Human Immunodeficiency Virus

1. Identification of the AIDS Virus

(a) Opportunistic infections observed in homosexual men (all had T4 helper cell depletion) -> termed Acquired Immune Deficiency Syndrome; Summer 1981 - CDC got reports of unusual pneumonia (*Pneumocystis carinii* rare, usually harmless protozoan rarely associated with pneumonia), Kaposi’s sarcoma (rare tumor of blood-vessel tissue often found in patients with AIDS), and chronic fatigue syndrome (CAS). Patients with persistent Lymphadenopathy Syndrome (LAS) had characteristics and SF lymphomatosus leukemia (Lymphoma). CDC & ECO reported of unusual pneumonia (*Pneumocystis carinii* rare, usually harmless protozoan rarely associated with pneumonia), Kaposi’s sarcoma (rare tumor of blood-vessel tissue often found in patients with AIDS), and chronic fatigue syndrome (CAS). Patients with persistent Lymphadenopathy Syndrome (LAS) had characteristics.

(b) Injections adequate probable, possibly a virus such as CMV or EBV and SF lymphomatosus leukemia (Lymphoma).

(c) HTLV transforms lymphocytes whereas the AIDS virus lysed lymphocytes.

(d) RT activity could be found in patients with LAS reported for HTLV (human T-cell Leukemia virus).

(e) A retrovirus agent similar to HTLV was be isolated from AIDS-infected patients. HTLV transforms lymphocytes whereas the AIDS virus lysed lymphocytes.

(f) A retrovirus agent similar to HTLV was be isolated from AIDS-infected patients.
The virus was later reclassified as the Human Immunodeficiency Virus (HIV), a member of the Lentivirus family (it is not oncogenic).

What are possible mechanisms for virus detection? How do they differ from AIDS surveillance? What is the real problem here?

Does this virus cause AIDS?

HIV (Human Immunodeficiency Virus) is not the AIDS virus — HTLV-III (French LAV)
Virus Distribution

a) current distribution of HIV-infected adults (12/99)
AIDS in the U.S. in 1998
Annual Number of Deaths in the U.S.
b) Spread of virus: sexual contact, blood, mother to infant

1) Infection via virus
2) Infection via virus-infected cells
3) Hallmark of HIV infection is the decline in T4 helper cells

HIV
HIV disease progression:

1) Initial acute infection (usually quite mild; flu-like)
2) Gradual reduction in T4 cell counts
3) Accumulation of opportunistic infections (these will eventually kill the patient, not the direct effect of HIV replication)

(d) HIV disease progression:

HIV
### Table 5.6: Pathology and Opportunistic Infections of Patients with HIV/AIDS

<table>
<thead>
<tr>
<th>STAGE OF HIV/AIDS</th>
<th>SYNDROME/SYMPTOMS</th>
<th>TYPE OF INFECTIOUS AGENT</th>
<th>ORGANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD4+ T-cell count 200-500 cells/µl</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin lesions</td>
<td>Viral</td>
<td>Molluscum contagiosum</td>
</tr>
<tr>
<td></td>
<td>Oral lesions</td>
<td>Fungal</td>
<td>Candida albicans</td>
</tr>
<tr>
<td></td>
<td>Basal cell carcinomas of skin</td>
<td>Viral</td>
<td>Human papilloma virus</td>
</tr>
<tr>
<td></td>
<td>Lung disease (Tuberculosis)</td>
<td>Bacterial</td>
<td>Reactivation of Mycobacterium tuberculosis</td>
</tr>
<tr>
<td></td>
<td>CNS disease of PBMC, etc.</td>
<td>Viral</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td><strong>CD4+ T-cell count &lt;200 cells/µl</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>Protozoan</td>
<td>Pneumocystis carinii</td>
</tr>
<tr>
<td></td>
<td>Severe diarrhea</td>
<td>Protozoan</td>
<td>Isospora belli</td>
</tr>
<tr>
<td></td>
<td>Cryptosporidia</td>
<td>Protozoan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chronic diarrhea</td>
<td>Protozoan</td>
<td>Cryptosporidia</td>
</tr>
<tr>
<td></td>
<td>Diseases of the CNS</td>
<td>Fungal</td>
<td>Cryptococcus neoformans</td>
</tr>
<tr>
<td></td>
<td>Generalised lymphadenopathy</td>
<td>Viral</td>
<td>JC polyomavirus</td>
</tr>
<tr>
<td></td>
<td>Generalised weight loss</td>
<td>Viral</td>
<td>Generalised lymphadenopathy</td>
</tr>
<tr>
<td></td>
<td>Headache, fever, malaise</td>
<td>Viral</td>
<td>Generalised lymphadenopathy</td>
</tr>
<tr>
<td></td>
<td>AIDS dementia complex</td>
<td>Viral</td>
<td>HHV-8</td>
</tr>
<tr>
<td></td>
<td>Generalised weight loss</td>
<td>Viral</td>
<td>Generalised lymphadenopathy</td>
</tr>
</tbody>
</table>

**Other Syndromes**

- Aseptic meningitis
- AIDS dementia complex
- Shingles
- Headache, fever, malaise
- Generalised lymphadenopathy

**Abbreviations used:** CNS = central nervous system; PML = progressive multifocal leukoencephalopathy; PCP = Pneumocystis carinii pneumonia; HHV-8 = human herpesvirus eight.

Adapted from Coffin et al. (1997) Table 1 on page 597.
Two major glycoproteins:

a. synthesized as 160kD precursor that is processed by cellular protease
b. gp41 is a transmembrane protein that contains an external domain required for fusion with target cells
c. gp120 protrudes from envelope and binds to CD4, contains most neutralization epitopes
HIV Pathogenesis

T cells are the major site of infection, but macrophages, dendritic cells, monocytes have low levels of CD4 and may harbor HI-1 infection of microglial cells may cause AIDS-related neurological problems.

Langerhans cells
Dendritic cells
BM precursor cells
Monocyte cell lines
Kupffer cells
Primary monocytes/macrophages
B cells
T cells

Brain capillary endothelium
Retinal neural cells
Glia cell lines
Microglia
Liver sinusoid epithelium
Colon carcinoma cells
Rhabdomyosarcoma cells

Others

a) Hematopoietic/Immune cells:

Cell types that can be infected by HIV:

b) Brain/glial cells
What are the coreceptor molecules?

**Fusion Process**

- a. Chemokine receptor - CXCR4, also known as Fusion: 7-Tmembrane receptor protein
- b. Chemokine receptor - CCR5: 7-Tmembrane receptor protein

T-tropic viruses use this co-receptor: these strains infect primary CD4+ T cells

M-tropic viruses use this co-receptor: these strains infect primary and established CD4+ T cells

They represent 90% of the sexual transmission of HIV
Chemokine Receptors
Mechanisms for HIV Induced Cell Death

I. HIV infection and cell death due to viral propagation

II. GP120 expressed on surface of infected cell binds to the CD4 receptor on uninfected cell. The cells fuse and the resultant giant cell dies.
Mechanisms for HIV-Induced Cell Death

Infected T cells expressing GP120 on their surfaces can be killed by the normal immune response by antibodies, NK cells, or cytotoxic T cells. Free GP120 from the bloodstream can bind to the CD4 molecule on healthy T cells and make them appear to be infected by the body's immune system and subsequently killed by NK, T cells, and antibodies.
Autoimmunity

Autoimmunity: HIV disturbs the balance of the immune system and autoimmune disorders accompany the virus. a. Autoimmune responses may be potential cofactors in HIV pathology.

General conclusions on potential importance of autoimmune responses (see Fig).

2. Illustrates a potential danger in vaccination with HIV proteins.

1. Autoimmune responses may be potential cofactors in HIV pathology.

2. It is unclear if AVA play a significant role in HIV biology.

1. EX: Autoantibodies to gp120 antibodies inhibit induced autoimmune antibodies against CD4.

2. Anti-HIV lymphocytes (AVA) antibodies to anti-anthrax antibodies may mirror images of the epitope against which the initial antibody was produced.

3. Anti-HIV lymphocytes may mirror images of the antibody, cause a disease called lymphocytopenia.

4. EX: Cross reactive antibodies that recognize a chicken and HIV gp120.

5. Sometimes this can elicit immune responses that cross react with normal cells.

d. Molecular mimicry: Viral gene(s) and/or gene product(s) sharing sequence homology with a normal cellular component.

b. HIV induced cells or reactive macrophages.

c. B-cell proliferation with resultant antibody production: probably results for IL-6 production by

p. T-cell dysregulation:

a. Over-reacting immune system: Autoantibodies to a large number of normal cellular antibodies

Autoimmunity: HIV disrupts the balance of the immune system and autoimmune disorders.
### Autoimmunity

<table>
<thead>
<tr>
<th>Region of HIV that resembles normal cellular function</th>
<th>Associated Clinical Condition</th>
<th>Antibodies directed in HIV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 18</td>
<td>Autoimmune disorders that resemble normal cellular function</td>
<td>Autoantibodies directed against normal cellular proteins</td>
</tr>
</tbody>
</table>

#### Table 18.7

<table>
<thead>
<tr>
<th>Region</th>
<th>Associated Clinical Condition</th>
<th>Antibodies directed in HIV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 18</td>
<td>Autoimmune disorders that resemble normal cellular function</td>
<td>Autoantibodies directed against normal cellular proteins</td>
</tr>
</tbody>
</table>
Antibody Response
Steps of HIV Pathogenesis

1. Enhanced cellular host range
2. Rapid kinetics of replication
3. CD4+ cell cytopathicity
4. Viral variants of the virus replicate to higher levels and destroy a large number of CD4+ cells
5. More virulent variants of the virus replicate to higher levels and destroy a large number of CD4+ cells
6. CD4+ cells then decrease steadily during the persistent period (for unknown reasons)
7. The individual begins to develop symptoms when CD4+ cells drop below 300 cells per µl and HIV is in blood increase.
8. Over the ensuing months to years, CD8+ cell number remains slightly elevated. Viruses
9. In the initial days following infection, high level of virus will take place in the lymph nodes
10. Virus initially enters an individual primarily by infecting either activated T cells, resident
11. Macrophages, or mucosal cells in the Peyer’s patch, intestinal crypts
12. This eliminates the ability of the body to mount any immune response to infection
13. At the same time there is a reduction in antral CD8+ cells
14. Symptomatic infection develops, the virus has characteristics distinct from the virus
15. Enhanced cellular host range
16. Viral kinetics of replication
HIV Genome

Genomic RNA (gag mRNA)
(gag-pro-pol mRNA)

An CAP
vif mRNA
An CAP
vpr mRNA
An CAP
tat mRNA
An CAP
rev mRNA
An CAP
vpu, env mRNA
An CAP
nef mRNA

An RRE
Proviral DNA
LTR

ORFs
1
2
3
4
5
6
7
8
9

HIV Genome
<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>mRNA</th>
<th>SIZE kD</th>
<th>FUNCTION</th>
<th>POST-TRANSLATIONAL MODIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>p17 (MA)</td>
<td>nef</td>
<td>27 kD</td>
<td>Matrix protein</td>
<td>myristylated at Gly-2</td>
</tr>
<tr>
<td>p7</td>
<td>eif/rev mRNA</td>
<td>8p 41 (TM)</td>
<td>RNA-binding protein</td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>eif/rev mRNA</td>
<td>8p 120 (SN)</td>
<td>RNA binding protein</td>
<td></td>
</tr>
<tr>
<td>gag</td>
<td>rev mRNA</td>
<td>61 kD</td>
<td>Capsid structural protein</td>
<td></td>
</tr>
<tr>
<td>pro</td>
<td>vpr mRNA</td>
<td>515</td>
<td>Reverse transcriptase</td>
<td>associates with P7</td>
</tr>
<tr>
<td>p10 (PR)</td>
<td>vif mRNA</td>
<td>23 kD</td>
<td>Reverse transcriptase</td>
<td>domain present in p66</td>
</tr>
<tr>
<td>p66/p51 RT</td>
<td>pol *</td>
<td>30 kD</td>
<td>Reverse transcriptase</td>
<td>P66/p51 RT</td>
</tr>
<tr>
<td>vpr</td>
<td>genome RNA</td>
<td>15 kD</td>
<td></td>
<td>associates with P7</td>
</tr>
<tr>
<td>vpu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>env</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Levy (1994) p. 8.
HIV Relatives

SIV: simian immunodeficiency virus
   a. discovered by analyzing sera from macaques: they had antibodies that cross-reacted with HIV proteins (gag)
   b. virus is 50% related to HIV-1 at the nucleotide level
   c. not a direct precursor to HIV-1

HIV-2: human immunodeficiency virus - 2
   a. discovered by analyzing sera from high-risk humans from West Africa -> cross-reacted with both gag and env proteins of SIV
   b. more closely related to SIV than to HIV-1
   c. produces symptoms similar to HIV-1 although less pathogenic
HIV Replication

I. Virus attachment and fusion
   a. gp120 interacts with domain 1 of CD4 (4 Ig-like domains)
   b. The interaction causes a conformational change that exposes a binding site for the chemokine coreceptor
   c. This interaction results in exposure of the TM fusion peptide and a conformational change in the host cell membrane

II. Virus-cell fusion
   a. HIV enters through a pH-independent route
   b. HIV entry (and subsequent replication) downregulates CD4 expression on the cell surface

III. Synthesis of viral DNA and integration
   a. Once the virus has entered the cell through the viral core, synthesis of proviral DNA begins
   b. Many current HIV drugs are nucleotide analogs that block synthesis of viral DNA
   c. Integration of the provirus into the host cell chromosome appears random and is mediated by the viral encoded integrase protein; once integrated, the viral DNA remains permanently associated with the host genetic material
VI. Intracellular control of HIV replication

a. The big question: What controls the HIV latency period?

b. Review of general retrovirus transcription

c. HIV has many different mRNAs which result from alternative splicing

d. The initial rate of transcription from the HIV 5' LTR is controlled by host cellular proteins (transcription factors) which require availability of TTFs.

- NF-κB
- AP-1
- CREB
- Sp1

3. The ability of HIV to replicate in particular cell type may be dependent on the relative abundance of the required cellular factors.

- Ex: Macrophages vs. T cells
- Ex: Viral gene expression vs. T cell

b. Viral gene expression is also required

- Nef, Tat, Rev

2. Promoter activity is regulated by cellular transcription factors (see figure)

- In most cell types, HIV LTR promoter is weak
- The initial rate of transcription from the HIV 5' LTR is controlled by host cellular proteins (transcription factors)

- HIV has many different mRNAs which result from alternative splicing

- Review of general retrovirus transcription

- The big question: What controls the HIV latency period?
VII. HIV transcription is tightly regulated and split into two segments:

a. Early mRNAs correspond to the regulatory proteins tat, rev, and nef

1. The balance of these regulatory proteins play a key role in controlling HIV transcription is tightly regulated and split into two segments:

   a. Early mRNAs correspond to the regulatory proteins tat, rev, and nef
   b. Tat: A viral transactivator is a viral encoded regulatory protein.
      a. Found in the nucleus
      b. Binds to a stem-loop structure (called 'tar') at the 5' end of the nascent RNA (see figure)
      c. Binding of tat increases the stability and frequency of the polymerase which is transcribing viral genes and permits synthesis of full-length RNAs
      d. The full-length viral RNA transcript contains multiple splicing sites which are utilized to produce multiple spliced viral mRNAs
      e. Binding of cellular proteins to Tat or Tar may play a role in regulating activity
      f. Tat is a positive regulator of transcription

   b. Nef: A negative regulator
      a. Also found in the nucleus
      b. Nef suppresses HIV replication: mechanism is not clear
         1. Could directly down regulate transcription from the LTR
         2. Nef may inhibit NF-κB induction
         3. Nef may have an anti-rev activity
      c. Nef may be acting through interactions with cellular protein (via phosphorylation)
      d. Expression of Nef may be critical to maintain HIV cellular latency
5. Conclusion

Both viral and cellular factors, working together, affect HIV replication.

By-pass the splicing machinery.

2. Binding of Rev to RRE may prevent the viral RNA to

I. Binding of Rev to RRE may directly inhibit cellular

I. The mechanism of Rev activity is unclear.

I. Binding region termed the "RRE": Rev responsive

Envelope coding region (see figure)

e. Rev binds to a complex stem-loop structure, within the

c. Rev is also located in the nucleus.

viral mRNAs can accumulate in the cytoplasm

b. In the absence of Rev, only small multiply spliced viral

RNA can be produced. In the absence of Rev, only small multiply spliced RNAs

produce virions. Rev is a key regulatory factor in balancing Tat and Nef and the

4. Rev: Plays a key regulatory role in balancing Tat and Nef and the

3. Nef: A negative regulator

a. Also found in the nucleus

b. Nef suppresses HIV replication: mechanism is not clear

1. Could directly down regulate transcription from the LTR

2. Nef may inhibit NF-κB induction

3. Nef may have an anti-rev activity

c. Nef may be acting through interactions with cellular protein (via phosphorylation)

d. Expression of Nef may be critical to maintain HIV cellular latency

4. Rev: Plays a key regulatory role in balancing Tat and Nef and the

2. Rev controls expression of late gene products and progeny

viral mRNAs. Rev has a key regulatory role in balancing Tat and Nef and the

4. Rev: Plays a key regulatory role in balancing Tat and Nef and the

2. Rev controls expression of late gene products and progeny

viral mRNAs. Rev has a key regulatory role in balancing Tat and Nef and the
b. Late regulatory genes: facilitate virus release and increase virus particle infectivity
   1. Vif: made from a singly spliced early transcript
      a. Plasmid effects: can increase or decrease virus replication
      b. Reduces expression of MHC class I and CD4
   4. Nef: Produced from multiply spliced early transcripts
      a. Causes G2 arrest; facilitates nuclear entry of premembrane complex
      b. Prevents transport of CD4 to plasma membrane
      c. Required for proper maturation of virus particles
      d. Prevents transport of CD4 to plasma membrane
      e. Required for proper maturation of virus particles
   a. AQP: Facilitates the export of virus particles from the cell
      a. AQP is transcribed from the singly spliced env mRNA.
      b. AQP is translated from the singly spliced env mRNA.
   2. Ypu: Facilitates the export of virus particles from the cell
      a. Ypu acts as a diphthymic RNA that contains sequences for vpu at the 5' end.
      b. AQP acts as a diphthymic RNA that contains sequences for vpu at the 5' end.
   3. Vpr: A small regulatory protein found in the virion
      a. Causes G2 arrest; facilitates nuclear entry of premembrane complex
   d. Immunization of macaques with a nef-deficient SIY virus did not prevent their offspring from developing AIDS-like disease.
Integrase
Assembly of the virus capsid

VIII. Assembly of the virus capsid

a. Review: structural proteins are translated by free cytoplasmic ribosomes as a polyprotein precursor.

b. Frameshifting is used to express replication proteins (RT and INT).

c. The capsid precursor protein and the capsid replicative precursor proteins co-assemble at the inner surface of the cell membrane.

1. These proteins insert themselves into the cellular membrane via a myristic acid fatty acid which is attached to the amino terminus of the capsid protein precursor.

2. Cleavage results in recognition of the capsid proteins and capsid.

1. This results in the cleavage of the capsid proteins and capsid.

2. Budding through the cellular membrane occurs.

3. The complex of capsid protein, replicative enzyme precursor, and viral RNA assemble into a closed spherical virus particle.

a. The site is referred to as the packaging sequence (Psi).

b. The cytoplasmic RNA which binds to the viral RNA near its 5'-end.

c. The capsid protein precursor contains two copies of a conserved cysteine.

d. The capsid precursor protein.

1. These proteins insert themselves into the cellular membrane via a myristic acid fatty acid which is attached to the amino terminus of the capsid protein precursor.

2. Budding through the cell membrane occurs.

3. Viral cleavage results in recognition of the capsid proteins and capsid.

1. Review: structural proteins are translated by free cytoplasmic ribosomes as a polyprotein precursor.