Objectives

- Characterize HIV-1 (classification, structure and life cycle)
- Describe the clinical course and immunopathology of untreated HIV infection
- Explain the principles of antiretroviral therapy
- Describe the clinical course and immunopathology of treated HIV infection
Outline

• HIV-1 Virology
  • Classification / Structure / Life Cycle
• Natural History of untreated HIV-1 Infection
  • Stages of acute and chronic HIV Infection
  • Factors modifying disease progression
• Antiretroviral Therapy
  • Principles
  • Reservoirs and Kinetics
• Natural History of treated HIV-1 Infection
  • Scope of Immune Reconstitution
  • Obstacles to Cure
# Retrovirus Classification

**Family: Retroviridae**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Features</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alpharetrovirus</td>
<td>Simple, Onco</td>
<td>Avian leucosis virus, RSV</td>
</tr>
<tr>
<td>2. Betaretrovirus</td>
<td>Simple, Onco</td>
<td>Mouse Mammary Tumor Virus</td>
</tr>
<tr>
<td>3. Gammaretrovirus</td>
<td>Simple, Onco</td>
<td>Murine leukemia virus (Moloney, Harvey)</td>
</tr>
<tr>
<td>4. Deltaretrovirus</td>
<td>Complex, Onco</td>
<td>Bovine Leukemia, Human T Cell Leukemia (HTLV)</td>
</tr>
<tr>
<td>5. Epsilonretrovirus</td>
<td>Complex, Onco</td>
<td>Walleye Dermal Sarcoma</td>
</tr>
<tr>
<td>6. Lentivirus</td>
<td>Complex</td>
<td>SIV, HIV, Visna, EIAV</td>
</tr>
<tr>
<td>7. Spumavirus</td>
<td>Complex</td>
<td>Simian Foamy Virus</td>
</tr>
</tbody>
</table>
Natural SIV hosts are the origin of HIV-1 and HIV-2

Slide courtesy of Beatrice Hahn
Natural SIV hosts are the origin of HIV-1 and HIV-2
Origins of HIV-1 families: chimps and gorillas

- SIV neg
- SIV neg
- SIV?
- SIVcpz Ptt in west-central African chimpanzees
- SIVgor in western Lowland gorillas
- SIVcpz Pts in eastern chimpanzees

- Pan troglodytes verus
- Pan troglodytes ellioti
- Pan troglodytes troglodytes
- Pan troglodytes schweinfurthii
- Gorilla gorilla gorilla
- Pan paniscus
A few words about SIV

- One of three important simian retrovirus families (also: STLV I, II, IV, SFV=Simian foamy virus)
- Simian "Immunodeficiency" Viruses: largest and most diverse family of mammalian viruses
- 45/73 African monkey species infected with their specific SIV species (many recombinant species)
  - Many can be cultivated in human PBMCs
- SIV prevalence in African Green Monkeys (most common primate in Africa) is 40-50%
- Not found in Asian or American monkeys but on island off the coast of Africa that separated > 40’000 y ago – phylogenetically likely millions of years old
A few words about SIV

- *Infection generally asymptomatic in natural hosts, immunodeficiency extremely rare*
- 40 different species (8 recombinants)
  - Many can be cultivated in human PBMCs
- Cross-Species transmission overall very rare
- 3 types of genomes (vpr, vpu, vpx gene)
- Transmission sexually > perinatal (+ bites).
Transfer of SIV to Humans

- SIVcpz was transferred to humans through hunting and handling of chimpanzees → HIV-1 clade M (~1908)
- SIVsm → HIV-2 (~1932)
- Oldest Human sample 1959
- The epidemic required urbanization and increased population mobility
- “Human error” theory (Edward Hooper, “The River” 2000)
  - Oral polio vaccine used in West Africa during the late 1950s may have been contaminated with SIV
  - SIV has not been recovered from this vaccine in subsequent studies
Classification of HIV-1

- HIV-1 groups
  - **M (major)**: cause of current worldwide epidemic
  - O (outlier) and N,P (Cameroon): rare HIV-1 groups that arose separately

- HIV-1 M subgroups (**clades**)
  - >10 identified (named with letters A to K)
  - One clade tends to dominate in a geographic region
  - Different clades have different clinical and biologic behavior
  - Several common clades (e.g., A/G ad A/E) are recombinants
Genomic Organization of HIV-1

10 kb in length

6 accessory genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>gag</td>
<td>Core and matrix proteins</td>
</tr>
<tr>
<td>pol</td>
<td>Enzymes</td>
</tr>
<tr>
<td>env</td>
<td>Transmembrane glycoprotein binds CD4 and coreceptors</td>
</tr>
<tr>
<td>tat</td>
<td>+ Transcriptional activator</td>
</tr>
<tr>
<td>rev (=regulator of virion)</td>
<td>Allows export of unspliced transcripts from nucleus</td>
</tr>
<tr>
<td>vif (=viral infectivity factor)</td>
<td>Neutralizes cellular APOBEC3G and 3F</td>
</tr>
<tr>
<td>vpr</td>
<td>Aides transport of preintegration complex into nucleus</td>
</tr>
<tr>
<td>vpu</td>
<td>Enhances viral budding by degrading cellular Tetherin, downregulates SNAT1 (→ impaired proliferation)</td>
</tr>
<tr>
<td>nef (=negative factor)</td>
<td>Promotes T-cell activation, downregulates MHC-1, CD4, and SERINC3/5 (→↑ infectivity)</td>
</tr>
</tbody>
</table>
• Flexible lipid bilayer composed of host cell surface membrane
Capsid: provides structure to virus and protects the genome

- p17 is associated with viral membrane
- p24 condenses to form shell

Multiple functions during entire life cycle.
• Protease assembles new viral proteins

• Integrase incorporates viral DNA into host DNA
• RNA genome present in 2 copies

• Reverse transcriptase: dual enzyme composed of DNA polymerase and ribonuclease
Binding to Cellular Receptors

New Eng J Med 1996; 335: 1528-1530
gp120 Summary

- Selective transmission:
  - HIV-1 viral envelope is critical target for host immune responses, but is very poorly immunogenic.
  - Early viral population have genetically less diverse envelopes with less glycolysation than viral population in source (or throughout course later in disease).
  - Mucosally transmitted virus is exclusively CCR-5 tropic.
  - HIV-1 present in early infection is predominantly CCR5-tropic (regardless of route of transmission).
Reverse Transcription

1. **Viral** RTase-DNA polymerase makes ssDNA copy of RNA genome

2. **Viral** ribonuclease degrades RNA

3. **Viral** DNA polymerase replicates ssDNA -> dsDNA

4. dsDNA migrates to nucleus using host cytoskeleton (up to 20 μM, several minutes)
PREINTEGRATION COMPLEX-HOST DNA BINDING

LEDGF/p75

Host DNA

From: HIV Integration. HIV Web Study (www.HIVwebstudy.org)
STRAND TRANSFER

Reaction Catalyzed by HIV Integrase

From: HIV Integration. HIV Web Study (www.HIVwebstudy.org)
From: HIV Integration. HIV Web Study (www.HIVwebstudy.org)
STRAND TRANSFER

Host DNA

HIV DNA

Host DNA

From: HIV Integration. HIV Web Study (www.HIVwebstudy.org)
From: HIV Integration. HIV Web Study (www.HIVwebstudy.org)
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
The ENV genes are translated and transported through the ER and Goligi 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.

**GAG polyprotein** (matrix, capsid)

**GAG-POL fusion protein** (matrix, capsid, reverse transcriptase, integrase, protease)
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
HIV Virus Maturation

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).
Influence of Other Host Genetic Factors on HIV-1 life cycle

- Host Restriction Factors: APOBEC3G

TRIM 5 alpha

Emerson M. PNAS, 2006.
Tetherin ⇔ vpu

- CD317: IFN-alpha-inducible membrane protein
- Induces a requirement for Vpu during HIV-1 particle release.
- Causes retention of virions on membranes and in CD317-positive compartments after endocytosis
- CD317 depletion abrogates requirement for Vpu
- Tetherin may form part of the IFN-alpha-induced antiviral defense.
Particles tethered at cell surface in absence of Vpu

Courtesy Paul Spearman
Stages of HIV-1 Infection

- Viral Transmission
- Primary HIV Infection (acute HIV infection)
- Seroconversion
- Clinically Latent Period
- Early Symptomatic Period (CDC “Class B”)
- AIDS / Advanced HIV Infection (CD4 < 50)
CD4 values predict risk for OIs
AIDS Related Conditions According to CD4 Cell Counts

- **CD4 Count**
- **Years**
- **HIV RNA**

- Acute retroviral syndrome
- Seroconversion
- TB
- CD4 count in Blood
- Train’s position:
- CD4 count in Blood
- Train’s speed:
- Viral Load in Blood
- Pneumococcal Pneumonia
- H. Zoster
- ITP
- PCP
- Thrush
- KS
- Toxo Lymphoma
- Cryptococcus
- Candida esophagitis
- Pneumococcal Pneumonia
- MAC
- CMV
- PML
- Toxo Lymphoma
- Cryptococcus
- Candida esophagitis
- Pneumococcal Pneumonia
- MAC
- CMV
- PML
### CDC Stage Definition

<table>
<thead>
<tr>
<th>CD4 cell categories</th>
<th>Clinical categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td></td>
<td>Asymptomatic, PGL or acute HIV infection</td>
</tr>
<tr>
<td>&gt;500/mm³ (&gt;29 percent)</td>
<td>A1</td>
</tr>
<tr>
<td>200-499/mm³ (14-28 percent)</td>
<td>A2</td>
</tr>
<tr>
<td>&lt;200/mm³ (&lt;14 percent)</td>
<td>A3</td>
</tr>
</tbody>
</table>

- AIDS indicator conditions: revised in 1993 to include bacterial pneumonia, invasive cervical neoplasia, pulmonary TB
- B Symptoms: Reflecting immunosuppression, or if clinical course complicated by HIV infection: Oral thrush, Cervical dysplasia, CIN, Bacillary angiomatosis, Oral hairy leukoplakia, multidermatomal zoster
Routes of Transmission of HIV

Sexual Contact:  Genital-genital  
                  Genital-Rectal  
                  Oral-Genital

Blood Exposure:  Intravenous Drug Use  
                  Occupational exposure  
                  Transfusion of blood or blood products

Maternal/Fetal:  In utero  
                  Peripartum  
                  Breastfeeding
## HIV Transmission Risk

### Table 2

The risk of HIV transmission following an exposure from a known HIV-positive individual

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Estimated risk of HIV transmission per exposure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion (one unit)</td>
<td>90–100⁵</td>
</tr>
<tr>
<td>Receptive anal intercourse</td>
<td>0.1–3.0⁶,⁷</td>
</tr>
<tr>
<td><strong>Receptive vaginal intercourse</strong></td>
<td>0.1–0.2⁷⁻¹²</td>
</tr>
<tr>
<td><strong>Insertive vaginal intercourse</strong></td>
<td>0.03–0.09¹⁰</td>
</tr>
<tr>
<td>Insertive anal intercourse</td>
<td>0.06¹³</td>
</tr>
<tr>
<td>Receptive oral sex (fellatio)</td>
<td>0–0.04¹³</td>
</tr>
<tr>
<td>Needle–stick injury</td>
<td>0.3 (95 CI 0.2–0.5)¹⁴⁻¹⁶</td>
</tr>
<tr>
<td>Sharing injecting equipment</td>
<td>0.67¹⁷</td>
</tr>
<tr>
<td>Mucous membrane exposure</td>
<td>0.09 (95 CI 0.006–0.5)¹⁸</td>
</tr>
</tbody>
</table>

Genetic bottleneck during transmission of HIV

Diverse virus population in chronically infected “donor”

Transmission

Re-emergence of viral diversity

Factors that influence sexual Transmission of HIV-1

- **Viral Load:**
  - Primary HIV Infection (high VL) accounts for ~50% of all new infections worldwide

- **STDs increase susceptibility 2-5-fold**
  - HSV-2, *Neisseria gonorrhoea*, *T. pallidum*, *BV*

- **Type of exposed mucosa:**
  - Rectal > Cervicovaginal > penile

- **Circumcision:**
  - nearly 2-fold reduction in male infection risk (heterosexual)

- **Hormonal contraceptives or pregnancy appears to increase risk of HIV infection**

- **First time sex partner > habitual sex partner**
  - Local immunity to HLA antigens from partner (present in viral lipid rafts)
Primary HIV Infection (PHI)

- Symptoms appear in 40-80% of acutely-infected pts 10-30 days after exposure. Usually lasts 14 d.
- Fever, rash, lymphadenopathy predominate (mononucleosis-like syndrome).
- Less common symptoms include headache, oral ulcers, pharyngitis, myalgias, meningitis, GI symptoms
- Immune system reigns in, but does not eliminate virus replication
  - Cellular immunity is most important; Antibody appears later
  - Virus set-point established determines subsequent rate of CD4 cell loss
- Patients with PHI symptoms have faster progression to AIDS
PHI: Window Period

Primary HIV infection

Acute HIV syndrome

Asymptomatic

antibody

circumstance

PCR

P24

ELISA

Time from a to b is the window period

0 2 3 4

Weeks since infection

Source: S Conway and J.G Bartlett, 2003
PHI: Massive CD4 T-cell Depletion in GALT

Peak viremia within the first 5-21 days of infection depletes host of 50% memory CD4+ T-cells. Peak VL up to 100 Mio cop/ml.

Limited subsequently by appearance of HIV-specific immune response (and disappearance of readily available target cells).

As GALT is rich in effector memory cells, their massive depletion may precipitate loss of mucosal integrity, leading to microbial translocation and systemic immune activation.

Viral Factors Affecting Disease Progression

- Co receptor usage:
  - CCR5-virus associated with slower loss in CD4 T cells
  - CXCR4-virus associated with rapid loss in CD4 T cells and development of clinical AIDS

- Accessory gene function (nef)
  - Virus with deleted nef gene leads to low viral load and slow loss of CD4 T cells

- Clade D (East Africa) associated with faster disease progression
Host Factors Affecting Disease Progression

- Co-receptor expression
  - CCR5
    - CCR5Δ32 (homozygous population = ‘HIV resistant’)
    - CCR5 promoter mutations (linked to CCR2)
    - CCL3L1 mutations (=CCR5 ligand)
    - RANTES, MIP1A mutations (=CCR5 ligands)
  - CXCR4
    - SDF-1 gene polymorphisms (=CXCR4 ligand)
Host Factors Affecting Disease Progression

- SNPs for IL-10, IFN-g, IL-18, IL-2
- Amount of β-chemokines production by CTLs
  - Granzyme, Perforin
- HLA Genotypes
  - Slow disease progression:
    HLA A2, B*27, HLA B*57, haplotype DRB1*13
    - Associated with strong cellular immune responses against a limited number of gag epitopes
  - Fast disease progression:
    B7, B*08, B*35
Immune Response to HIV: CD4+ T-cells

- Paradox: The virus elicits a broad immune response which is not protective.
- T Helper Cells:
  - Weak or absent HIV-specific response in most patients. Reasons:
    - Early clonal deletion of activated, expanding, HIV-specific CD4 T helper cells
    - Induction of anergy of T cells due to high-level antigen burden, antigen-induced apoptosis, or a defect in antigen presentation rendering HIV-1-specific T helper cells non-functional, PD-1 expression
  - Qualitative defect precedes quantitative loss
  - High HIV-specific CD4 response in long-term non-progressors
Immune Response to HIV: CD8+ T-cells

- Present in high numbers in HIV infection (CD4/CD8 ratio shifted)
- Involved in initial control of viremia
- Decline with progressive CD4 cell loss
- Anti-viral effect of CTL
  - Experimental depletion leads to increased viral loads (SIV)
  - Lysis of virus-infected cell before mature virions are released
  - Inhibition of viral replication (interferon-\(\gamma\))
  - Inhibition of viral entry into surrounding cells:
    - MIP-1a, MIP-1b, RANTES:
      - Blocks CCR5 usage
      - Produced and released within cytotoxic granules
Immune Response to HIV: B-cells

- Overall TH-1 → TH-2 shift of CD4-cells:
  → humoral response favored (hyper γ–globulinemia)

- Neutralizing antibodies
  - Exact role still unclear in HIV (unlike most viral infections)
  - Arise late after acute infection (3-6 months)
  - Directed at V3 loop (that coreceptor), CD4 binding site on gp120, coiled-coil structure on gp41 c. Only effective against lab strains, not primary isolates of patients.
  - A minority of heavily exposed, but uninfected subjects have been found to have neutralizing IgG or mucosal IgA

- ADCC antibodies
  - Lyse virus-infected cells
  - Importance unclear
HIV Escape of Host Immune Response

- High viral replication in conjunction with error-prone RT→mutations, including CTL epitopes (peptides presented by MHC-I)
- nef downregulates MHC-I and CD4 expression on infected cells
- Neutralizing antibody targets prone to constant escape mutations or not exposed until binding of gp120-gp41 complex to CD4
Enigma of HIV Pathogenesis

- Why do CD4+ T-cells go out of stock?
- What causes the (persistent) immune activation?
- Why do natural hosts of SIV not develop immunodeficiency?
Causes of CD4+ T cell depletion and disease progression

- Loss of CD4+ T cells:
  - Chronic immune activation: Activation induced cell death
  - Direct lysis of cells by HIV.
  - Lysis of infected CD4+ T cells by immune response (CTL, AICD).
  - Only 1/10^6 peripheral CD4+ T cells infected at any given time.

- Inability to replace lost CD4+ T cells
  - Thymic dysfunction
  - Bone marrow dysfunction
  - Limited ability of T cells to expand in the periphery (telomere shortening)
Microbial translocation causes systemic immune activation in chronic HIV

Microbial translocation as a cause of AIDS?
Few (!) human viremic controllers
CCR5/CD4 co-expression in natural vs. unadapted hosts

Brenchley, Silvestri, Douek: Nonprogressive and Progressive Primate Immunodeficiency Lentivirus Infections Immunity 2010, 32:737-42
Target Cell Restriction

A
Non-natural hosts: Humans; *Macaca sp.*

- Naïve CD4+ T cells
- Increased infection and loss of CD4+ Tcm
- CD4+ T cell depletion
- Upregulation of CCR5 upon differentiation to memory cells
- High frequency of CCR5+CD4+ T cells (57)

B
CD4+ Naive T cell
CD4+ Tcm
CD4+ Tem
CD3+ CD4+ CD8+ DN or CD3+CD4+ CD8αβ

Chahrouri et al. Science 2012 335, 1188-93
Target Cell Restriction

Lower frequency of CCR5+ CD4+ Tcm (30)
Decreased infection of CD4+ Tcm (30)
CD3+CD4-CD8- (DN) cells with T-helper-like function (60)
Low frequency of CCR5+CD4+ T cells (57)

Natural host: Sooty Mangabeys

Preserved Tcm pool
Stable CD4+ T cell levels
Summary: HIV Disease Pathogenesis

- Early, rapid and massive memory CD4 T cell infection and depletion in acute infection
- Chronic activation imposes homeostatic strain on maintenance of vulnerable CD4 T cell pools
- Destruction of the lymph node microenvironment preferentially affects CD4 T cell homeostasis
- Suppression of thymic output preferentially affects CD4 T cell reconstitution

Douek. CROI. Boston 2005
Immune Response to HIV: Determinants of Outcomes

- The human immunodeficiency virus (HIV)-mediated immune response may be beneficial or harmful, depending on the balance between expansion of HIV-specific T cells and the level of generalized immune activation.
- Very few individuals (<5%) are long-term non-progressors and achieve partial control of HIV replication is associated with high levels of HIV-specific IL-2 and IFN-γ CD4 T cells and typically low levels of T-cell activation.

Deeks, S et al., J Virol 2005