HIV and AIDS

LECTURE 1 -
• Incidence of the disease
• Course of infection

LECTURE 2 -
• Clinical feature of AIDS
• Details of the viral lifecycle
• Diagnosis and treatment

Clinical Features of AIDS

1) Affect on lymphoid tissue

ASYMPTOMATIC phase -
   persistent generalized lymphadenopathy (PGL)

AIDS -
   Destruction of lymphoid tissue

AIDS

Consequent problems -

2) Opportunistic infections
   • Especially common in lungs -
     Pneumocystis jiroveci
     Also - mycobacterium tuberculosis
     mycobacterium avium
   • Also - human cytomegalavirus
     - fungal infections
AIDS

3) CANCER

A) KAPOSI SARCOMA - HHV8

B) B CELL LYMPHOMA - EBV

C) CERVICAL CANCER - Papilloma Virus

AIDS

4) Neurological problems

1/3 OF HIV infected individuals develop neurological disorders

2/3 OF AIDS patients develop sub-acute encephalitis
(Also called AIDS dementia)

Develops slowly over last year
Mean survival time from onset of severe symptoms - 6 months
HIV enters the brain early in infection
(Both in plasma and infected macrophages and T cells)

Neuropathy caused by two effects:

Infection of glial cells (CD4+)
Changes in production of signaling molecules
**Structure of HIV-1**

- **Structural proteins** - gag, pol, env
- **Regulatory proteins** - tat, rev
- **Accessory proteins** - vif, vpr, vpu, nef

**Viral Entry**

MAKE dsDNA + INTEGRATE

TRANSCRIPTION

PROCESSING + TRANSLATION

**SU and TM Allow Attachment + Trigger Fusion**

The host cell infected is determined by:

- The sequence of the viral SU protein,
- The expression of the co-receptors.
This a cellular protein that is packaged into the virus (1 molecule per 10 of capsid protein).

Cyclophilin A destabilizes the nucleocapsid releasing the retroviral core.

**Viral Entry**

MAKE dsDNA + INTEGRATE

**Transcription**

PROCESSING + TRANSLATION

Structure of HIV-1

Proteins encoded by the pol gene are critical for synthesis, nuclear uptake and integration of dsDNA.
The reverse transcriptase is expressed as a fusion protein with RNase H.

**Production of functional RT and RNase H is dependent on the action of the viral protease**

RT and RNase H are critical for generation of ds DNA.

Primer is a cellular tRNA that is incorporated into the viral particle.

Virion contains approx. 50-100 molecules of RT.

The RNaseH activity is generated by separate domain of RT.
REVERSE TRANSCRIPTION

• RT does NOT have an editing activity to excise mispaired nucleotides

• Frequency of mis-incorporations varies between 1 in 70 and 1 in 10^6 copies

• The rate of mis-incorporation depends on the position of the nucleotide (i.e. there are hot spots for mutation)

  Mutation rate of viral genome \( 3 \times 10^{-3} \)

INTEGRATION INTO HOST CELL GENOME

Step 1: Nuclear uptake

• Retroviral DNA synthesis occurs in cytoplasm

• Integration requires nuclear entry

• The pre-integration complex (dsDNA bound to IN) is too large (60S) to enter the nucleus passively

• For most retrovirus, integration is dependent upon the breakdown of the nuclear membrane during cell division

• HIV-1 expresses an accessory protein, called Vpr, that binds the nuclear pore proteins and facilitates docking and import of the preintegration complex

  Normal import pathway

• This accounts for the ability of HIV to infect non-dividing cells.

• This is a general feature of the Lentiviruses
INTEGRATION INTO HOST CELL GENOME

Step 1: Insertion into genome

VIRAL ENTRY

MAKE dsDNA + INTEGRATE

TRANSCRIPTION

PROCESSING + TRANSLATION

Transcriptional initiation occurs in the LTR.
NFkB is particularly important in T cells.

TAR is first open reading frame downstream of the LTR.

Short (60bp) products

This is transcribed very efficiently during the early stages of viral infection because you get premature termination.

The TAR RNA participates in the pre-initiation complex.
Structure of HIV-1

The Tat protein also participates in the pre-initiation complex.

Tat is only produced when long transcript is produced.

Production of the long transcript is inefficient in early infection because of premature termination.
Tat association with the pre-initiation complex blocks premature termination and promotes production of the long transcript.

Production of the full-length transcript has two goals
1) It can be spliced to allow expression of regulatory proteins that control the infection process
2) It is a full length viral genome that can be packaged for viral production.
The ratio of mRNA production:viral genomes changes during the course of infection.

Early - ratio 20:1
  This favors expression of regulatory proteins

Late - ratio 1:1
  This favors genome and viral production

• This switch is controlled by REV
• REV regulates mRNA export

REV recruits the host cell export machinery
Additional key HIV accessory proteins

**VIF**
- Viral infectivity factor
- Increases infectivity approx. 1000 fold
- VIF must be present during synthesis of viral particle
- In absence VIF, get viral entry and initiate replication but don’t produce dsDNA

**NEF**
- Greatly increases HIV pathogenesis and promotes progression to AIDS
- NEF acts to down-regulate the expression of CD4 and MHC on virally infected cells by promoting endocytosis

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**Summary of role of HIV accessory proteins**

<table>
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<th>Role</th>
<th>Accessory Protein</th>
<th>Function</th>
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<tr>
<td>15</td>
<td>VIF</td>
<td>Increases infectivity</td>
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<td>27</td>
<td>Vif</td>
<td>Adenylate for virus assembly</td>
<td>Cell membrane</td>
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<td>Vpr</td>
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Matrix and capsid proteins are encoded by Gag. Gp120 (SU) and gp41 (TM) are encoded by Env. Packaging of the virus is dependent upon the viral protease.

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HIV infection is usually diagnosed by

1) testing serum for antibodies against HIV proteins using ELISA assays

2) Confirmatory western blots
PCR-based testing can detect virus earlier

**PRIMERS USED IN NESTED H-POL POLYMERASE CHAIN REACTION**

First round: 4235–4538 (outer primers)
Second round: 4327–4481 (inner primers)

* M or O sense primer (replace 4327)
HAART - Highly Active Anti-Retroviral Therapy

A WAY TO EXPLAIN TROUGHS, MUTANTS AND STAUNCH COMPLIANCE

Drug level in blood

Drug level at which virus can replicate

Morning dose Afternoon dose Night dose Mixed dose Mixed dose Night dose

Drug resistant viruses

Drug-sensitive viruses

Before treatment begins, viral populations are a mix of mostly drug-sensitive virus, plus a range of drug-resistant viruses

The long term memory cell problem!
Executive Summary AIDS Vaccine Research Working Group Report

Since the first AIDS cases were reported in the US in 1981, more than 65 million people worldwide have been infected with HIV-1. To control the global HIV-1 pandemic, development of a safe, practical and effective vaccine is urgently needed. The problems that continue to face HIV vaccine development are the:

1. lack of an immunogen that will induce antibodies that broadly neutralize HIV primary isolates,
2. lack of an immunogen that will induce effective T cell responses against diverse HIV isolates,
3. lack of an immunogen, adjuvant, carrier or delivery strategy that will enhance the duration of immune responses enough to make a vaccine practically useful over time,
4. lack of an immunogen that induces protective immune responses with one or two primes/boosts,
5. lack of an immunogen or strategy to induce protective innate immunity, and
6. lack of an immunogen that is accessible and affordable worldwide.