AIDS: acquired immunodeficiency syndrome

- causative agent: HIV (human immunodeficiency virus), a retrovirus (contains RNA not DNA as its genetic component)
- HIV attacks CD4-positive T lymphocytes leading to relentless destruction of the immune system
- patients are subject to opportunistic infection with microbes normally easily handled by healthy people; neoplastic complications
- has resulted in the deaths of over half of its victims

International AIDS statistics

42 million people are estimated to be living with HIV/AIDS. Of these, 38.6 million are adults. 19.2 million are women, and 3.2 million are children under 15.

An estimated 5 million people acquired HIV in 2002, including 2 million women and 800,000 children under 15.

During 2002, AIDS caused the deaths of an estimated 3.1 million people, including 1.2 million women and 610,000 children under 15.

Women are becoming increasingly affected by HIV. Approximately 50%, or 19.2 million, of the 38.6 million adults living with HIV or AIDS worldwide are women.

Over 95% of HIV-infected persons live in developing countries

U.S. AIDS statistics (816,000 cumulative cases as of 12/2001)

http://www.cdc.gov/hiv/stats.htm
Story of HIV discovery

- Dr. Robert Gallo of the National Cancer Institute had discovered the first two human retroviruses, HTLV-I and HTLV-II

- in 1984, research groups led by Dr. Gallo, Dr. Luc Montagnier at the Pasteur Institute, and Dr. Jay Levy at UCSF, all identified a retrovirus as the cause of AIDS

- each group called the virus by a different name: HTLV-III, LAV, and ARV

- in 1987, the president of the United States and the prime minister of France announced a joint agreement on the issue (the first time a medical research question had reached level of political negotiation)

Transmission of HIV

- HIV is present in highest amounts in genital secretions and blood; appearance of HIV in saliva, urine, tears and sweat is of no major clinical importance

- rate of HIV transmission with sexual intercourse is much lower than other STDs: 0.3% per sexual contact with an HIV-infected person (some people, however, have been infected after a single encounter)

- health care workers with percutaneous exposure to HIV-containing blood are infected fewer than 1 in 300 times (screening of blood products for HIV has almost eliminated this means of transmission)

- congenital AIDS occurs, on average, in one-fourth of babies born to HIV-1 infected mothers (actual rates of transmission vary between 7 to 71% depending on risk factors)
Typical clinical course following infection with HIV: 3 phases

- decrease in CD4+ T lymphocytes count is primary diagnostic measure in assessing progression of AIDS

HIV virus: artistic representation
Diagram of HIV virus

- two short strands of RNA - 9749 bases long with associated proteins
- outer lipid envelope with 72 projections containing glycoprotein antigens

Genome of HIV

- contains three major genes -- gag, pol, and env

• env - encodes the major structural components of HIV
  gp160 - glycoprotein precursor of two envelope glycoproteins
    gp41 - transmembrane glycoprotein
    gp120 - outer envelope glycoprotein (binds to CD4 on lymphocytes)

• gag - encodes core nucleocapsid proteins (p55, p40, p24: core antigen; p17: matrix; p7: nucleocapsid)

• pol - encodes HIV enzymes (p66 and p51: reverse transcriptase; p11: protease; p32: integrase)
Accessory genes of HIV

- tat gene produces a transactivator protein which speeds up transcription of the HIV provirus

- rev gene encodes for a regulatory protein which switches the processing of viral RNA to a pattern that predominates with established infection, leading to production of viral structural and enzymatic proteins

- nef gene produces a regulatory protein that modifies the infected cell to make it more suitable for producing HIV virions; plays a role in down-regulating cell surface molecules like CD4 and MHC I thereby preventing immune detection

- vif, vpr, and vpu genes encode proteins that appear to play a role in generating infectivity and pathologic effects

Vif: viral infectivity factor

a cellular cytidine deaminase called APOBEC3G is packaged into virions; when the virus infects a new cell and replication of the provirus begins, this enzyme will attack the minus strand of newly-synthesized reverse transcripts and lead to conversion of cytosine to uracil and G to A transitions on the plus strand. (Vif binds to APOBEC3G and blocks its action)

Vpu: this accessory factor is exclusively found in HIV-1 (this may relate to the weaker severity of infection with HIV-2 and SIV)

- mediates degradation of CD4 by linking ER-derived CD4 to the proteasome

this newly-synthesized CD4 is often bound to gp160 and its degradation allows gp160 to continue to the surface

- enhancement of viral particle release

- has structural homology to the influenza protein M2, which is an ion channel; (the role of Vpu as an ion channel has not been determined)
Compact organization of the HIV genome

viral cycle: fusion and cell entry
- viral particle is attracted to a cell with appropriate CD4 receptor molecules where it either attaches by fusion or is endocytosed into cell
- attachment to certain cells also requires additional receptors called chemokine receptors
Chemokine receptors fall into two families:

- CXC family (CXCR1 to CXCR5) and CC family (CCR1 to CCR9)

- Their presence on cells can aid binding to HIV envelope glycoprotein, gp120; initial binding of HIV to CD4 receptor is mediated by conformational changes in gp120 subunit but these changes are not sufficient for fusion.

- Chemokine receptors produce a conformational change in the gp41 subunit of HIV which allows fusion.

Differences in chemokine receptors on different cells explain HIV tropism; the ability of different strains of HIV to infect cells selectively.

- T-tropic strains: interact with CXCR4 chemokine coreceptor to infect lymphocytes.
- M-tropic strains: interact with CCR5 coreceptor to infect macrophages.
- CCR8: cofactor which permits infection by either strain (dual tropic HIV strains have also been identified).

--> over time, mutations in HIV may increase the ability of the virus to infect cells via these routes.

* Infection with cytomegalovirus may enhance HIV infection because CMV encodes a chemokine receptor similar to human chemokine receptors.
chemokine coreceptor mutations and HIV resistance

- four mutational chemokine variants including CCR5-delta 32 have been discovered and may account for resistance in certain individuals and explain differences in infectivity within populations

viral cycle: integration of genome

- once within the cell, the viral particle uncoats from its envelope and releases its RNA

- HIV reverse transcriptase that is bound to the HIV RNA synthesizes host cellular proviral DNA

- proviral DNA is then inserted into the host cell genomic DNA by the integrase enzyme
viral cycle: formation and release of new virions

- viral replication begins in infected cells with the synthesis of new viral RNA and assembly of the capsid envelope by the secretory apparatus of the host cell
- the HIV large gene products (i.e. gag and pol) are expressed as long polypeptide chains and are processed into their active forms by the HIV protease enzyme (necessary step for production of infectious virions)
- new viral particles can be released in two ways:
  1) by budding from the host cell membrane
  2) lysis of cells and release of viral particles

Overview of the HIV viral cycle:

1. Free Virus
2. Binding and Fusion: Virus binds to cell surface receptor sites
3. Infected Cell: Virus penetrates cell, contents emptied into cell
4. Reverse Transcription: Single stranded viral RNA is converted into double stranded DNA by the reverse transcriptase enzyme
5. Integration: Viral DNA is integrated into the cell DNA by the integrase enzyme
6. Transcription: When the infected cell nucleus, the viral DNA is 'read' and long chains of proteins are made
7. Assembly: Parts of viral protein chains come together
8. Budding: Viral particles bud off the infected cell, leaving virus cell envelope behind
9. Maturation: Protein chains in the new viral particle are cut by the protease enzyme into individual proteins that combine to make a working virus
Spread of HIV in the body

- following initial entry of HIV into host cells and establishment of infection, HIV virions released from infected cells enter circulation and are carried to widespread sites in the body

- cells responsible for innate immunity (mononuclear phagocytes like macrophages)

- liver and spleen

- gut associated lymphoid tissue

*in period of clinical latency, virus is not detectable in blood but viral replication continues in lymphoid tissue*
Reservoirs of HIV virions

- HIV binds to multiple receptors; distribution of these receptors permits infection of other cells like dendritic cells and macrophages as well as CD4+ lymphocytes

- DCs and macrophages have many unique characteristics that allow HIV-1 to exist in various replication states and tissue compartments; they are rarely destroyed by HIV infection and may allow virus to survive despite host immune responses and antiretroviral therapy

Macrophages as covert viral reservoirs

- a considerable amount of viral assembly in macrophages occurs in multivesicular bodies inside the cells

- virions in these compartments are stable and are less visible to cytotoxic T cells since most of the viral proteins are located inside the cell

Dendritic cells as “trojan horses” of HIV

- dendritic cells promote HIV replication in two important ways:

  1) DCs are susceptible to direct infection

  2) DCs capture virions and pass them along to CD4+ T cells (following capture, DCs also send signals to T cells that promote their ability to replicate the virus)
- DC-SIGN captures HIV-1 at sites of entry by binding to gp120, enabling its transport to lymphoid tissues

- once in lymphoid tissues, efficiently transmits low amounts of HIV-1 to T cells

- captured virions are internalized into a low pH, non-lysosomal compartment but are not degraded

DC-SIGN represents a novel class of HIV receptors: it does not allow viral infection but binds to HIV-1 and enhances its infection of T cells in trans

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**HIV can lead to the death of host cells by multiple mechanisms:**

- cell lysis or direct viral cytopathic effects

- fusion of infected cells to form syncytia (giant multinucleated cells)

- cytotoxic immune response by other lymphocytes (CD8+ cytotoxic T lymphocytes)

- autoimmune mechanisms

- disruptive interaction of HIV envelope proteins with cell membrane

- immune clearance from alteration of antigenicity of the host cell

- activation of apoptosis
HIV genome encodes pro-apoptotic proteins

- Tat: upregulation of Fas/FasL pathway, increased caspase activity

- Vpr: cell-cycle arrest in G2

- Protease: activation of caspases, proteolytic degradation of BCL-2, an anti-apoptotic protein

- Nef: upregulates FasL on surface of host cells; this leads to induction of apoptosis in Fas-expressing T cells

  it might be beneficial for HIV to inhibit cellular apoptosis until high levels of progeny virus are produced

HIV proteins also exhibit anti-apoptotic activity

*before destroying the immune system through activation of apoptosis, HIV ensures its survival by manipulating the apoptotic machinery to its advantage in infected cells*

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HIV has adapted ways of manipulating the immune system in its favor

the imbalance in the T\[{\text{H}}\] response to a predominantly T\[{\text{H}}\]2 response is mediated by HIV proteins gp120 and Tat which trigger the release of cytokines necessary for a T\[{\text{H}}\]2 response (more antibody production)

increased serum IgE antibody levels suggests that the switch has occurred and predicts a poorer prognosis
Opportunistic infections

- the lung is the main site for a variety of these infections
- *Pneumocystis carinii* pneumonia (PCP) - most frequent and severe o.i. in patients with AIDS (over half of all AIDS patients will have at least one episode during their clinical course; mortality rate from a single episode ranges from 10% to 30%)
- *Cryptococcal neoformans* pneumonitis and meningitis
- *Mycobacterium tuberculosis*
- *Toxoplasma gondii*

Other complications associated with AIDS

*HIV wasting syndrome* - progressive, involuntary weight loss, malabsorption of nutrients due to opportunistic infections in the GI tract

*Lipodystrophy* - changes in the distribution of fat in the body; occurs following treatment with protease inhibitors (up to 2/3 of patients taking PI)

- loss of facial fat and abdominal distension

metabolic consequences of PIAL: increased insulin resistance, increased serum cholesterol, increased serum triglyceride

*HIV encephalopathy* - also called AIDS dementia complex

1) impaired memory and concentration
2) motor deficits such as ataxia and tremor
3) behavior disturbances ranging from apathy to frank psychosis
Treatments for HIV infection

targets for which effective therapies to control viral production have been produced

HIV RT (reverse transcriptase)
HIV protease
HIV integrase (IN)
viral entry and fusion

none of these therapies will completely eliminate HIV from infected persons

Impact of new therapies to treat HIV infection on the death rate of AIDS in the US

although deaths are declining in US, AIDS is still an epidemic in Africa
NRTIs: nucleotide reverse transcriptase inhibitors

- nucleoside analogs that bind in the active site of RT

- Zidovudine (ZDV), AZT, 3’-azido-3’deoxythymidine was the first pharmacological agent developed that had significant effectiveness

![AZT molecule]

AZT can act as a competitive inhibitor of RT as well as a chain terminator if it is incorporated

AZT is a thymidine analog that is phosphorylated to its active triphosphate form (AZTTP) by cellular thymidine kinases

conversion of AZT to AZTMP is rapid but AZTMP is a poor substrate for the next enzyme, thymidylate kinase; this leads to the accumulation of AZTMP inside cells and may be associated with cytotoxicity

AZT monotherapy has been shown to prolong the lives of treated patients by:

1) decreasing frequency and severity of opportunistic infections

2) partially suppressing HIV replication

3) transiently increasing CD4 lymphocyte counts
AZT cytotoxicity
- the two major side effects associated with AZT treatment are myopathy and bone marrow suppression with anemia
- other side effects include nausea, vomiting, headache and liver toxicity

- myopathy is believed to be due to effects on mitochondrial DNA synthesis (AZTTP is a good inhibitor of the mitochondrial DNA polymerase)
- the mechanism behind bone marrow suppression is not clear but may have to do with effects of AZT on glycosylation
- AZTMP can act as an inhibitor of nucleotide-sugar import thereby limiting the amount of glycosylation precursors in the Golgi lumen (defective glycosylation can lead to failure of hematopoietic precursors to differentiate into mature blood cells)

Protease Inhibitors (PI)
- HIV protease is necessary to cleave multiprotein molecules into separate subunits and for the development of infectious virions
- protease inhibitors are synthetic analogues of the HIV protein
- protease inhibitors may also function by decreasing CD4 lymphocyte apoptosis through decreased caspase 1 expression
- problems with PIs include development of resistance and the ability to interfere with normal cellular proteases
- numerous side effects are apparent due to the high effective doses of these drugs (lipodystrophy)
One intriguing benefit of protease inhibitors is their ability to inhibit the formation of AIDS-associated malignancies such as Kaposi sarcoma.

*Patients taking a PI had lower risk of AIDS-related cancers*

- Use of protease inhibitors can affect diverse processes such as angiogenesis, tumor growth and invasion by inhibition of cell invasion and matrix metalloproteinase activity and modulation of NFκB pathways.
Mechanism of action of enfuvirtide: a 36 amino acid peptide derived from the extracellular domain of gp41

prevents the conformational change of gp41 needed for fusion of virus to host cell