HIV INFECTION: An Overview

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VJ TEMPLE
What is HIV?

- HIV: Human Immunodeficiency Virus;
- HIV belongs to group of viruses called Retrovirus;
- Re-Tro-Virus:
  - Re = Reverse,
  - Tr = Transcription (Viruses that reversibly transcribe their genetic material, from RNA \(\Rightarrow\) DNA)
- HIV causes AIDS;
- HIV damages the immune system in humans,
- Two types of HIV: **HIV-1** & **HIV-2**; both types of HIV cause AIDS;
What is AIDS?

• **AIDS**: Acquired Immune Deficiency Syndrome;
• **AIDS** is collection of signs and symptoms (Syndrome) resulting from many diseases in a patient with HIV;
• HIV specifically affect the T-Helper Cells,
• HIV breaks down the immune system exposing the patient to “Opportunistic Infections”;
How long after HIV infection does an individual develops AIDS?

• HIV is a Lentivirus,

• Patient recently infected with HIV may not develop AIDS immediately;

• In some individuals:
  • Decline in T-cell counts and opportunistic infections that signal AIDS may develop soon after HIV infection;

• Most individuals may not develop symptoms for 10 to 12 years after infection,

• A few individuals may remain free of symptoms for much longer;
How is HIV related to AIDS?

• HIV mainly enters the White blood cells (Immune cells);
• Over time HIV causes progressive damage of T-helper cells (CD4 cells);
  • Reducing ability of the immune system to protect the body from infections;
• Ultimately patient becomes vulnerable to various opportunistic infections and other diseases;
• According to WHO and Centers for Disease Control and Prevention (CDC USA) Clinical diagnosis of AIDS may be made if a patient:
  
• Tested positive for HIV (using approved confirmatory test methods),

• Meets one or both of the following conditions:
  • Presence of one or more AIDS-related infections or illnesses;
  • CD4 (T-cell) Count fallen below 200 Cells per cubic millimeter of blood (CD4 count ranges from 450 to 1200 in healthy individuals);
IMPORTANT TO NOTE

• Some AIDS related infections or illnesses include:
  • Fungus infection – Candidiasis (Thrush),
  • Skin infections,
  • TB,
  • Herpes Zoster (Shingles),
  • Patient may have frequent Diarrhea,
What is the basic structure of HIV?

- **Viral Envelope (VE):** lipid bilayer that forms the outer membrane of HIV;
- Embedded in VE are complex HIV proteins Env (Spikes);
- Each Env is made up of:
  - Glycoprotein 120 (GP 120), and
  - Transmembrane Glycoprotein 41 (GP 41);
- Within VE is **Matrix** made up of Glycosaminoglycans (GAG) and Viral Protein (P 17);
- Within Matrix is **Viral Capsid** made up of subunits: Capsomeres (Viral Protein, P 24);
• Within Capsid are **Two Single Strands (Diploid) HIV RNA (Viral RNA Genome),**
• Each Viral RNA has complete copy of **HIV genes;**
• Within Capsid are **Three Enzymes:**
  • Reverse Transcriptase (P 66),
  • Integrase (P32),
  • Protease (P 11);
• Fig. 1: Schematic diagram of basic HIV structure
Fig. 1: Schematic diagram of basic structure of HIV
What is the basic structure of the Genome of HIV?

• HIV genome consists of 2 (Diploid) RNA Molecules (9.8 kilobases in length),

• Genome contains 9 different genes:
  • Three Structural Genes,
  • Six Regulatory Genes,

• The 3 Structural Genes encode for major structural proteins: Gag, Pol, Env;
  • To make structural proteins for new virus particle

• Example: Env gene codes for a large protein GP 160 that is cleaved by viral enzyme to form GP120 and GP41, which are components of Viral Envelope;
• The **6 Regulatory Genes** encode for:
  • Regulatory Proteins: **Tat and Rev,**
  • Accessory Proteins: **Nef, Vif, Vpr, Vpu;**

• **Nef** (Negative Factor) encoded protein is necessary for HIV to replicate efficiently;

• **Vpu**-encoded protein influences release of new virus particles from infected cells;

• **Vif** encoded Protein interacts with antiviral defense protein in host cells (APOBEC3G),
  • It causes inactivation of antiviral effect, thus enhancing HIV replication;
• Both ends of each Strand of Diploid RNA genome contain RNA sequence: **Long Terminal Repeat (LTR)**

• Specific regions in LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or host cell;
What receptor on host cells interacts with HIV?

• To infect host, genetic material of HIV must enter cells;
• HIV interacts with **Specific Cell Surface Receptor** and **Co-receptors** on host cells;
• **CD4**: major Specific Cell Surface Receptor on host cells,
  • CD4: Large Glycoprotein located on surface of, Helper T cells, Regulatory T cells, Monocytes, Dendritic cells;
• **CD4** is the receptor that assists T Cell Receptor (TCR) to activate T Cell following interaction with Antigen Presenting Cell (APC);
• **CD4**: **Primary Receptor used by HIV to gain entry into Host T Cells**;
• {CD = Clustered Differentiation}
What co-receptors on host cells interact with HIV?

- **Co-receptors** on host cells are: **CCR5** or **CXCR4**,  
  - Proteins on surface of Lymphocytes or Monocytes that bind to **GP120** protein of HIV and with CD4 facilitate entry of diploid Viral RNA and proteins into Host cells,

- **CCR5** binds Macrophage-Tropic, Non-syncytium-inducing (R5) Viruses, associated with Mucosal and Intravenous Transmission of HIV infection;

- **CXCR4** binds T-cell-tropic, Syncytium-inducing (X4) Viruses, which are frequently found during the later stages of AIDS;
Naturally occurring deletion of 32 base pairs in CCR5 gene results in Mutant CCR5 Co-Receptor;
  • Individuals homozygous for this mutation are almost completely resistant to HIV infection;
• It indicates the role of CCR5 in spread of HIV and suggests that small molecules that prevent HIV interaction with CCR5 might form promising new class of antiretroviral drugs;
How does Fusion of the HIV with T-Cells occur?

• **GP 120** on envelope binds **CD4** Receptor on Host Cell;

• Co-receptor (CCR5 or CXCR4) on host cell participates in Fusion of GP 120 with CD4 receptor; (**Fig. 2**)

• Fusion separates GP120 from GP 41, thus releasing Spike on GP 41;

• Spike (GP 41) like a spring-loaded lancet pierces the cell membrane of the Host Cell; (**Fig. 3**)

• **NOTE:** Fusion Inhibitors (T-20 and T-1249) can prevent Fusion by blocking conformational changes resulting in the release of the spike
Fig. 2: Interaction of HIV with target cell in Host
Fig. 3: Fusion and release of HIV Spike
How does the viral genome enters the Host cell?

- Piercing of Host membrane by GP 41 is followed by injection of HIV Capsid into Cytoplasm of Host cell,
- Viral Envelope does not enter the Host cell,
- Host enzymes hydrolyze Viral Capsid, releasing contents:
  - Diploid Viral RNA genome,
  - Lysine Transfer RNA (tRNA-Lys)
    - Primer for Reverse Transcription,
  - Viral Reverse Transcriptase (P66),
  - Integrase (P32),
  - Protease (P11),
  - Viral Protein R (Vpr),
  - Other Viral proteins
Fig. 4: Release of HIV Capsid into Host cell
How is HIV genome Reversibly Transcribed?

• Reverse Transcription:

  Viral RNA ===> double stranded viral DNA

• Reverse Transcriptase (P66) is a complex viral enzyme that uses one of the Viral RNA strands as template for formation of DNA;

• DNA Polymerase is part of Reverse Transcriptase complex;

• Lysine Transfer RNA (tRNA-Lys) acts as Primer for Reverse Transcription;
• DNA Polymerase uses the Primer to synthesize a Single Stranded DNA copy using the Viral RNA as template,
  • Forming a DNA/RNA hybrid molecule; \textbf{(Fig. 5)}
• RNA and DNA are separated;
• RNA template is destroyed by Ribonuclease;
• Single Stranded Viral DNA Replicates forming a Double-Stranded Viral DNA molecule;
Fig. 5: Reverse Transcription of HIV RNA genome in host
How is Viral DNA Integrated into the Host DNA? (Formation of Provirus)

- Integration of newly formed Double-Stranded Viral DNA into Host DNA is catalyzed by Viral Integrase (P32) made up of three enzymes;

- Process can be separated thus:
  - Exonuclease removes 2 Nucleotides from each 3′-end of the Viral DNA Duplex;
  - Double-Stranded Endonuclease cleaves Host DNA at integration site;
  - Ligase generates a single covalent linkage at each end of the Proviral DNA, thus forming the HIV Provirus within the Host DNA; (Fig. 6)
Fig. 6: Integration of double stranded Viral DNA into host DNA to form Provirus
What is Dormancy period or Transcriptional Latency?

• After formation of Provirus, a relatively long period may occur during which infected individuals show no signs or symptoms and may not be aware of their HIV status;

• **Integration is followed by latent forms of infection or Transcriptional Latency;**

• Transcriptional latency explains inability of antiviral therapies to eradicate HIV from the body;

• **Silent pro-viruses are reservoir for re-emergence of HIV when the body's defenses grow weaker;**

• Understanding latency and developing approaches to target latent virus are essential for control of HIV;
How are new viruses formed from Proviral DNA?

• Activation causes Transcription of Proviral DNA to RNA;
• Multiple copies of Viral RNA are produced and released into the Cytoplasm,
• Viral RNA acting as Messenger RNA attaches to Ribosome in the Host cell for Translation to occur;
• Translation of Viral RNA produces Viral Proteins:
  • Core,
  • Matrix and Envelope proteins,
  • Transmembrane and Control proteins,
  • Reverse Transcriptase,
  • Integrase and Protease;
• Transmembrane Glycoproteins are formed and migrate into host cell membrane;
• Viral particles are assembled via self arrangement of Capsid around Viral RNA and enzymes;
• Each Viral unit buds from host cells, collecting Envelope with GP 120;
How is HIV transmitted from infected person?

• HIV is in body fluids (blood, semen, vaginal secretions, breast milk)
• HIV can be transmitted when these fluids enter the bloodstream of another person;
• Sharing needles or syringes with someone who is HIV infected; (needles or syringes used to inject drugs),
• Other types of needles, such as those used for body piercing and tattoos, can also carry HIV;
• Laboratory studies show that infectious HIV can survive in used syringes for a month or more;
• During childbirth, or breast-feeding;
Laboratory Test for HIV / AIDS

• Laboratory methods can be used for:
  • Screen Blood,
  • Diagnose Infection, and
  • Monitor disease progression

• Tests can be used to:
  • Detect Antibody;
  • Identify Antigen;
  • Detect or Monitor Viral RNA,
  • Estimate T-lymphocyte numbers
    • Cell Phenotyping);
• Tests to detect Antibody to HIV are classified as:
  • **Screening Assays**, designed to detect infected individuals,
  • **Confirmatory** (supplemental) assays, designed to identify individuals who are not infected but who have reactive screening test results;

• **Screening tests** possess a **high degree of Sensitivity**,  
• **Confirmatory Assays** have **a high Specificity**;
• Technical errors may occur, and there are biologic factors that can limit the accuracy of HIV tests;
• **Effective QA Programs** are needed in all HIV labs;
• Lab tests are used to supplement Clinical diagnosis;
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