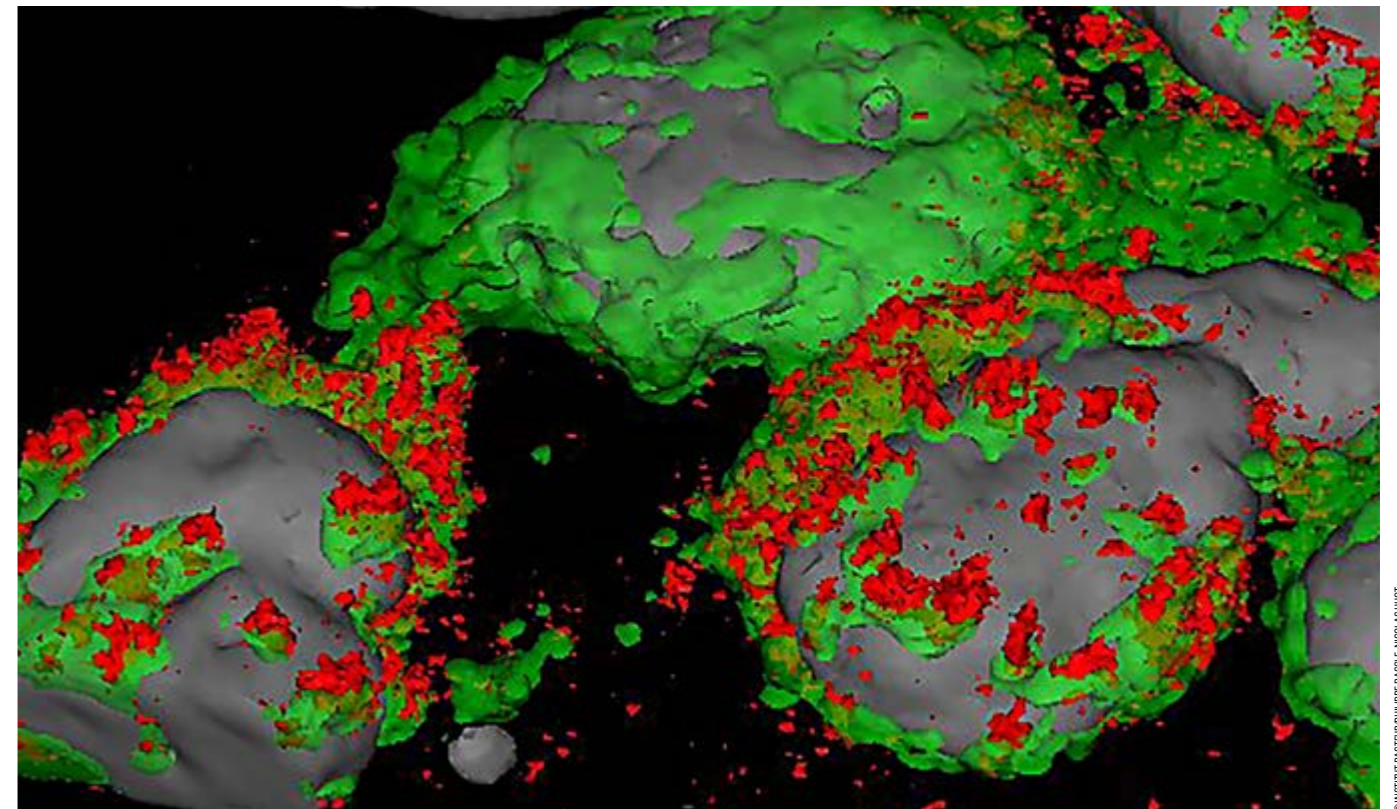
Cytokine IL-15 (in purple), produced for example by cells in the gut (as shown here) and lymph nodes improves the survival and cytotoxicity of NK cells.



NK cells are visible here (in green) inside a B cell follicle in the lymph nodes of a green monkey infected with SIV. The blue color delineates the nuclei and red MHC-E of the cells.



NK cells (in green) communicating with B cells (in red) in the lymph nodes of a green monkey infected with SIV.



3D reconstruction of CD4 cells infected with SIV. The virus is shown in red, the cell nuclei in gray and the CD4 receptor in green.



- 1 - [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(99\)00211-1](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(99)00211-1)
- 2 - <https://www.nature.com/articles/ng.3980/>
- 3 - <https://www.jci.org/articles/view/23006>
- 4 - <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005774>
- 5 - <https://www.nature.com/articles/nm.4421>
- 6 - <https://www.nature.com/articles/s41467-021-21402-1>
- 7 - <https://www.nature.com/articles/s41467-021-23189-7>



# AND OVER THE NEXT 40 YEARS?



*I hope we will eradicate AIDS by 2030. Everyone has got behind that idea. But it won't be easy, especially with the recent COVID epidemic, which had a negative impact on screening and access to HIV prevention. To reach that target, we need to step up HIV testing so that we can start treatment as early as possible in infected individuals and reduce transmission. Around 30% of people are diagnosed when the infection is at an advanced stage. And access to treatment also needs to be improved – 25% of people with HIV are still unable to access treatment.*

**FRANÇOISE BARRÉ-SINOUSSE**

A RETROVIROLOGIST AT THE INSTITUT PASTEUR, LAUREATE OF THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE AND PRESIDENT OF THE FRENCH HIV/AIDS CHARITY SIDACTION



*There are two obstacles to the eradication of HIV: the first is biological with viral reservoirs, and the second is a societal barrier with stigma and discrimination. Research is giving us increasingly effective tools to stop the epidemic, but our society has not made sufficient progress and this is preventing vulnerable people from having access to these tools.*

**ASIER SÁEZ-CIRIÓN**

HEAD OF THE INSTITUT PASTEUR'S VIRAL RESERVOIRS AND IMMUNE CONTROL UNIT



*Stigma is still a major issue. People still find it hard to say that they have HIV, whereas they have no problem saying they have COVID or cancer. But in scientific terms, we have made huge progress with the possibility of single-cell genome sequencing, the discovery of broadly neutralizing antibodies, messenger RNA vaccine technology, etc. mRNA technology won't be enough on its own to develop an HIV vaccine, but it will speed up vaccine research. I think we will get there one day.»*

**MICHAELA MÜLLER-TRUTWIN**

HEAD OF THE INSTITUT PASTEUR'S HIV, INFLAMMATION AND PERSISTENCE UNIT



*I am optimistic that we can eradicate the viral reservoir. I am probably a little less optimistic when it comes to a vaccine, but in science we build up knowledge for years and then suddenly an innovation leads to a key discovery. I am also convinced that major discoveries are possible if we adopt an interdisciplinary approach.*

**FRANCESCA DI NUNZIO**

HEAD OF THE INSTITUT PASTEUR'S ADVANCED MOLECULAR VIROLOGY UNIT



*We can hope for progress over the next 40 years. But this will only happen if we continue to research virus-host interaction mechanisms. That's what we are doing at the Institut Pasteur.*

**OLIVIER SCHWARTZ**

HEAD OF THE INSTITUT PASTEUR'S VIRUS AND IMMUNITY UNIT



*Over the past 40 years, I have had the satisfaction of seeing a laboratory discovery – a broadly neutralizing antibody (bNAb 10-1074) – being tested in humans in several clinical phases. So I am hopeful that 40 years from now, one or more vaccines will offer protection for the population and that new therapies that will greatly benefit patients will have been discovered.*

**HUGO MOUQUET**

HEAD OF THE INSTITUT PASTEUR'S HUMORAL IMMUNOLOGY UNIT

*Once we have developed a better understanding of the mechanisms that a vaccine needs to trigger to confer effective protection – and that will require a great deal of basic immunology research –, we may be able to envisage the possibility of an HIV vaccine. And undoubtedly also a vaccine for other diseases like tuberculosis or malaria, for which there are currently no effective vaccines. I also believe that in future there will be combined therapies enabling patients to control their virus themselves without treatment – in other words, therapies that lead to sustained remission. That would be a major breakthrough at global level.»*

**FRANÇOISE BARRÉ-SINOUSSE**

A RETROVIROLOGIST AT THE INSTITUT PASTEUR, LAUREATE OF THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE AND PRESIDENT OF THE FRENCH HIV/AIDS CHARITY SIDACTION

# HIV RESEARCH AT THE INSTITUT PASTEUR

At the Institut Pasteur, extensive research is being conducted in different units on virus-host interactions, treating HIV infection, and vaccine candidates.

## PARIS

- **Epidemiology of Emerging Diseases Unit**, Arnaud Fontanet
- **Oncogenic Virus Epidemiology and Pathophysiology Unit**, Antoine Gessain
- **Imaging and Modeling Unit**, Christophe Zimmer
- **Innate Immunity Unit**, James Di Santo
- **Humoral Immunology laboratory**, Hugo Mouquet
- **Innovation lab: vaccines** Armelle Phalipon
- **MISTIC Group** (*Mucosal Immunity and Sexually Transmitted Infection Control*), Elisabeth Menu
- **Viral Reservoirs and Immune Control Unit**, Asier Sáez-Cirión
- **HIV, Inflammation and Persistence Unit**, Michaela Müller-Trutwin
- **Advanced Molecular Virology Unit**, Francesca Di Nunzio
- **Structural Virology Unit**, Félix Rey
- **Virus and Immunity Unit**, Olivier Schwartz
- **Institut Pasteur Medical Center**, Fabien Taieb
- **National Reference Center for Mycoses**, Fanny Lanternier

# AND IN THE PASTEUR NETWORK

The Pasteur Network members are also actively involved in HIV and AIDS research. The fact that there are **Pasteur Network** institutes in Africa and South-East Asia in particular is especially important given that over 90% of people living with HIV are in the southern hemisphere. The vast majority of Pasteur Network institutes in Africa and South-East Asia carry out serological and molecular diagnosis of HIV infection and provide immunological and virological monitoring for patients, whether or not they are receiving antiretroviral therapy.

## CENTRAL AFRICAN REPUBLIC

- **Widespread** availability of HIV viral load testing

## VIETNAM

- **Study of the use** of blotting paper to measure viral load (MOVIDA study)
- **Study on high-risk injection practices** in HIV transmission (social sciences, ANRS, NIHE)
- **Project on methadone substitution for drug addiction** (Hanoi, Haiphong). Project DRIVE ANRS 12353/NIDA RO1 DA041978 aiming to demonstrate the feasibility of eradicating HIV transmission through community intervention; project DRIVE-C ANRS 12380 on hepatitis C; and project DRIVEMIND ANRS 12410 also assessing a strategy for community-based treatment of mental disorders
- **The ANRS** is also supporting a phase II clinical trial on HIV and tuberculous meningitis co-infection implemented in both Haiphong and Hanoi
- **Project on substitution** with methadone and novel drugs for drug addiction (Institut Pasteur in Ho Chi Minh City/NIDA and Institut Pasteur)

## CAMBODIA

### HIV molecular biology

- **Therapeutic trials** of second-and third-line ARVs (ANRS)
- **Immunological study** in patients infected with HIV and/or tuberculosis
- **Study of mother-to-fetus transmission** of hepatitis B (ANRS)
- **ANRS DATURA project** – Determination of adequate tuberculosis regimen in adults and adolescents hospitalized with HIV-associated severe immune **suppression**
- **ANRS12394 LILAC-TB**, use of immunological biomarkers for early prediction of response to tuberculosis treatment

## CAMEROUN

- **Improving** the long-term follow-up of HIV-infected children who received early treatment (ANRS-Pediacam cohort)
- **Looking for specific metabolic signatures** using nuclear magnetic resonance-based metabolomics in correlation with markers of inflammation or immune activation and the risk of developing neurocognitive disorders (ANRS-MIND)
- **Research into the pathophysiological mechanisms** responsible for inducing and maintaining negative serology via EIA tests in HIV-infected children who received early antiretroviral multitherapy (ANRS-PEDIACAMNEG)
- **Observatory** for phylogenetic and genotypic diversity in non-M HIV-1 in Cameroon (ANRS Obsocam)

## CÔTE D'IVOIRE

- **Use of drugs** other than antiretroviral therapy by HIV-positive patients (ANRS-MOTUHS project)



*When I was working with countries in Africa and South-East Asia, I discovered the Pasteur Network. And if I had come across it earlier in my career, before the discovery of HIV, I would have liked to work there for a few years. Life and daily struggles for scientists in the Pasteur Network are completely different from those on the Paris campus, and I think that it can be a very rewarding experience for early career scientists.*

**FRANÇOISE BARRÉ-SINOSSI**, A RETROVIROLOGIST AT THE INSTITUT PASTEUR, LAUREATE OF THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE AND PRESIDENT OF THE FRENCH HIV/AIDS CHARITY SIDACTION

# SCIENTIFIC CONFERENCE

To mark the 40<sup>th</sup> anniversary of the discovery of HIV, the Institut Pasteur, in partnership with the ANRS, is organizing an international scientific conference entitled «40 years of HIV science,» which will look at the major scientific advances and discoveries in the field in recent decades. The conference will be attended by HIV specialists from all over the world and take place from November 29 to December 1, 2023 at the Institut Pasteur's Scientific Information Center (CIS).

## NOVEMBER 29 (OPENING ADDRESS)

SPEAKERS: STEWART COLE, ANTHONY FAUCI

## NOVEMBER 30

- Session 1: Host cell-virus interactions

SPEAKERS: WESLEY SUNDQUIST, FRANCESCA DI NUNZIO, OLIVIER SCHWARTZ, FLORENCE MARGOTTIN

- Session 2: Host responses to HIV infection

SPEAKERS: ZAZA NDHLOVU, HUGO MOUQUET, NICOLAS HUOT

- Session 3: Social/implementation Sciences

SPEAKERS: LORRAINE SHERR, BEATRIZ GRINSTEJN, GABRIEL GIRARD, JOSEPH LARMARANGE

- Session 4: Viral Persistence and Viral control

SPEAKERS: XU-YU, ASIER SÁEZ-CIRIÓN, VICTOR GARCIA, MORGANE BOMSEL, MONSEF BENKIRANE

## DECEMBER 1

- Session 5: HIV Prevention

SPEAKERS: MIKE COHEN, JEAN-MICHEL MOLINA, BILL SCHIEF, GLENDA GRAY

- Session 6: New therapeutic strategy against HIV

SPEAKERS: MICHEL C. NUSSENZWEIG, JOE ERON, MIREILLE MPOUDI, KAMEL KHALILI

KEYNOTE: FRANÇOISE BARRÉ-SINOUSI

# GLOSSARY

## ADAPTIVE (OR ACQUIRED) IMMUNITY:

specific immunity involving T cells and B cells. It develops later than the innate immune response but offers more lasting protection by establishing immune memory. If the body is infected again by the same pathogen, the response will be faster.

## AIDS:

acronym for acquired immunodeficiency syndrome. The term «immunodeficiency» refers to the weakening of the immune system, and the term «acquired» indicates that it is not an inherited condition.

## ANTIBODIES OR IMMUNOGLOBULINS:

proteins secreted by B cells that are part of the adaptive immune system. They are specific to foreign substances; they neutralize viruses and destroy infected cells with the help of innate immune cells (e.g. macrophages, NK cells, the complement system).

## ANTIGEN:

a foreign substance (bacterium, virus, toxin, etc.) that can induce the formation of antibodies.

## ANTIRETROVIRAL DRUG:

a drug designed to control HIV multiplication in the body by acting on RNA viruses, known as retroviruses, such as HIV.

## B CELL FOLLICLE:

a region in the lymph node where B cells proliferate and differentiate.

## B LYMPHOCYTES:

also known as B cells, they specialize in antibody production. The surface of each B cell has a type of immunoglobulin that can recognize a specific antigen, for example an antigen characteristic of a given pathogen. Antibodies are produced when immunoglobulins bind to the corresponding antigen.

## CHEMOKINES: see cytokines.

## COMPLEMENT SYSTEM:

a group of proteins involved in innate immunity. The complement system stimulates inflammation, lyses pathogenic or infected cells, and recruits B cells (initiating the adaptive response) and macrophages.

## CYTOKINES:

proteins secreted by a large number of immune cells to communicate among themselves and participate in immune defense. They include interferons, interleukins and chemokines.

## CYTOTOXIC:

capable of destroying living cells.

## HIV:

acronym for human immunodeficiency virus.

## IMMUNOTHERAPY:

treatment aimed at strengthening or changing the body's immunity.

## INNATE IMMUNITY:

immune responses involving macrophages, dendritic cells, NK cells, etc. The innate immune system acts immediately to defend the body against pathogen attacks. Innate immunity is considered to be non-specific, although recent studies have shown it to be much more «plastic» than previously thought.

## INTERFERONS:

see cytokines.

## INTERLEUKINS:

see cytokines.

## LYMPH NODE:

site where immune cells are produced and differentiate. The lymph nodes are situated along the lymphatic system and grouped at certain strategic points (the neck, abdomen, thorax, etc.).

## LYMPHOCYTES:

a type of white blood cell responsible for the body's defense. There are many types of lymphocyte including NK cells, part of the innate immune system, and also B cells and T cells, involved in adaptive immunity.

## MACROPHAGE:

a large cell belonging to the innate immune system that is capable of phagocytizing damaged or old cells, foreign particles and bacteria.

## NATURAL KILLER (NK) CELLS:

large granular lymphocytes in the innate immune system with cytotoxic activity able to kill tumor cells and infected cells. NK cells also secrete cytokines, which are involved in guiding the adaptive immune response.

## RETROVIROLOGIST:

a scientist who studies retroviruses.

## RETROVIRUS:

a virus whose genome, composed of RNA, is reverse-transcribed into DNA before integration into the host cell genome. HIV is a retrovirus.

## RNA:

ribonucleic acid, a biological molecule very similar to DNA that is found in nearly all living organisms, including certain viruses. HIV is a virus whose genetic information is carried by RNA.

## T LYMPHOCYTES:

also known as T cells, they play an important role in the adaptive immune response (T stands for thymus, the organ where T cells mature). CD8 T cells, for example, destroy infected cells, whereas CD4 T cells play a role in coordination by stimulating the amplification or differentiation of other lymphocytes. CD4 T cells are the main target of HIV.

# NOTES



A series of horizontal lines for writing notes, starting with a solid line and followed by multiple dashed lines.

A large, faint, circular graphic of a virus particle with spikes, centered on the page. The graphic is a light grey color and serves as a background for the contact information.

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GRAPHIC DESIGN : TOM & FRED

Extract from the laboratory board of Jean-Claude Cherman and Françoise Barré-Sinoussi, 1987.



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