Lentiviruses: HIV-1 Pathogenesis

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Replication cycle for a Retrovirus (HIV, Rous sarcoma virus)
Part I: HIV virology

HIV-1 is a lentivirus

- Lentiviruses comprise a genus of the family Retroviridae

- Lentiviruses = slow viruses, since they have long incubation periods.

- The following lentiviruses are found in mammals:
  
<table>
<thead>
<tr>
<th>Virus</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1,2</td>
<td>Human Immunodeficiency Viruses</td>
</tr>
<tr>
<td>SIV</td>
<td>Simian Immunodeficiency Virus (monkey)</td>
</tr>
<tr>
<td>EIAV</td>
<td>Equine Infectious Anemia Virus (horse)</td>
</tr>
<tr>
<td>FIV</td>
<td>Feline Immunodeficiency Virus (cat)</td>
</tr>
<tr>
<td>CAEV</td>
<td>Caprine Arthritis Encephalitis Virus (goat)</td>
</tr>
</tbody>
</table>

- Lentiviruses (like other retroviruses) are enveloped viruses, ~100-120 µm in size for HIV-1.

- The HIV-1 genome is a positive single stranded RNA of ~10,000 nucleotides.

- Lentiviruses have more complex genomes than most retroviruses.

- They can infect non-dividing cells.
Life cycle of HIV

1. Virion binding to CD4 and chemokine receptor
2. Fusion of HIV membrane with cell membrane; entry of viral genome into cytoplasm
3. Reverse transcriptase-mediated synthesis of proviral DNA
4. Integration of provirus into cell genome
5. Cytokine activation of cell; transcription of HIV genome; transport of spliced and unspliced RNAs to cytoplasm
6. Synthesis of HIV proteins; assembly of virion core structure
7. Expression of gp120/gp41 on cell surface; budding of mature virion

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 20-5

Courtesy of Helen Fernandes, Ph.D.
NJMS, UMDNJ
Structure of human immunodeficiency virus

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 20-3

Courtesy of Helen Fernandes, Ph.D.
NJMS, UMDNJ
Schematic representation of a mature human immunodeficiency virus 1 (HIV-1) virion.
(Adapted from ref. 206, with permission.)
HIV-1 encodes 3 groups of proteins:

- **Structural polyproteins**: Gag, Pol, Env
  - Gag-Pol precursor is digested to give the three viral enzymes:
    - reverse transcriptase, protease and integrase
  - Gag is digested to give matrix, capsid and nucleocapsid proteins
  - Env is digested to give gp120 and gp41

- **Regulatory proteins**: Tat, Rev

- **Accessory proteins**: Nef, Vif, Vpr, Vpu
Function of HIV-1 proteins, DNA and RNA

LTR Integration of viral DNA into host cell genome; binding site for host transcription factors

**gag** Nucleocapsid core and matrix proteins  p17  p24  p7  p6

**pol** Reverse transcriptase, protease, integrase, and ribonuclease

**env** Viral coat proteins (gp120 and gp41) mediating CD4 and chemokine receptor binding and membrane fusion

**vif** Enhances infectivity of viral particles. Prevents encapsidation of APOBEC proteins

**vpr** Promotes nuclear import of viral DNA; G2 cell cycle arrest

**tat** Required for elongation of viral transcripts; also TAR- a regulatory RNA element

**rev** Promotes nuclear export of incompletely spliced or unspliced viral RNAs

**vpu** Downregulates host cell CD4 expression and enhances release of virus from cells

**nef** Downregulates host cell CD4 expression and enhances release of virus from cells; downregulates host cell class I MHC expression

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 20-4

Courtesy of Helen Fernandes, Ph.D. NJMS, UMDNJ
Part II: AIDS

Where does HIV / AIDS come from?

I. Recognition of AIDS

**Late 1970s-1980:** Patients with immunological dysfunction seek advice and treatment.

  - **Symptoms:** generalized lymphadenopathy, opportunistic infections, unusual cancers
  - **Lab finding:** depletion of CD4+ T-lymphocytes

**1981:** CDC describes high-risk groups affected with AIDS:

  - Male homosexuals
  - Intravenous drug users
  - Hemophiliacs
  - Blood transfusion recipients
  - Recent Haitian immigrants
  - Sexual partners and children of people in these groups.

Suggested a novel pathogen transmitted via contaminated blood or sexual intercourse.
II. Discovery of HIV-1

1983: *Retrovirus* was isolated from patient’s lymph node in the Pasteur Institute in Paris, France.

2008 winners of the Nobel prize in physiology or medicine: *Francoise Barre-Sinoussi* and *Luc Montagnier* for their discovery of HIV-1

1984: *Retrovirus* and antibodies reactive with this virus were isolated from AIDS patients’ blood and also from asymptomatic (healthy) individuals in the USA.

1986: The name *HIV*, human immunodeficiency virus, was agreed. HIV-2 was discovered and HIV was re-named *HIV-1*.

III. Origin of the human disease

1959: The oldest known HIV-1 virus is strain ZR59, isolated from a serum sample of an African male (Democratic Republic of Congo).

1969: HIV entered the US from Africa via Haiti.

1990: Wild-born chimpanzee was found to harbor a closely-related virus, *SIVcpz* (contains *vpu* - an HIV-1 specific gene). Chimpanzee communities in Cameroon are the most likely originators.
IV. Groups of HIV-1

Group M: from a single trans-species transmission into the African population in the 1940s or early 1950s.

Groups N and O: from separate introductions of SIVcpz into the human population.

Phylogenetic analysis of HIV-1 sequences

Analyzing the sequence of DRC60 (1960) and comparing it to ZR59 (1959), dates the transmission into the African population near the beginning of the twentieth century.

### Global summary of the AIDS epidemic, December 2007

#### Number of people living with HIV in 2007

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Adults</th>
<th>Women</th>
<th>Children under 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.2 million [30.6 – 36.1 million]</td>
<td>30.8 million [28.2 – 33.6 million]</td>
<td>15.4 million [13.9 – 16.6 million]</td>
<td>2.5 million [2.2 – 2.6 million]</td>
</tr>
</tbody>
</table>

#### People newly infected with HIV in 2007

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Adults</th>
<th>Women</th>
<th>Children under 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 million [1.8 – 4.1 million]</td>
<td>2.1 million [1.4 – 3.6 million]</td>
<td>420 000 [350 000 – 540 000]</td>
<td></td>
</tr>
</tbody>
</table>

#### AIDS deaths in 2007

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Adults</th>
<th>Women</th>
<th>Children under 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.1 million [1.9 – 2.4 million]</td>
<td>1.7 million [1.6 – 2.1 million]</td>
<td>330 000 [310 000 – 380 000]</td>
<td></td>
</tr>
</tbody>
</table>
Sub-Saharan Africa is the highest infected region
~ 69% of people living with HIV are in sub-Saharan Africa.

South and southeast Asia region is the second highest
How is HIV-1 transmitted?

I. Stability

HIV-1 is unstable outside the body.

Infectivity is reduced rapidly upon air drying.

The virus is inactivated by exposure to heat (60°C, 30 min.), germicides and pH extremes.

II. Routes of transmission
(established by Epidemiology)

1. Sexual contact
2. Blood
3. Mother to child

• HIV-1 enters the body through mucosal surfaces or broken skin.

• The probability of transmission is a function of exposure frequency and virus concentration in the body fluid.
III. Common modes of transmission

1. Heterosexual sex
2. Homosexual sex
3. Intravenous drug use
4. Infected mother to child
   (at the time of delivery or via infected breast milk).

- Modes of transmission vary in different geographical locations largely due to behavioral differences.

The efficiency of transmission is influenced by the concentration of the virus in the body fluid to which the individual is exposed.

### Table 18.4 Isolation of infectious HIV-1 from body fluids

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Virus isolation</th>
<th>Estimated quantity of virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>33/33</td>
<td>1–5,000&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tears</td>
<td>2/5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ear secretions</td>
<td>1/8</td>
<td>5–10</td>
</tr>
<tr>
<td>Saliva</td>
<td>3/55</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Sweat</td>
<td>0/2</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feces</td>
<td>0/2</td>
<td>ND</td>
</tr>
<tr>
<td>Urine</td>
<td>1/5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Vaginal-cervical</td>
<td>5/16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Semen</td>
<td>5/15</td>
<td>10–50</td>
</tr>
<tr>
<td>Milk</td>
<td>1/5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>21/40</td>
<td>10–10,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infected cells</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC&lt;sup&gt;f&lt;/sup&gt;</td>
<td>89/92</td>
<td>0.001–1%&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saliva</td>
<td>4/11</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Bronchial fluid</td>
<td>3/24</td>
<td>ND</td>
</tr>
<tr>
<td>Vaginal-cervical fluid</td>
<td>7/16</td>
<td>ND</td>
</tr>
<tr>
<td>Semen</td>
<td>11/28</td>
<td>0.01–5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adapted from Table 2.1 of J. A. Levy (ed.). *HIV and the Pathogenesis of AIDS*, 2nd ed. (ASM Press, Washington, D.C., 1998), with permission.

<sup>b</sup>Number of samples positive/number analyzed.

<sup>c</sup>For cell-free fluid, units are infectious particles per milliliter; for infected cells, units are percentages of total cells capable of releasing virus. Results are from studies in the laboratory of J. A. Levy. ND, not determined.

<sup>d</sup>High levels associated with acute infection and advanced disease (~5 × 10<sup>6</sup> PBMCs per ml in blood).

<sup>e</sup>ND, none detected.

<sup>f</sup>PBMC, peripheral blood mononuclear cells.
What are the events occurring after HIV-1 infection?

- Primary Infection
- Acute HIV syndrome
- Wide dissemination of virus
- Seeding of lymphoid organs
- Clinical Latency
- Death
- Opportunistic Diseases
- Constitutional Symptoms

CD4+ T Lymphocyte Count (cells/mm³)

HIV RNA Copies per ml Plasma
The course of HIV-1 infection

From Flint, Enquist, Krug, Racaniello & Skala: Principles of Virology. ASM Press, 2000. Fig. 18.9.
What do we know about the dynamics of HIV-1 replication?

- **Continuous and highly productive replication** of HIV-1 occurs in all infected individuals, although the rates of virus production vary by up to 70-fold in different individuals.

- An average of $2 \times 10^9$ new **CD4+ T cells** are produced each day.

- ~$10^8$ newly infected **CD4+ T cells** are responsible for producing most of the plasma virus.

- $10^9-10^{10}$ **virions** are produced per day.

- **Turnover** of plasma virus is very fast: a particle's half-life is **28-110 min**.

- Thus, contrary to what was initially believed, there is a very dynamic HIV infection cycle in HIV-infected people involving **continuous infection, destruction and replacement of CD4+ T cells**. Billions of new CD4+ T cells are produced, infected and killed each day.

- Distribution of HIV in the body:
  - 2% of the virus is in the circulation
  - 98% of the virus replicates at different rates in various compartments
    - lymph nodes, liver, spleen, and neural tissue.

- Viruses circulating in the blood are from all of the compartments in which HIV replicates.
How can HIV infection be prevented?

How can it be controlled?

How can it be cured?
Multiple potential targets for antiviral compounds

Antivirals in use

RT inhibitors
- Nucleoside analogs
  Compete with nucleotides for incorporation into proviral DNA. Cause chain termination.
- Nonnucleoside inhibitors
  Bind to hydrophobic pocket close to the active site on RT and inhibit the enzyme function allosterically.

PR inhibitors
Mimic protease cleavage site in the HIV protein substrate

Fusion inhibitor
Binds to viral gp41 and prevents fusion of viral membrane with cell membrane

Integrase inhibitor
Inhibits DNA strand transfer from the viral genome to the host genome.


HAART, Highly Active Anti-Retroviral Therapy
Development of HIV-1 drug resistance

Mutations in the viral genome appear when reverse transcriptase synthesizes the double-stranded proviral DNA.
- Reverse transcriptase is a highly error-prone enzyme (error rate ~ 1 in 10,000).
- More than one particle can infect a cell and multiple recombination events occur.

Every HIV genome is likely to have at least 1 mutation.

HIV-1 viruses in the patient’s body are quasispecies - reservoirs of diversity available for selection.

Note the mutations already existing in untreated patients that would result in drug resistance.

In patients’ plasma:

- 48% are R5 viruses
- 2% are X4 viruses *
- 50% are dual tropic viruses

* X4 viruses are associated with rapid disease progression

“Host tropism” or “cell tropism” refers to the way in which different viruses/pathogens have evolved to preferentially target specific host species, or **specific cell types** within those species.
Why does HIV-1 infection give rise to chronic disease?

Half-life of various forms of HIV

Free virion
Productively infected CD4+ T lymphoblasts
Resting CD4+ T cells with unintegrated HIV-1 DNA
Virion on FDC (follicular dendritic cells)
Infected macrophage
Resting CD4+ T cells with integrated HIV-1 DNA (half-life ? years)

Half-life (days)

Finzi D, Siliciano R. Cell 1998
The establishment of cellular latency in memory T cells
The establishment of cellular latency in memory T cells
(a) **drug-resistant variants** can emerge from the latent reservoir through activation.

(b) **low-level residual viremia** during HAART can lead to viral rebound if therapy is stopped.
**Part III: Virus-Cell interaction**

Virus-cell interactions have been described for many stages of the infectious cycle.

<table>
<thead>
<tr>
<th>PROCESS</th>
<th>VIRAL COMPONENT</th>
<th>CELLULAR COMPONENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane binding and entry</td>
<td>gp120, gp41</td>
<td>CD4, CCR5/CXCR4</td>
</tr>
<tr>
<td>Integration</td>
<td>Integrase</td>
<td>LEDGF/p75</td>
</tr>
<tr>
<td>Transcription</td>
<td>Tat, TAR</td>
<td>P-TEFb</td>
</tr>
<tr>
<td>Splicing and transport</td>
<td>Rev, RRE</td>
<td>CRM1/Exportin 1</td>
</tr>
<tr>
<td>Assembly</td>
<td>Gag</td>
<td>PI(4,5)P2*</td>
</tr>
<tr>
<td>Release</td>
<td>Gag, Vpu</td>
<td>Tsg101 and ubiquitin</td>
</tr>
<tr>
<td>Countering host-defense</td>
<td>Nef</td>
<td>MHC class-I</td>
</tr>
</tbody>
</table>

*phosphoinositide phosphatidylinositol (4,5) bisphosphate*
Mechanism of HIV entry into cells

HIV virion

CD4

Host cell membrane

Chemokine receptor

HIV membrane

gp41

gp120

CD4

T cell membrane

CCR5/CXCR4

HIV gp120 binds to T cell CD4

Conformational change in gp120 promotes binding to chemokine receptor

Conformational change in gp41 exposes fusion peptide, which inserts into T cell membrane

Fusion of viral and cell membranes

T-20 peptidomimetic

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 20-6
Landmarks in human immunodeficiency virus (HIV) research. Important discoveries and technical or conceptual advances are noted on either side of the central time line.
Challenges 2008

- **Prevention** – increased efficacy
- **Vaccine** – none currently available
- **Antivirals** – development of resistance
  - side effects
- **Latency** – proviral reservoir
- Deciphering **viral replication**
Assignment

Pick one of the 6 HIV regulatory and accessory proteins: Tat, Rev, Vif, Vpr, Nef, Vpu

Describe its structure (briefly),
its function in the virus life cycle, and
its interactions with cellular components
(or its chief cellular partner)