Immune Response to Pathogens

Zakaria Hmama, PhD
UBC - Department of Medicine
1- History of HIV & AIDS.
2- Structure of HIV and its genome.
3- HIV attachment and entry into host cell.
4- AIDS pathogenesis.
5- Control and prevention of the spread of AIDS.
History of AIDS & HIV

**AIDS** was first recognized by the U.S. CDC in 1981 and the causative **HIV** was identified in 1983;

AIDS is a disease that leaves individuals susceptible to **opportunistic infections** and tumors;

The **CD4** molecule’s role as an HIV receptor was identified in 1984;

HIV is transmitted through exposure of a mucous membrane or the bloodstream to a **biological fluid** containing HIV.
History of AIDS & HIV

Controversy over the discovery of HIV

Luc Montagnier
(Pasteur Institute, Fr)

Robert Gallo
(Univ Maryland, USA)


Popovic M, Sarngadharan MG, Read E, Gallo RC. (May 1984). "Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS". Science 224: 497–500
On April 23rd 1984, the US Health & Human Services Secretary declared:

*There would soon be a commercially available test able to detect the virus with essentially 100 percent certainty*

*We hope to have a vaccine [against AIDS] ready for testing in about two years*

However, the approaches that have been successful in the control of various viral infections have failed in the case of HIV.
History of AIDS & HIV

HIV Nomenclature

The AIDS virus was originally named as LAV (lymphadenopathy-associated virus) by the Montagnier’s group, and as HTLV-III (Human T-Lymphotrophic Virus III) by the Gallo’s group.

In 1986, an international committee consisting of several reputed virologists, including Montagnier but not Gallo, named the AIDS viruses as Human Immunodeficiency Virus.
History of AIDS & HIV

Controversy over the discovery of HIV

1987: Let’s share the HIV test patent
~ US$ 100 M/ year
History of AIDS & HIV

The Nobel Prize in Physiology or Medicine 2008

Luc Montagnier and collaborator Françoise Barré-Sinoussi,

*Robert Gallo was excluded*

More on the controversy over the discovery of HIV:

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HIV Today

Source: UNAIDS
In 2010, 34 million people were living with HIV

- 2.7 million new infection and 2 million death, including ~300,000 children, were reported
HIV is a spherical enveloped virus. 
~110-120 nm in diameter; ~ 60 times smaller than a red blood cell.
HIV-1 is composed of 2 (+) strand RNA enclosed by a conical capsid comprising the viral protein p24, typical of lentiviruses; 

**Nucleocapsid** proteins (NC) associate with genomic RNA (one molecule per hexamer) and protect the RNA from digestion by nucleases.
The capsid includes 2 molecules of RNA dependent DNA polymerase or reverse transcriptase (RT);

**Integrase (IN)** is an enzyme that enables HIV genetic material to be integrated into the DNA of the infected cell.
The capsid also includes **proteases** (PR) needed for the development of the virion;

A **matrix** (MA) composed of the viral protein **p17** surrounds the capsid ensuring the integrity of the virion particle.
The matrix is surrounded by the **viral envelope** which is composed of two layers of phospholipids taken from the membrane of the host cell;

Embedded in the viral envelope are about 70 copies of a complex HIV protein known as **Env**, which consists of trimeric glycoprotein called **gp120**, anchored to the viral envelope via **gp41** molecules.
HIV Attachment and Entry

1984-HIV receptor

Letters to Nature

*Nature 312*, 763-767 (20 December 1984) | doi:10.1038/312763a0; Accepted 13 November

The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus

Angus G. Dalgleish*, Peter C. L. Beverley†, Paul R. Clapham*, Dorothy H. Crawford†, Melvyn F. Greaves* & Robin A. Weiss*

After David Klatzmann and Luc Montagnier showed that HIV infected CD4 cells but not CD8 T-cells in vitro, we sought to identify the HIV receptor by screening all the cell surface molecules known at that time to be expressed on T cells.

Over 150 mAbs specific to these various proteins had been tested … only antibodies binding to CD4 blocked HIV infection.
However, CD4-expressing nonhuman T cells have revealed a defect of HIV infection and suggested the need of a human-specific cofactor required for cell infection.
A cofactor for HIV entry was identified on $CD4^+$ T cells. This protein, designated "fusion," is a putative G protein-coupled transmembrane receptor.

**C-X-C chemokine receptor type 4 (CXCR-4)** also known as fusin or CD184
Shortly after the identification of CXCR4, Berger’s group identified a different co-receptor on macrophages.

_CCC CKR5: A RANTES, MIP-1α, MIP-1β Receptor as a Fusion Cofactor for Macrophage-Tropic HIV-1_

Ghalib Alkhatib, Christophe Combadiere*, Christopher C. Broder*, Yu Feng*, Paul E. Kennedy*, Philip M. Murphy†, Edward A. Berger†

*C-C chemokine receptor type 5, also known as CCR5 or CD195*
HIV Attachment and Entry

CCR5- & CXCR4-tropic HIV

**CCR5**

**CXCR4**

**R5 viruses (M-tropic)**
Prevalent in early disease

**X4 viruses (T-tropic)**
Late disease; associated with CD4 decline and clinical progression
The first step of infection is the binding of gp120 to the CD4 receptor, followed by the binding of gp 41 to the chemokine receptor.
Once bound to CD4, the gp120 will change conformation;

The conformation change open a binding site for the co-receptor (i.e., chemokine receptor);

The gp120 binding to the co-receptor induces a conformation change in gp41;
Modified gp41 mediates the fusion between the virus envelope and the host cell plasma membrane and subsequent entry of the viral capsid;

The HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease and protease, are injected into the cell;
A 32-base-pair deletion within the coding region of CCR-5 results in a frame shift, and generates a non-functional receptor that does not support membrane fusion or infection by macrophage confirming that CCR-5 is the major co-receptor for primary HIV-1 strains.
HIV Attachment and Entry

Role of DC in HIV infection

Identification of DC-SIGN, a Novel Dendritic Cell-Specific ICAM-3 Receptor that Supports Primary Immune Responses

Teunis B. H. Geijtenbeek, Ruurd Torensma, Sandra J. van Vliet, Gerard C. F. van Duijnhoven, Gosse J. Adema, Yvette van Kooyk,* and Carl G. Figdor

DC-SIGN, a Dendritic Cell-Specific HIV-1-Binding Protein that Enhances trans-Infection of T Cells

1- Demonstration that ICAM-3 expressed by resting T cells is important for first contact with DC.

2- Discovery that instead of the common ICAM-3 receptors LFA-1, a novel DC-specific C-type lectin, DC-SIGN, binds ICAM-3 with high affinity.

3- Antibodies against DC-SIGN inhibit DC-induced proliferation of resting T cells.
HIV Attachment and Entry

1st paper: DC-T cell interaction

Model of DC-resting T cell interactions
(1). DC-SIGN/ICAM-3 interactions form a first contact between DC and T cell. This transient interaction facilitates the formation of low-avidity interactions in which LFA-1/ICAM-1 also participates giving rise to loose antigen-independent DC–T cell clustering (2). Only after TCR ligation (3), signals result in high-avidity LFA-1/ICAM-1 and CD2/LFA-3 interactions giving rise to a further stabilization of the immunological synapse in which the smaller TCR/MHC/peptide and CD2/LFA-3 form the center and LFA-1/ICAM-1 form the outer ring (4).
Nucleotide sequence analysis of the cDNA indicated that DC-SIGN is identical to a previously described HIV-1 gp120-binding C-type lectin isolated from a placental cDNA library.
HIV Attachment and Entry

2nd paper: DC-SIGN mediates HIV binding to DC in the absence of CD4

Binding of gp120-coated fluorescent beads
2nd paper: DC-SIGN mediates HIV infection in a DC-T cell co-culture

DC were preincubated with CD4 or DC-SIGN Ab or with a combination of CCR5-specific chemokines. Preincubated DC were pulsed with HIV-and unbound virus particles and mAb were washed away. Subsequently, DC were cocultured with PBMC for 9 days. Coculture supernatants were subjected to p24 levels measurement.
HIV Attachment and Entry

Role of DC in HIV infection

DC-SIGN mediates trans infection of T cells
HIV Attachment and Entry

The New York Times

Scientists Find New Receptor for HIV

By LAWRENCE K. ALTMAN
Published: February 11, 2008

Article

*Nature Immunology* 9, 301 - 309 (2008)
Published online: 10 February 2008 | doi:10.1038/ni1566

HIV-1 envelope protein binds to and signals through integrin $\alpha_4\beta_7$, the gut mucosal homing receptor for peripheral T cells

James Arthos$^{1,6}$, Claudia Cicala$^{1,6}$, Elena Martinelli$^{1,2,6}$, Katilyn Macleod$^1$, Donald Van Ryk$^1$, Danlan Wei$^1$, Zhen Xiao$^3$, Timothy D Veenstra$^3$, Thomas P Conrad$^3$, Richard A Lempicki$^4$, Sherry McLaughlin$^5$, Massimiliano Pascuccio$^1$, Ravindra Gopaul$^1$, Jonathan McNally$^1$, Catherine C Cruz$^1$, Nina Censoplano$^1$, Eva Chung$^1$, Kristin N Reitano$^1$, Shyam Kotttilil$^1$, Diana J Goode$^1$ & Anthony S Fauci$^1$
HIV Attachment and Entry

Uninfected CD4\(^+\) T cells cultured with CD4\(^+\) T cells infected with HIV-1, cultured with retinoic acid and stained with antibody specific for HIV-1 p24 (blue), \(\alpha_4\) (green), active LFA-1 (red) or CD4 (purple). Yellow, colocalization of \(\alpha_4\) and LFA-1; white, colocalization of \(\alpha_4\) and CD4.
Series 2: Fluorescence microscopy of CD4⁺ T cell conjugates treated with gp120 and stained with mAb specific for gp120, α₄, LFA-1 or CD4. Far right, colocalization of α₄, LFA-1 and gp120.

Series 3: Magnification of the series 2 conjugate and z-axis reconstructions of the cell-cell interface visualized with various combinations of mAbs.
Model of a Virological Synapse (VS) upon engagement of α4β7 by HIV-1 envelope.

(a): An HIV-1 infected cell encounters a highly susceptible target cell expressing high levels of α4β7.

(b) HIV-1 envelope on infected cell binds to α4β7 on the target cell and activates the downstream integrin LFA-1.

(c) LFA-1 binds to its ligand ICAM-1 and stabilizes a VS.
HIV replication cycle

1. Fusion of HIV to the host cell surface.
2. HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.
3. Viral DNA is formed by reverse transcription.
4. Viral DNA is transported across the nucleus and integrates into the host DNA.
5. New viral RNA is used as genomic RNA and to make viral proteins.
6. New viral RNA and proteins move to the cell surface and a new, immature HIV forms.
7. The virus matures by protease releasing individual HIV proteins.

Host Cell

Mature Virion

Co-receptor (CCR5 or CXCR4)

gp120

CD4

Preintegration complex
Clinical course of HIV infection

HIV infection has basically four stages: incubation period, acute infection, latency stage and AIDS.

The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks;

The stage of infection can be determined by measuring the patient's CD4+ T cell count, and the level of HIV in the blood.
AIDS Pathogenesis

Acute HIV infection

It is a period of rapid viral replication leading to an abundance of virus in the peripheral blood: ~ several million viruses/ml;

Virus replication is accompanied by a marked drop of circulating CD4$^+$ T cells;

Acute viremia is associated with activation of cytotoxic CD8$^+$ T cells, which kill HIV-infected cells;

The immune response leads to antibody production, or seroconversion … marking the start of the latency stage.
AIDS Pathogenesis

Clinical course of HIV infection

- Primary Infection: CD4+ T Lymphocyte Count (cells/mm³)
- Acute HIV syndrome: Wide dissemination of virus, Seeding of lymphoid organs
- Clinical Latency
- Opportunistic Diseases
- Constitutional Symptoms
- Death

Graph showing CD4+ T Lymphocyte Count (cells/mm³) over Weeks and HIV RNA Copies per ml Plasma over Years.
Acute HIV infection

Because of the nonspecific nature of the symptoms, they are often not recognized as signs of HIV infection;

Patients are often misdiagnosed as having one of the more common infectious diseases...such as Influenza.
Without treatment, about 9 out of every 10 persons with HIV will progress to AIDS after 10–15 years.

Treatment with anti-retrovirals increases the life expectancy of people infected with HIV.

Even after HIV has progressed to diagnosable AIDS, the average survival time with antiretroviral therapy is estimated to be more than 5 years.

Without antiretroviral therapy, death normally occurs within a year.

It is hoped that current and future treatments may allow HIV-infected individuals to achieve a life expectancy approaching that of the general public.
AIDS Pathogenesis

Can a Bone-Marrow Transplant Halt HIV?

The only person in the world who has been cured of HIV

Timothy Brown
“The Berlin Patient.”
I was attending school in Berlin in 1995 when I tested positive for HIV; in 2006, a bone marrow biopsy showed that I had acute myeloid leukemia; I had to stop my third round of chemotherapy half-way through when I developed sepsis…Bonne marrow transplant was the unique option; 

Dr. Huetter had a revolutionary treatment idea…The CCR5 receptor allows the HIV virus to attach to the T-cell and subsequently infect the cell….People with defective CCR5 appeared resistant to HIV infections; I thought: A possibility to be rid of cancer and HIV? At the same time?
In January of 2007 Dr. Huetter found a compatible donor who also had the CCR5 delta 32 mutation;

I underwent total body irradiation to wipe out my body’s immune system before receiving the transplant. _I last took my HIV medications on the day of my stem cell transplant;

It is now 5 years after my first stem cell transplant. I am still without leukemia, but then that’s not the reason why you are listening to me today. _It is that after five years without HIV medications, I still have no trace of HIV in my body._
Can a Bone-Marrow Transplant Halt HIV?

Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

Does HIV cause AIDS?

HIV does not meet any of Koch's four postulates.

Koch's postulates:
1. Isolate the pathogen (virus, microbe, etc.) from sick creature.
2. Grow the pathogen in the laboratory and obtain a pure culture.
3. Inoculate a healthy creature with a sample from the pure culture. The pathogen should cause the same disease symptoms that were seen in the first creature.
4. Reisolate the same pathogen from the second sick animal.
Does HIV causes AIDS?
http://rethinkingaids.com

Postulate one: Most people are diagnosed HIV-positive based solely on antibody tests…antibodies cannot be accepted as evidence of the presence of HIV;

Postulate two: The process of HIV culturing is not isolation or purification…HIV is never isolated in the sense of being purified. No electron micrographs of purified HIV exist;

Postulate three: Many healthy, HIV-positive people exist, but they are always subject to pressure to consume immunosuppressive medications. No group of initially healthy HIV-positive people who are not taking AIDS drugs, and who do not have any immune system risk factors have ever been followed for a lengthy period;

Postulate four: PCR tests do not necessarily indicate the presence of HIV…they are prone to false positives, and they cannot distinguish non-infectious RNA from an infectious virus particle.
Does HIV causes AIDS?

Peter H. Duesberg, Professor of Molecular and Cell Biology at the University of California Berkley.

Duesberg’s Hypothesis:
AIDS is caused by long-term consumption of recreational drugs and/or antiretroviral drugs...HIV is a harmless passenger virus.
Does HIV causes AIDS?
Does HIV causes AIDS?

Luc Montagnier

Winner of the 2008 Nobel Prize for the “discovery of HIV”.

Montagnier: “We can be exposed to HIV many times without being chronically infected. Our immune system will get rid of the virus within a few weeks if you have a good immune system.”

Interviewer: “If you have a good immune system, your body can naturally get rid of HIV?”

Montagnier: “Yes”

Interviewer: “If you take a poor African who’s been infected and you build up her immune system, is it also possible for them to also naturally get rid of HIV?”

Montagnier: “I would think so. It’s important knowledge, which is completely neglected. People always think of drugs and vaccine.”

Interviewer: “There’s no money in nutrition, right?”

Montagnier: “There’s no profit, yes.”
HIV is the cause of AIDS
http://www.avert.org/hiv-causes-aids.htm

Koch’s postulates ???.......HIV is not a bacteria !

The evidence that HIV causes AIDS is abundant and conclusive:

• AIDS does not occur without HIV;
• HIV infection is the only factor that predicts who will develop AIDS;
• Surveillance statistics support the HIV theory;
• Modern antiretroviral treatment is highly beneficial.
Prevention of AIDS

- Unprotected sexual intercourse with an infected partner
- Vertical transmission (from mother to child)
  - in utero
  - during delivery
  - breastfeeding
- Injection drug use (rare: infected blood/blood products)

HIV INFECTION
Prevention of AIDS

Simple, easy and costs less than lifetime treatment

STOP
Unprotected sexual intercourse with an infected partner

STOP
Vertical transmission (from mother to child)
- in utero
- during delivery
- breastmilk

STOP
Injection drug use (rare: infected blood/blood products)

HIV INFECTION