The HIV Epidemic:
Biology of the virus and modes of therapy
OVERVIEW

• History of the virus
  ➢ How was it discovered?
  ➢ Where did it come from?
• Clinical manifestation of HIV/AIDS
• HIV classification
• Genome and morphology of the virus
• Viral Life Cycle
• Host factors
• Therapeutics

Key Take-Home Ideas

• Why is it so difficult to eradicate HIV?
• Viral life cycle
• What is the relationship between HIV and the host cell?
• Evolutionary battle between HIV and host cell: Host factors and Restriction Factors
• HAART therapy: Why is it important to target multiple viral enzymes at once?
The Discovery of AIDS in the U.S.

- HIV first appeared in the U.S. in 1969 and likely entered the country through a single infected immigrant traveling from Haiti.

- The first clinical observation of AIDS was in 1981.

- By this time, physicians in New York, Los Angeles and San Francisco were reporting unusually high numbers of patients suffering from pneumocystis pneumonia (a rare fungal pneumonia) and Kaposi’s sarcoma (a rare skin cancer).

- Because PCP and Kaposi’s usually manifest in patients with immune deficiencies, the term AIDS - **Acquired Immune Deficiency Syndrome** - was coined.

- Although initially it was believed AIDS only affected the gay community, it became clear that virtually no one was immune to the disease as heterosexuals, blood transfusion patients, and even newborns were acquiring the disease.
HIV as the causative factor of AIDS

- In 1983 Robert Gallo and Luc Montagnier discovered a novel virus which appeared to be spreading among the population of AIDS patient.

- Initially, it was difficult to determine whether this virus was the causative agent of AIDS because by the time patients developed AIDS, they were often infected with other viruses and pathogens that could also lead to an immuno-compromised state.

- However, this novel virus was found to be the common link among all AIDS patients and was thus coined Human Immunodeficiency Virus – HIV. Further studies revealed that HIV had a high affinity for CD4+ T-cells, which were nearly depleted in all AIDS patients, and monkeys afflicted with AIDS were found to harbor a virus very similar in morphology to HIV.

- There are currently ~35 million people infected with HIV worldwide (UNAIDS, 2012) and thus far over 35 million people have died from AIDS-related disease.

- The highest prevalence of HIV/AIDS is in sub-Saharan Africa which harbors nearly 70% of all HIV/AIDS cases.

UNAIDS, 2009
Where did HIV come from?

- There were initially several theories on how HIV originated, including a theory that it was a man-made virus created in a government lab as an agent for biological warfare.

- Years of research into the origins of HIV now strongly support the theory that the virus originated from two variants of SIV which infected the same monkey and formed a hybrid strain that could not only be passed onto other monkeys, but could also pass the species barrier into humans.
Clinical progression of HIV/AIDS

Three stages of HIV progression:

- **Acute phase**: Viral load in patient blood undergoes an initial spike, concurrent with a drop in CD4+ T-cells (host cell).

- **Chronic phase/Latency**: Immune system begins production of antibodies against HIV to neutralize infection. During this “latent” phase, the viral load initially decreases as CD4+ counts increase. Viral replication continues at a low rate, eventually decreasing CD4+ counts.

- **AIDS**: When CD4+ T-cell counts reach below 200 cells/uL, patient is diagnosed with AIDS. Death is caused primarily by opportunistic infections.
How does HIV deplete T-cell reservoirs & Why is it difficult to eradicate the virus?

• For decades, scientists believed HIV infects T-cells and then kills them. But no one really knew how or why this happened.

• Recent studies published in 2013 in Science and Nature revealed that initially a small number of immune T-cells (HIV’s natural target cell) become infected. This is turn triggers an inflammatory/immune response which recruits more T-cells to kill the infected cells. In this way, HIV is able to couple infection with chronic inflammation which provides more immune cells for the virus to infect.

• Thus far it has been difficult to eradicate HIV for several reasons:
  
  ➢ HIV contains a highly error-prone reverse transcriptase which causes mutations in the viral genome, some of which may confer drug resistance.

  ➢ HIV has the ability to undergo a latency period once it has integrated into the host cell. Latently infected cells are difficult to target and eliminate as they do not appear infected.

  ➢ Attempts to develop an FDA approved HIV vaccine have failed thus far.
HIV Classification

**Retrovirus**: An enveloped virus carrying single stranded, positive sense RNA.

- RNA undergoes reverse transcription, by virally encoded reverse transcriptase, to produce double-stranded DNA which is then inserted/integrated into the host cell genome to form a “provirus.”

**Lentivirus**: Subgroup of the Retroviruses.

- Characterized by a long incubation/illness period and the ability to infect and integrate genome into non-dividing cells.

There are two species of HIV: HIV-1 and HIV-2.

- HIV-1 is the most globally prevalent form and is more infectious than HIV-2.

- HIV-1 can further be subdivided into three groups (M, N, O).

- Group M can be divided into ten clades (A, B, C, D, F1, F2, G, H, J, K)

- Clade C is the most globally prevalent, accounting for 50% of infections worldwide.
HIV Genome and Virus Structure

**HIV-1 Genome**

- **5’**
  - LTR
  - Gag
  - Pol
    - PR
    - RT
    - IN
  - MA
  - CA
  - NC
  - p17
  - p24
  - p7 + p6
- **3’**
  - LTR
  - Vif
  - Rev
  - Nef
  - Env
  - Vpr
  - Tat

**HIV-1 structure**

- SU (gp120)
- TM (gp41)
- MA (inner membrane)
- RT, IN, NC
- CA (viral core)

Immature HIV-1
Mature HIV-1

100 nm
The HIV Life Cycle

Early Events

- Cell Entry
- Reverse Transcription
- Capsid Disassembly

Late Events

- Maturation
- Integration into host genome
- Expression of viral proteins

Key Events & Inhibitors:

- Reverse Transcriptase inhibitors (AZT)
- Protease inhibitors (Darunavir)
- Integrase inhibitors (Raltegravir)
The HIV Life Cycle : Video Overview

https://www.youtube.com/watch?v=odRyv7V8LAE
The HIV Life Cycle: ENTRY

- HIV entry requires the viral envelope proteins gp120 and gp41.
- gp120 binds to the CD4 receptor on helper T-cells (or macrophages) and undergoes a conformational change, allowing it to bind to co-receptors, CCR5 or CXCR4.
- Once gp120 binds, it allows another conformational change allowing gp41 to insert into the host cell membrane.
- Insertion of gp41 into the cell membrane allows fusion of the viral and cell membrane, thus allowing entry of the viral capsid into the cytoplasm.
The HIV Life Cycle: UNCOATING

- The viral core is composed of ~1500 capsid proteins that form oligomers to produce a conical shape.

- Uncoating of the capsid core within the cytoplasm releases the viral RNA genome and associated proteins, including RT, IN, and PR.

- The rate at which the core disassembles is critical to HIV-1 replication. Mutations in the CA gene lead to subsequent defects in reverse transcription.

- Host factors have been shown to play a significant role in aiding uncoating.
The HIV Life Cycle: REVERSE TRANSCRIPTION

- Reverse transcription involves the transcribing of the single-stranded RNA genome into double stranded cDNA.

- This process requires Reverse Transcriptase, as part of a protein complex (that even includes some cellular proteins), as well as a host-provided tRNA primer.

- Reverse transcription is a highly error-prone process! What might be the virus’ advantage to this?
Once the cDNA is synthesized, it enters the nucleus as part of the “pre-integration complex” (PIC) which includes both viral (IN, RT, MA, Vpr) and cellular proteins.

Integration can be divided into three steps (3’ processing, strand transfer, and integration) to ultimately form the integrated provirus. Integration is usually targeted to transcriptionally active regions of the human genome. Why might that be?

The resulting provirus is what leads to prolonged illness/infection in the latency period.

Once integration has occurred, it is nearly impossible to flush out the virus!
Several different RNA species are transcribed from the provirus: Full-length mRNA which codes for Gag-Pol polyprotein, and several spliced species which each code for Env and various accessory proteins.

These mRNA species are exported from the nucleus into the cytoplasm with the help of several cellular proteins.

In addition to HIV-1 mRNAs being translated, some full-length viral RNA species are exported from the nucleus to be packaged into virions as the single-stranded RNA genome.
• HIV-1 protein assembly is driven primarily by Matrix which, as part of the Gag-Pol polyprotein, targets Gag-Pol to the plasma membrane where multimerization occurs.

• As enough Gag-Pol and other viral proteins (ie – Env, Vif, Nef, etc) aggregate at the membrane, the virion begins to “bud” out of the cell.

• Eventually, the immature virion is released from the cell, carrying with it the viral RNA genome, plasma membrane from the host cell, and even some cellular proteins (host factors).

• HIV-1 virion becomes a mature particles when viral protease cleaves the Gag-Pol polyprotein into its individual protein components and the capsid core forms in the center.
Cellular Host Factors Involved in HIV-1 replication

Early Events
- Plasma membrane
- Capsid (CA)
- Matrix (MA)
- Surface Glycoproteins
- Nucleocapsid (NC + RNA)
- Cell Entry
- Capsid Disassembly

Late Events
- Immature Virion
- Mature Virion
- Maturation
- Targeting of Gag to membrane
- Expression of viral proteins
- Reverse Transcription
- Integration into host genome
- Integration into host genome

Key Proteins:
- TRIM5α
- APOBEC
- TNPO3
- GEMIN2
- INI1; LEDGF
- Tetherin
## Cellular Host Factors Involved in HIV-1 replication

<table>
<thead>
<tr>
<th>Host Factor</th>
<th>Affected stage of viral life cycle</th>
<th>Proviral or Restrictive?</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4, CXCR4, CCR5</td>
<td>Entry</td>
<td>Proviral</td>
<td>Receptor and coreceptors for HIV-1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Entry</td>
<td>Proviral</td>
<td></td>
</tr>
<tr>
<td>Cyclophilin A</td>
<td>Uncoating</td>
<td>Proviral</td>
<td>Facilitates proper kinetics of core disassembly</td>
</tr>
<tr>
<td>Trim5α</td>
<td>Uncoating</td>
<td>Restrictive</td>
<td>Accelerates kinetics of core disassembly; leads to degradation of viral cDNA</td>
</tr>
<tr>
<td>APOBECs</td>
<td>Reverse transcription</td>
<td>Restrictive</td>
<td>Introduces a high degree of mutations in the HIV-1 genome during reverse transcription</td>
</tr>
<tr>
<td>RANBP2</td>
<td>Nuclear import</td>
<td>Proviral</td>
<td>Required for nuclear import of the PIC</td>
</tr>
<tr>
<td>LEDGF</td>
<td>Integration</td>
<td>Proviral</td>
<td>Required for targeting integration to transcriptionally active areas of host genome</td>
</tr>
<tr>
<td>INI1</td>
<td>Reverse transcription/integration/</td>
<td>Proviral/Restrictive</td>
<td>Evidence suggests INI1 can have proviral or restrictive abilities depending on the</td>
</tr>
<tr>
<td></td>
<td>transcription</td>
<td></td>
<td>stage of the viral life cycle</td>
</tr>
<tr>
<td>Tetherin/BST2</td>
<td>Budding/Particle release</td>
<td>Restrictive</td>
<td>Inhibits full virus release</td>
</tr>
</tbody>
</table>
INI1’s Dual Roles in HIV-1 Replication: Proviral factor and Restriction Factor

1. INI1 in producer cells is a **proviral host factor** that is required for virus production and IN1 is incorporated into HIV-1 virions as they are released from the cells.

2. INI1 within the virions is required for those virions to infect target cells (INI1+/+ or INI1-/-).

3. However, cells deleted for INI1 are much more susceptible to infection, suggesting that cellular INI1 in target cells may be a restriction factor for early events.
Methods by which HIV-1 overcomes Host Restriction Factors: Tetherin versus Vpu

• Tetherin is one of many antiviral factors produced by the innate immune system (interferons) during a viral infection.

• Tetherin functions as dimers found within the membrane of budding virions; As virions are released from the cell, tetherin dimers link to one another, thus “tethering” virions to the cell.

• Virions are eventually internalized by the cell via endocytosis and degraded by the cell.

EM image of HIV-1 virions linking together in the presence of cellular tetherin.
Methods by which HIV-1 overcomes Host Restriction Factors: Tetherin versus Vpu

• The antiviral effects of tetherin are overcome by the HIV-1 accessory protein, Vpu.

• Vpu is a membrane protein made up of 81 amino acids and largely α-helical in structure.

  - Vpu directly interacts with tetherin at the cell surface and targets tetherin for lysosomal degradation → Allows HIV-1 release from the cell membrane to subsequently infect neighboring cells and spread infection.

Host Cell: Tetherin +/+  
Virus: HIV-1 (deleted for Vpu)

Host Cell: Tetherin +/+  
Virus: HIV-1 (WT)
Methods by which HIV-1 overcomes Host Restriction Factors: APOBEC versus Vif

• APOBEC3G binds to viral RNA in the “producer cell” and is incorporated into virions. During reverse transcription in the “target cell”, A3G converts cytidines to uracils, which causes A → G mutations in the viral cDNA. The resulting viral proteins are rendered defective:

![Diagram](image1.png)

• Vif counteracts A3G by binding to it and a protein complex which adds Ub to A3G. Once A3G is ubiquinated, it’s targeted for degradation by the 26S proteasome:

![Diagram](image2.png)
HIV-1 Therapeutics

• **HAART**: Highly active anti-retroviral therapy is the current treatment for HIV-AIDS patients.

• HAART consists of a drug cocktail containing several drugs that target different key enzymes critical for viral replication (reverse transcriptase, integrase and protease).

• The most common drug regimen today includes drugs that inhibit protease and reverse transcription. Recently, a new class of drugs – integrase inhibitors – are proving to be effective as well.
HIV-1 Therapeutics: Inhibiting Reverse Transcription

**NRTIs and NNRTIs**

- **NRTI**: Nucleoside reverse transcriptase inhibitor. These are nucleoside/nucleotide analogs that bind directly to reverse transcriptase and terminate reverse transcription as soon as they are incorporated into the growing viral cDNA chain. AZT, a thymidine analog, was the first anti-HIV drug developed to combat infection.

- **NNRTI**: Non-nucleoside reverse transcriptase inhibitor. These are allosteric inhibitors that bind directly to reverse transcriptase and blocks its function. (Allosteric inhibitors bind to the enzyme outside of its active site to block enzyme activity).

AZT has 100x more affinity for reverse transcriptase than it does for DNA polymerase – which is very important!
HIV-1 Therapeutics: Mechanism of integrase inhibitors

• In 2007, Raltegravir was the first integrase inhibitor approved by the FDA to be used as part of HAART treatment for HIV/AIDS patients.

• Raltegravir is considered an Integrase-strand transfer inhibitor (INSTI); The drug binds to the active site of integrase when IN is bound to viral DNA and prevents the joining of viral cDNA into the host genome (strand transfer reaction).

• Because viral DNA is unable to integrate into the host genome, it is eventually metabolized by cellular enzymes, thereby blocking integration.

![Raltegravir structure and interaction with specific residues](image)

Raltegravir (pink) bound and interacting with specific residues (green) within the active site of Integrase.
Possible HIV-1 Therapies for the future...

- **Peptides to disrupt Virus-Host Factor interactions** and block various steps of the viral life cycle.

- **HDAC inhibitors to HIV-1 gene expression**
  - One of the biggest issues with “curing” HIV is that there’s always a latent reservoir of infected cells which is difficult to flush out because the genes from the provirus are not being expressed.
  - HDACs (histone deacetylases) are largely responsible for silencing gene expression by inducing DNA to wrap around histones, thereby preventing transcription factors from binding and expressing genes.
  - One possible new avenue for therapeutics is to treat HIV-1 patients with HDAC inhibitors that are specific for the HDACs which repress gene expression from the provirus. By inhibiting these HDACs, provirus expression can be resumed, the cell will appear infected and the immune system can purge the latent reservoir of virus.

- **Gene therapy using CCR5Δ32**
  - A small number of people carry a mutant allele of the CCR5 co-receptor which encodes for a truncated version of CCR5 where 32 amino acids are deleted.
  - These people are resistant to HIV-1 infection as the virus needs to bind the co-receptor for cellular entry.
  - Studies have shown that by introducing T-cells expressing CCR5Δ32 into a wild-type T-cell population infected with HIV-1, the infected cells eventually die and the CCR5Δ32 population takes over which is resistant to infection.

- **HIV-1 vaccines**