Retroviridae

The RNA genome is 5’-capped and 3’-polyadenylated.

Three genes groups are found in the genomes of all retroviruses:

- **GAG** genes code for capsid proteins
- **POL** genes code for enzymes critical to virus development:
  - reverse transcriptase
  - integrase
  - protease
- **ENV** genes code for envelope glycoproteins
Retroviridae

The Replication Strategy for Retroviruses is Very Different From Other RNA Viruses

- The virus attaches to a cell via a specific receptor
- This triggers fusion of the cell membrane and the viral envelope, which releases the nucleocapsid into the cytosol

Typical Retrovirus Replication Cycle
Typical Retrovirus Replication Cycle

- The nucleocapsid “uncoats” and the RNA genome is “reverse” transcribed into a duplex DNA copy.
- This is performed by the viral enzyme Reverse Transcriptase.

Typical Retrovirus Replication Cycle

- The DNA migrates to the nucleus and is integrated into the host chromosome by the enzyme integrase.
- The integrated DNA is transcribed by host RNA polymerase into viral mRNA.
**Typical Retrovirus Replication Cycle**

- The viral mRNA is translated into capsid proteins, enzymes and envelope glycoproteins.
- The viral mRNA also serves as a plus-sense viral genome.
- Nucleocapsid assembly occurs in the cytoplasm.
- The nucleocapsid then buds from the plasma membrane acquiring its envelope glycoproteins.

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**Typical Retrovirus Replication Cycle**

- Transcribe mRNA and HIV is made!!
**Human Immunodeficiency Virus (HIV)**

Infection with HIV is associated with a disease known as **Acquired Immuno Deficiency Syndrome (AIDS)**

HIV is a typical retrovirus

The nucleocapsid contains two copies of the RNA genome (capped and polyadenylated)

**HIV Virion Structure**

**ENV Genes - Surface Glycoproteins**

**Gp120**
- a peripheral membrane protein
- responsible for binding to cell surface receptor

**Gp41**
- an integral membrane protein
- responsible for membrane fusion
HIV Virion Structure

**GAG Genes - Internal Structural Proteins**

**Matrix Protein**
- membrane associated
- lines the inner surface of viral membrane

**Capsid Protein**
- forms bullet-shaped shell (core)

**POL - Viral Enzymes**

**Viral Protease**
- required for maturation of the GAG and GAG-POL polyproteins

**Reverse Transcriptase**
- RNA-dependent RNAP
- DNA-dependent RNAP
- RNase H

**Integrase**
- inserts viral DNA into host genome
HIV Cell Entry

The viral gp120 glycoprotein binds to the **CD4 receptor** to initiate virus infection (found on T lymphocytes).

The CD4 receptor is necessary, but not sufficient for infection.

- gp120 binding to a **chemokine “co-receptor”** promotes a conformational change in the viral gp41 protein.

- This promotes fusion of the viral envelope with the cell plasma membrane, releasing the nucleocapsid into the cytoplasm.
**HIV Cell Entry**

The **CCR5** is a chemokine co-receptor found in **macrophages**

*Macrophage-trophic* strains are associated with mucosal and intravenous transmission of HIV

They are less virulent and rarely form *syncytia*

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**HIV Cell Entry**

**CXCR4** is a chemokine co-receptor found in **T-cells**

CXCR4 (a.k.a. fusin) is a G protein-coupled receptor; it promotes the fusion of CD4+ cells leading to syncytium formation

A syncytium is a multinucleated mass of cytoplasm that is not separated into individual cells

Syncytium formation allows spread of the infection without any free virus

*T cell-trophic* strains are more virulent and are frequently found during the later stages of disease
HIV Cell Entry

Initially infection typically occurs with macrophage-trophic strains that infect (CD4$^+$ CCR5$^+$) macrophages.

The virus readily mutates during infection and are transformed into T cell-trophic strains that infect (CD4$^+$ CXCR4$^+$) T-cells.

HIV Reverse Transcription

Upon entry into the cell, the nucleo-capsid partially uncoats.

RT uses the plus-sense RNA strand as a template to synthesize a RNA-DNA hybrid duplex (RNA-dependent DNA polymerase).

RT then degrades the RNA strand (RNase H).

Finally, RT uses the DNA strand as a template to synthesize duplex DNA (DNA-dependent DNA polymerase).
HIV Reverse Transcription

The cytoplasmic concentration of nucleotides may be a limiting factor in reverse transcription, especially in non-dividing cells

*Hydroxyurea*, an inhibitor of ribonucleotide reductase, has been used to inhibit HIV replication

The dsDNA formed by reverse transcription is known as a **provirus**

The provirus migrates to the nucleus and is integrated into the host chromosome, a reaction catalyzed by **DNA integrase**

Proviral DNA is copied along with cellular DNA during cell replication

At this stage the provirus is just like a normal gene
**HIV Genome Synthesis**

Full length, genomic RNA (plus sense vRNA) is copied from integrated proviral DNA by host RNA polymerase II.

Expression of vRNA is regulated by both cellular and viral factors (transcription factors).

- infection
- production of inflammatory cytokines
- cellular activation

**HIV Protein Synthesis**

The ENV genes are translated and transported through the ER and Golgi where they are glycosylated.

gp120 and gp41 are transported to the plasma membrane.

A GAG polyprotein and a GAG-POL fusion polyprotein are translated from the vRNA.
HIV Virus Assembly

The GAG-POL polyprotein binds to viral RNA and initiates GAG protein assembly into a nucleocapsid structure that buds from the plasma membrane.

After the virus has budded from the cell, the viral protease, which is part of the GAG-POL polyprotein, cuts itself free.

The protease completes the cleavage of the GAG-POL polyprotein, which releases reverse transcriptase and integrase.

The protease finally cleaves the GAG polyprotein into the structural proteins that form the bullet-shaped core (matrix and capsid).
Anti-Retroviral Drug Therapy

There are currently three HIV targets for anti-HIV therapy

• Reverse Transcriptase Inhibitors
• Viral Protease Inhibitors
• gp41 Membrane Fusion Inhibitors

Nucleoside Reverse Transcriptase Inhibitors

These drugs share a common mechanism

They are competitive inhibitors of RT and additionally act as DNA chain terminators (remember acyclovir)

Nucleoside RT Inhibitors

Azidothymidine (AZT)
Dideoxycytosine (DDC)
Dideoxyinososine (DDI)
Tenofovir

Azidothymidine (AZT)
Dideoxycytosine (DDC)
Dideoxyinososine (DDI)
Tenofovir
Nucleoside RT Inhibitors

Abacavir  Didehydrothymidine (d4T)  2'-deoxy-3'-thiacytidine (3TC)

Non-Nucleoside RT Inhibitors

The high rate of RT mutation and resistance to the nucleoside inhibitors led to the development of non-nucleoside inhibitors.

These drugs are non-competitive inhibitors of reverse transcriptase.

The idea is that mutations in RT leading to resistance to nucleoside inhibitors would be different than those leading to resistance of the non-nucleoside inhibitors.

Thus, the nucleoside and non-nucleoside RT inhibitors could be used in combination therapy.
Non-Nucleoside RT Inhibitors

Efavirenz

Nevirapine (NVP)

Delavirdine (DLV)

Viral Protease Inhibitors

A viral protease is critical to replication of HIV
Several protease inhibitors are in clinical use today
All of these drugs mimic the peptide substrates for the enzyme

Saquinavir (SQ)
Ritonavir
Viral Protease Inhibitors

Nelfinavir
Amprenavir
Lopinavir
Indinavir

Viral Fusion Inhibitors

Enfuvirtide (INN) is peptide that binds to gp41 and inhibits membrane fusion
HIV Infection

Initial studies indicated that HIV had a specificity (tropism) for helper T lymphocytes that express the CD4 (monocytes, dendritic cells and brain microglia also express CD4)

Infection of CD4⁺ T lymphocytes results in cell lysis, or induces apoptosis, causing profound immunosuppression

In contrast, other HIV-infected cells do not show a cytopathic effect and the virus may continually bud from the cell

In particular, follicular dendritic cells (FDC’s) of the lymph node may be infected, but not killed; approximately 20% of an individual’s T-cells go through the lymph nodes daily and many are infected

FDC’s may thus serve as a reservoir for further infection

Cellular Latency

CD4⁺ lymphocytes replicate HIV only when they are activated

Activated T cells produce large amounts of virus resulting in cell death

Viral and bacterial infections can activate infected resting CD4⁺ lymphocytes resulting in virus replication and viremia

Activation of infected cells usually leads to cell death; however, a small proportion of cells revert to the resting memory state where the virus again becomes dormant

This is called *cellular latency*

This minority of resting cells may provide a reservoir of integrated virus that cannot be eliminated by chemotherapy
AIDS - Clinical Course

From the original infection, there is usually a period of 8-10 years before the clinical manifestations of AIDS occur.

Approximately 10% of patients succumb to AIDS within 2 to 3 years.

**Acute Infection**

- self-limiting mild disease
- viremia after 4-11 days that continues for a few weeks
- mononucleosis-like symptoms (fever, rash, lymphadenopathy)
- a decrease in the number of CD4$^+$ cells (helper T-cells)
- an increase in the number of CD8$^+$ cells (killer T-cells)

AIDS - Clinical Course

**Cell-Mediated and Humoral Immune Response**

Cytotoxic B and T lymphocytes mount a strong defense that partially clears the viremia.

Remaining infected cells continue to produce virus, but are destroyed, either by the immune system or by the virus.

However, the rate of production of CD4$^+$ cells compensates for destroyed cells and a steady state is achieved.

In this state, only a small fraction of the resting memory CD4$^+$ cells carry an integrated HIV genome.

The virus disseminates to other regions, including lymphoid and nervous tissue.
**AIDS- Clinical Course**

**Clinical Latency**

The strong immune defenses significantly decreases the viremia, and the patient enters clinical latency.

Viruses are found in the bloodstream or in peripheral blood lymphocytes.

Initially, the number of blood CD4\(^+\) cells is only slightly decreased, but the virus persists in other tissues.

Particularly important is the infection of follicular dendritic cells in lymph nodes.

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**AIDS- Clinical Course**

**Loss of CD4\(^+\) Cells and Immunodeficiency**

The immune system fails to control HIV infection because the CD4\(^+\) T helper cells are the target of the virus.

There is a profound loss of the immune response:

- The CD4\(^+\) cells that proliferate in response to the virus are infected and killed by it.
- Epitope variation (gp120 mutations) can lead to escape from the immune response.
- Activated T cells are susceptible to apoptosis, including uninfected CD4\(^+\) and CD8\(^+\) T cells.
- The number of follicular dendritic cells falls over time, resulting in diminished capacity to stimulate CD4\(^+\) cells.
AIDS- Clinical Course

Thus, there is a dramatic decrease in CD4\(^+\) cell number, especially those specific to HIV. This occurs from the very beginning of infection and is permanent.

Near the end stage of AIDS, CD8\(^+\) cell numbers also decline precipitously.

Note that during the course of HIV infection, most CD4\(^+\) cells are never actually infected; they nevertheless die by other means.

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**Onset of AIDS**

Clinical latency varies from 1-2 years to more than 15 years.

Eventually, the virus can no longer be controlled as helper CD4\(^+\) cells are destroyed.

Once the T4 cell count falls below 200/mm\(^3\), virus titers rise rapidly and immune activity drops to zero.

The loss of immune competence enables opportunistic parasites (fungi, protozoa, etc.) to cause infections.

Once AIDS develops, patients rarely survive more than two years without chemotherapeutic intervention.
AIDS - Clinical Course