A PROJECT ON

HIV

INTRODUCED BY

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Introduction:

- Three groups of nations have been identified in which the epidemiology of HIV(Human Immunodeficiency Virus) varies:
  1- Group I (Europe, USA, and Australia). The cases are homosexuals and numbers are decreasing.
  2- Group II (Central and East Africa), where virus is Spread heterosexually and by infection of babies.
  3- The most explosive outbreaks are occurring now in India, Thailand, and North Africa, where the spread is pre-dominantly among heterosexuals.
- *Long-term survivors* are a group of HIV-positive individuals who after 17 years have not developed AIDS (Acquired immunodeficiency syndrome).

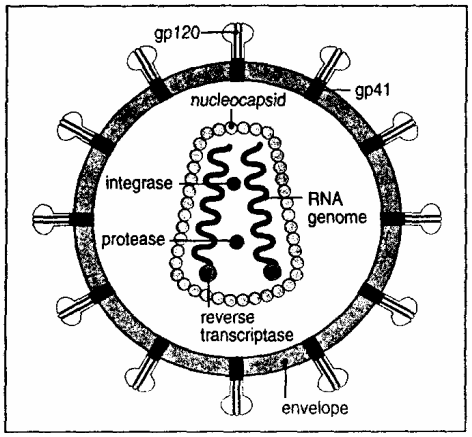
HIV is a retrovirus that infects CD4 T cells, dendritic cells, and macrophage

Structure:

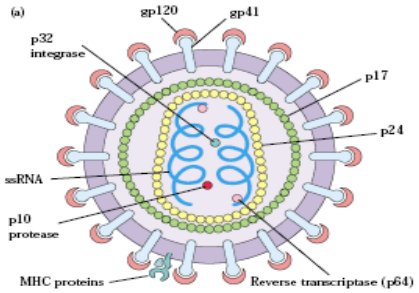
- HIV is an enveloped retrovirus **(**carry its genetic information in the form of RNA**).** It establishes a latent infection in which the provirus remains quiescent. Each virus particle (Virion) contains two copies of an RNA genome related with 3enzymes (reverse transcriptase, integrate, and protease) and a complex of two covalently associated glycoproteins (gp120 and gp41) in the viral envelope.
- HIV genome consists of nine genes flanked by long terminal repeats (LTR). LTR is required for the integration of the provirus into the host cell DNA and contains binding sites for regulatory proteins that control the expression of the viral genes. The three major genes are:
  1- Gag: encodes the structural proteins of the viral core.
  2- Pol: encodes the enzymes involved in viral replication and integration.
  3- Env: encodes the viral envelope glycoproteins.
- HIV-2 is less pathogenic in humans than HIV-1. It infects certain non-human primates that are not infected by HIV-1.

Classification :

- Lent virus including HIV-1 and -2 (from the Latin *lentus* = *slow;* because of the gradual course of the disease that they cause). They are characterized by the presence of cone-shaped nucleoid and absence of oncogenicity.
- BLV-HTLV retrovirus including HTLV-1 and -2 (Human T-cell leukemia virus). They are able to cause tumors (adult T-cell leukemia / lymphoma, ATLL) rather than immuno-suppression.
- Spumaviruses (Latin, *spuma*=foam). They are characterized by foamy appearance in infected cell and they are not pathogenic.



VISUALIZING CONCEPTS



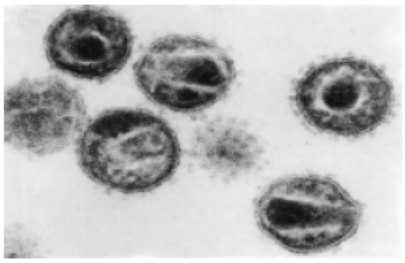


FIGURE 19-8 Structure of HIV. (a) Cross-sectional schematic diagram of HIV virion. Each virion expresses 72 glycoprotein projections composed of gp120 and gp41. The gp41 molecule is a transmembrane molecule that crosses the lipid bilayer of the viral envelope. Gp120 is associated with gp41 and serves as the viral receptor for CD4 on host cells. The viral envelope derives from the host cell and contains some host-cell membrane proteins, including class I and class II MHC molecules. Within the envelope is the viral core, or nucleocapsid, which includes a layer of a protein called p17 and an inner layer of a protein called p24. The HIV genome consists of two copies of single-stranded RNA, which are associated with two molecules of reverse transcriptase (p64) and nucleoid proteins p10, a protease, and p32, an integrase. (b) Electron micrograph of HIV virions magnified 200,000 times. The glycoprotein projections are faintly visible as "knobs" extending from the periphery of each virion. *[Part (a) adapted from B. M. Peterlin and P. A. Luciw, 1988,* AIDS *2:S29; part (b) from a micrograph by Hans Geldenblom of the Robert Koch Institute (Berlin), in R. C. Gallo and L. Montagnier, 1988,* Sci. Am. *259(6):41.]*

Transmission

- Common means of transmission include homosexual and heterosexual intercourse, needle drug abuse, transfusion with contaminated blood products, and via placenta (maternal-fetal transmission).
- The risk for HIV infection appears in:
  1- Patients who received blood transfusions and hemophiliacs who received blood products before tests for HIV in the blood supply were routinely used.
  2- Exposure to infected blood among intravenous drug users who normally share hypodermic needles.
  3- Infants born to mothers who are infected with HIV-1(at birth or through breast milk).
  4- Casual contact with or touching an infected person.
  5- Other sexually transmitted infections (especially those that cause genital ulcers).
- The long period after infection represents a factor contributing to the spread of HIV, during which, no clinical signs may appear but the infected individual may infect others.
- The Virus is carried in infected CD4 T cells, dendritic cells and macrophages, and as a free virus in blood, semen, vaginal fluids, or mother's milk.

Stages of HIV-1 disease (Clinical features of AIDS):

1- Asymptomatic phase (Influenza-like illness), around 3-4 weeks.
2- AIDS-related complex (with persistent lymphadenopathy, night sweats, fever, weight loss, and diarrhea).
3- Full-blown AIDS (with a plethoral of opportunistic infections).

Asymptomatic period:

- This is a period preceding the developing of AIDS, after which, opportunistic infections begin to appear.

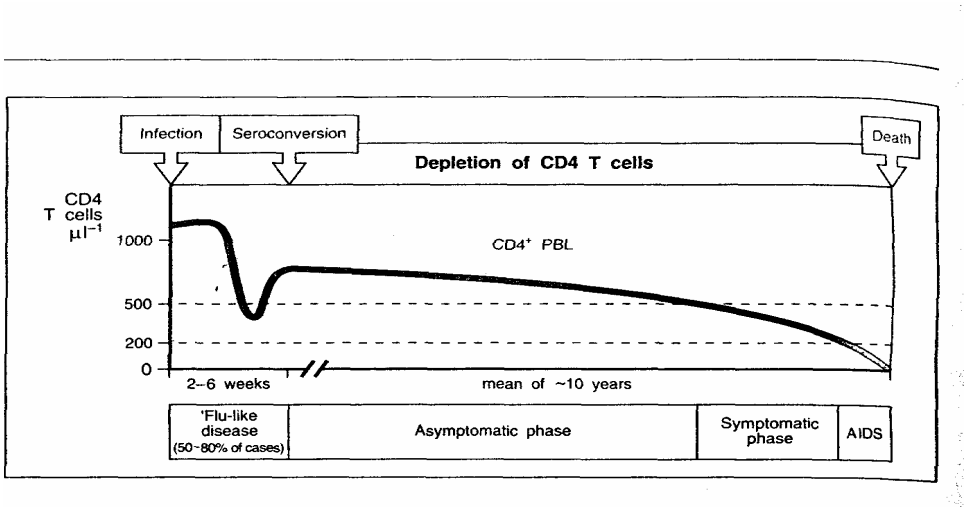
- It is not silent (along which, persistent replication of the virus and a gradual decline in the function and numbers of CD4 T-cells occur).

Symptoms of primary infection:

- An influenza-like illness (up to 80% of cases).
- Abundance of virus in the peripheral blood.
- Marked decrease in the numbers of circulating CD4 T-cells.

Symptoms of secondary infection:

- Swollen lymph nodes
- Fever, chills, and night sweats
- Diarrhea
- Weight loss
- Coughing and shortness of breath
- Persistent tiredness
- Skin sores
- Blurred vision and headaches
- Development of other infections, such as certain kinds of Pneumonia

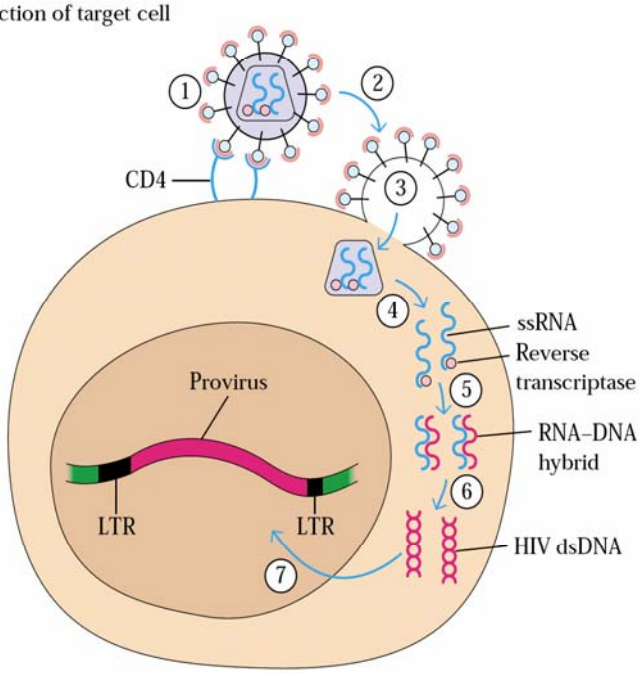


The typical course of untreated infection with H1V. The first few weeks are typified by an acute influenza-like viral illness, called seroconversion disease, with high titers of virus in the blood. An adaptive immune response follows, which controls the acute illness and restores levels of CD4 T cells ($CD4^+$ PBL) but does not eradicate the virus. Opportunistic infections and other symptoms become more frequent as the CD4 T-cell count falls, starting at about 500 cells. The disease then enters the symptomatic phase. When CD4 T-cell counts fall below 200 cells, the patient is said to have AIDS.

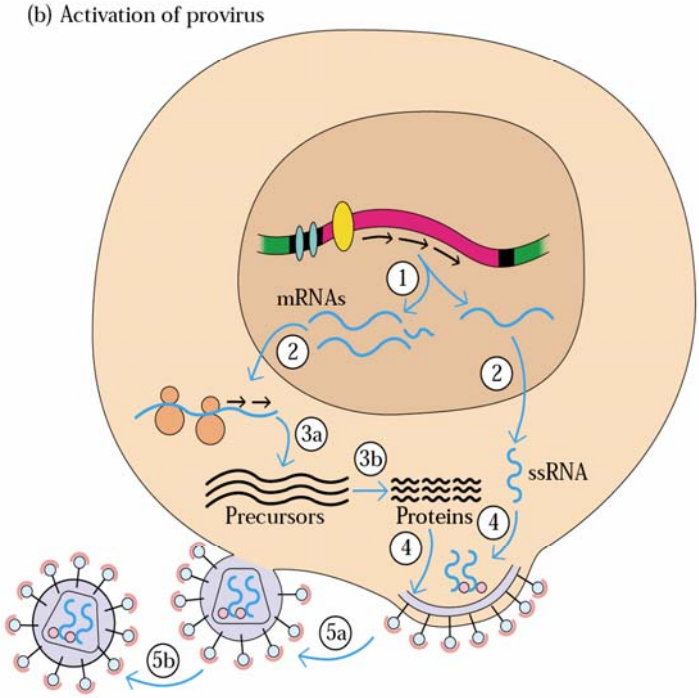
<u>Life Cycle of HIV-1 in T-Cell</u>

- The Cellular tropism of the virus (the ability of the HIV to enter particular types of cell) is determined by the expression of specific receptors for the virus on the surface of those cells.
- HIV-1 infects T cells that carry the CD4 antigen on their surface. The preference for CD4+ cells is due to a high-affinity interaction between the coat (envelope) protein of HIV-1 and the cell-surface CD4.
- First, the gp120 portion of the glycoprotein complex binds to the cell- surface molecule CD4. Before fusion process, gp120 must also binds to a co-receptor (Chemokine receptor; G-protein-coupled receptor with seven transmembrane-spanning domains), since the interaction alone between the viral envelope and CD4+ is not sufficient for entry.
- The major types of Chemokine receptors for HIV are:
  1- CCR5 (expressed on dendritic cells, macrophage and CD4 T-cells).
  2- CXCR4 (expressed on activated T-cells).
- Determination of the Chemokines receptor determines the cell types infected by different variants of HIV: -
  1- R5 viruses (macrophage-tropic): Infect dendritic cells, macrophage, and T-cells in *vivo*. They use CCR5 as a co-receptor.
  2- X4 viruses (lymphocyte-tropic):Infect CD4 T-cell in *vivo*. They use CXCR4 as a co-receptor.
- When HIV-1 enters the cell, the RNA is reverse transcribed to DNA by a virally enzyme, reverse transcriptase (RT), and makes a DNA copy of the viral RNA genome called a **provirus**.
- **The Provirus** is integrated into the cell genome and replicated with the host cell DNA. It may remain latent in the cell until regulatory signal starts the expression process.

(a) Infection of target cell



(b) Activation of provirus



(1) HIV gp120 binds to CD4 on target cell.

(2) Fusogenic domain in gp41 and CXCR4, a G-protein–linked receptor in the target-cell membrane, mediate fusion.

(3) Nucleocapsid containing viral genome and enzymes enters cells.

(4) Viral genome and enzymes are released following removal of core proteins.

(5) Viral reverse transcriptase catalyzes reverse transcription of ssRNA, forming RNA–DNA hybrids.

(6) Original RNA template is partially degraded by ribonuclease H, followed by synthesis of second DNA strand to yield HIV dsDNA.

(7) The viral dsDNA is then translocated to the nucleus and integrated into the host chromosomal DNA by the viral integrase enzyme.

(1) Transcription factors stimulate transcription of proviral DNA into genomic ssRNA and, after processing, several mRNAs.

(2) Viral RNA is exported to cytoplasm.

(3a) Host-cell ribosomes catalyze synthesis of viral precursor proteins.

(3b) Viral protease cleaves precursors into viral proteins.

(4) HIV ssRNA and proteins assemble beneath the host-cell membrane, into which gp41 and gp120 are inserted.

(5a) The membrane buds out, forming the viral envelope.

(5b) Released viral particles complete maturation; incorporated precursor proteins are cleaved by viral protease present in viral particles.

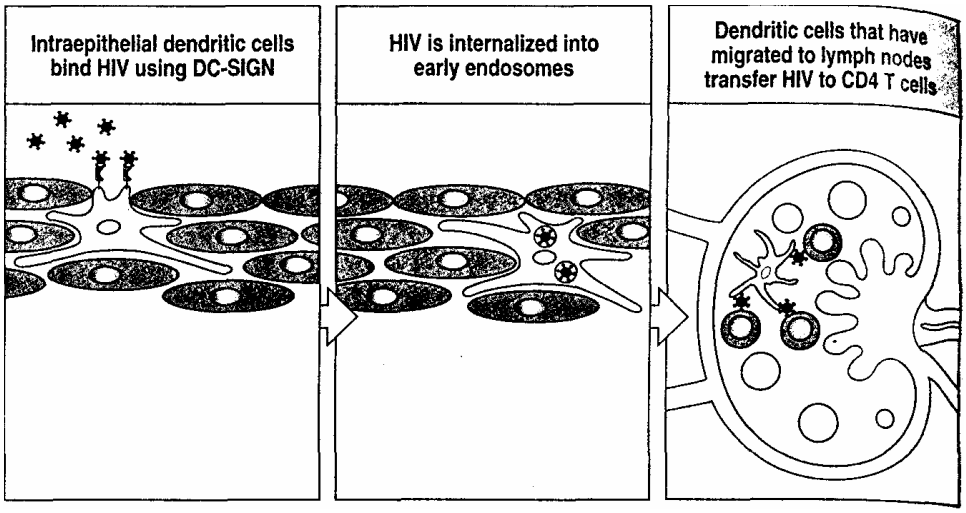
Q) Why HIV binds CD4 receptor specifically?

- CD4 is an integral membrane glycoprotein, containing four Immunoglobulin-like extra-cellular domains. Normally, CD4+ T-cell recognize the major Histocompatability Complex (MCH) Class II molecules on the surface of antigen presenting cells, APC. MCH Class II molecules contain an antigen in the form of a peptide that is recognized by the Receptor of the CD4 cell. Once an antigen is recognized, CD4 cells employ the immune response by: Proliferating more T-cells, activating B cells to

secrete antibodies, and releasing cytokines to increase action of phagocytes. Instead of APC recognizing the CD4 glycoprotein, HIV uses this receptor as a binding site specifically and infects those cells. HIV not only utilizes CD4 cells to replicate, but it destroys these cells and reduces the immune response to other viral antigens.

Epithelial cells and viral infection

The mucosa of the vagina, penis, cervix, and anus is covered by a stratified squamous epithelium. Dendritic cells within such cells initiate infection by binding HIV and transporting it to lymphoid tissue.



Which cells are infected?

- HIV virus infects $CD4^+$, macrophages & T cells but mainly $CD4^+$ T-cell lines are the main target. Most infections take place through the mucosal surface of the genital tract. It is thought that the virus binds to the surface of the dendritic cells, but does not enter or infect. The dentritic cells then migrate carrying the virus to lymph nodes, where the virus is transferred to and infects an activated $CD4^+$ T cell.

- The rate in which the plasma virus levels decline is much slower reflecting the very slow decay of virus production from cells that provide a longer-lived reservoir of infection, such as dendritic cells, tissue macrophages and latently infected memory CD4 T cells.
- The major reservoir of the HIV infection is the lymphoid tissue, in which infected CD4 T cells, monocytes, macrophages and dendritic cells are found.
- The decline in plasma viremia is accompanied by a steady increase in CD4-lymphocyte counts in the peripheral blood.
- The major target for HIV infection is CD8 cytotoxic T cells & CD4 T cells , CD4 T cells also have an important role in the host response to HIV infected cells as illustrated by 3 evidences :
  1- A correlation was found between the strength of CD4 T-Cell proliferate responses to HIV antigen AND viral load.
  2- Some patients who did not progress to AIDS long after infection by HIV showed strong CD4 T-cell proliferative responses .
  3- Early treatment with anti-retroviral drugs of acutely infected individuals was associated with are recovery in CD4 proliferative responses to HIV antigens.
- Three complementary mechanisms have been established for the recovery in CD4 T- Cell numbers:
  a- A redistribution of CD4 T memory cells from lymphoid tissues into the
  b- Circulation as viral replication is controlled.
  c- The reduction in the abnormal levels of immune activation as the HIV infection is controlled associated with reduced cytotoxic T-lymphocyte killing of infected CD4 T cells.
  d- The emergence of new native T cells from the Thymus.

- **The role of cytotoxic T -cells & T- helper cells**

  - Both C8 cytotoxic T cells and CD4 T_H cells specifically responsive to infected cells are associated with the decline in detectable virus after the initial infection.
  - These T-cell responses are unable to clear the infection completely and it can also cause some pathology, the T-cell Reponses to reduce viral spread therefore on balance, reduce the pathology of the disease.
  - The role of CD8 cytotoxic T cells, Cd4 T cells and the antibody ultimately fails to contain the infection.
  - Cytotoxic T cells can be seen to invade sites of HIV replication and they are responsible for killing many productively infected cells before any infectious virus can be released.

How HIV affects on CD4 T-cells?

There are three dominant mechanisms for the loss of CD4 T cells in HIV infection:

a- Direct viral killing of infected cells.

b- There is increased susceptibility to the induction of apoptosis in infected cells.

c- There is killing of infected CD T cells by CD8 cytotoxic lymphocytes that recognize viral peptides.

When CD4 T cell numbers decline below a critical level, cell mediate immunity is lost (CD4 T- cell count drops toward zero) and infections with a variety of opportunistic microbes appear which finally leads to death

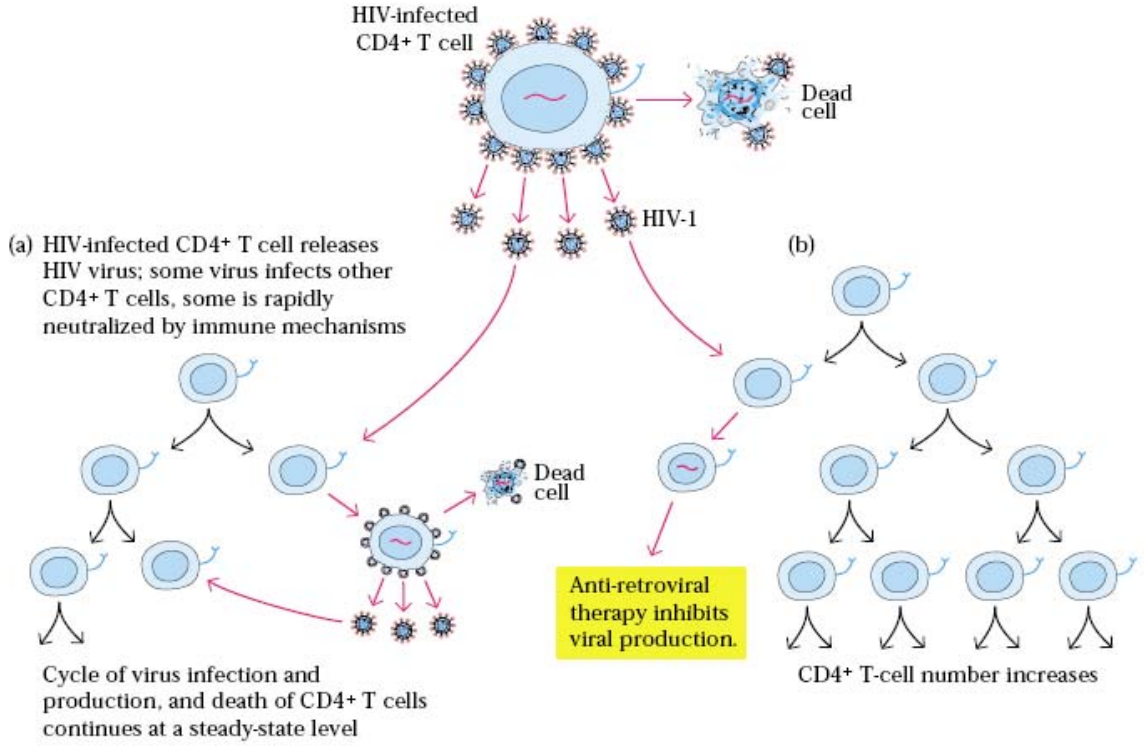
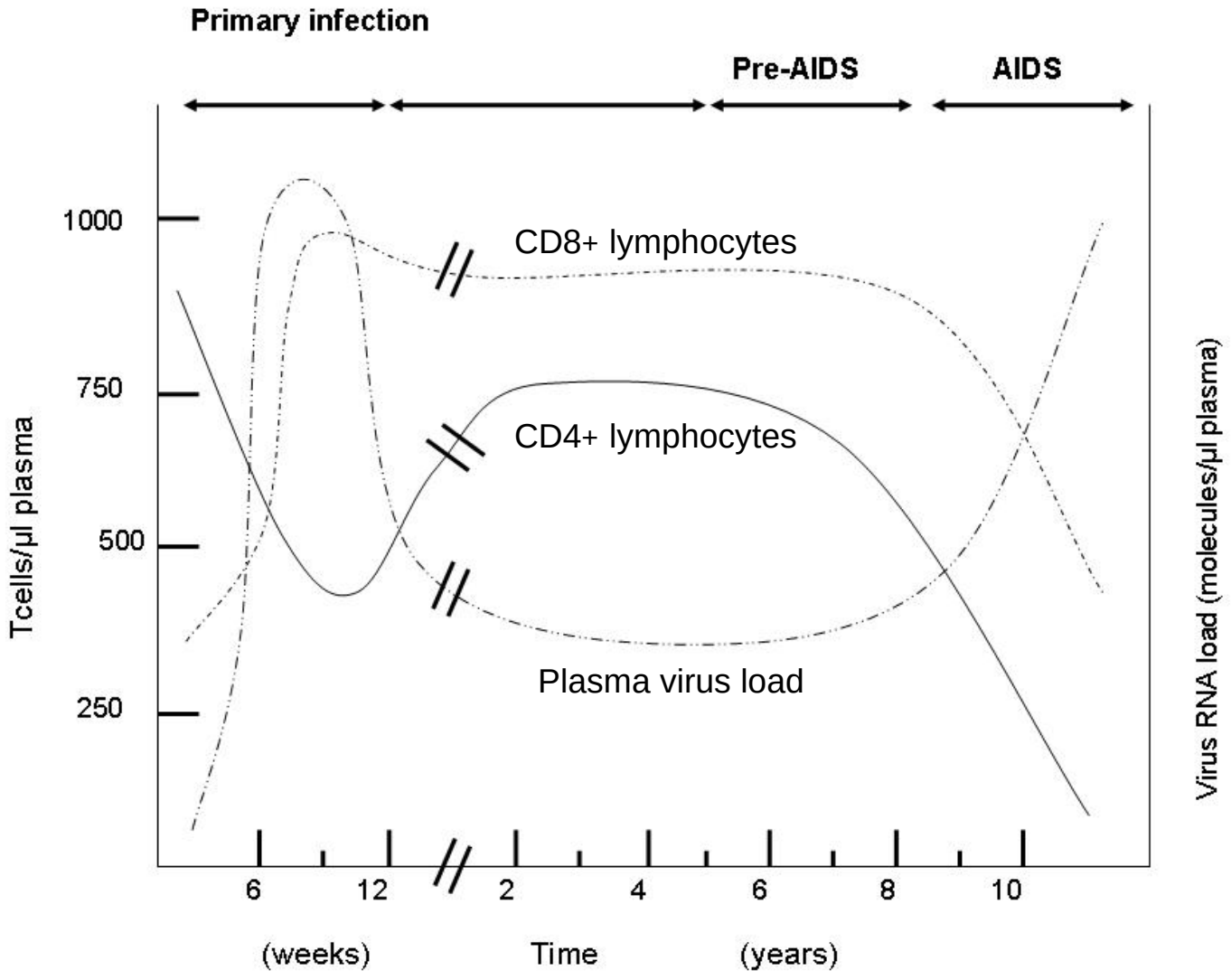


FIGURE 19-13 Production of virus by $CD4^+$ T cells and maintenance of a steady state of viral load and T-cell number. (a) A dynamic relationship exists between the number of $CD4^+$ cells and the amount of virus produced. As virus is produced, new $CD4^+$ cells are infected, and these infected cells have a half-life of 1.5 days. In progression to full AIDS, the viral load increases and the $CD4^+$ T-cell count decreases before onset of opportunistic infections. (b) If the viral load is decreased by anti-retroviral treatment, the $CD4^+$ T-cell number increases almost immediately.



Immunological abnormalities

The loss of $CD4^+$ T cells is the major result of HIV infection; also there are many immunological and non immunological abnormalities.

- Diagnostic abnormalities

$CD4^+$ T cell deficiency.

Reduction in levels of all lymphocytes.

Lowered cutaneous delayed-type hypersensitivity.

Non-specific elevation of immunoglobulin concentration in serum.

- Other abnormalities

Decreased proliferation responses to antigen.

Decreased cytotoxic responses to all antigens.

Decreased response to new immunogens.

Decreased $CD8^+$ T-cell cytotoxic response to HIV.

Decreased macrophage function.

Decreased of autoantibody.

Decreased natural killer cell activity.

Decreased dendritic cell number and activity.

Decreased of lymph node structure.

HIV accumulates many mutations in the course of infection in a single individual resulting outgrowth of drug resistant variants of the virus

- The rapid replication of HIV coupled with a mutation rate of approximately $3x10^{-5}$ per nucleotide base per cycle of replication, leads to the generation of many variants of HIV in a single infected patient in the course of a day.
- Reverse transcriptase lacks the proofreading mechanism associated with the cellular DNA polymerases and the RNA genomes of the Retrovirus are therefore copied into DNA with relatively low fidelity.
- When antiviral drugs are administrated, variants of the virus those carry mutations conferring resistance to their effects emerge and expand until former levels of plasma virus are regained.

Methods of HIV Treatment

- **Drugs that block HIV replication (CHEMOTHERAPY)**

-These drugs leads to a rapid decrease in titer of infectious virus and an increase nCD4 T cells in which two viral proteins in particular have been the target aimed at nteresting viral replication . These are:

a- The viral transcriptase , which is required for the synthesis of the provirus

b- The viral protease, which cleaves the viral polyproteins to produce the virion proteins and viral enzymes.

-Inhibitor of these enzymes prevent the establishment of further infection in uninfected cells,

-Cells that are already infected can continue to produce virions because once the provirus is established, reverse transcriptase is not needed to make new virus particles while the viral protease inhibition does not prevent the virus form being released. However the released virions are not infectious and further cycles of infection and replication are prevented.

-Treatment with the chemotherapy of HIV infection has two main aims:

1- To pevent the progression of infection to AIDS.

2- To clear the infection completely.

- The first objective has been achieved through the treatment of HAART which now usually means triple drug therapy and under the best conditions can reduce plasma virus to undetectable levels.

-However it does not completely restore the $CD4^+$ memory cell population and function and does not clear virus from resting $CD4^+$ memory cells in which the virus is latent ((and possibly other reservoirs).

-Chemotherapy must be continuous. It is calculated that, with the natural turnover of the pool of resting $CD4^+$ memory cells, it will take 10-60 years for the latently infected cells to disappear.

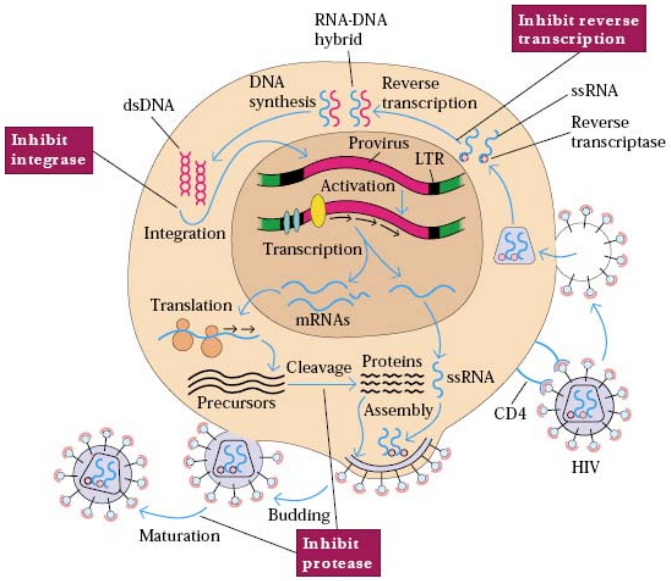


FIGURE 19-14 Stages in the viral replication cycle that provide targets for therapeutic antiretroviral drugs. At present, the licensed drugs with anti-HIV activity block the step of reverse transcription of viral RNA to cDNA or inhibit the viral protease necessary to cleave viral precursor proteins into the proteins needed to assemble a new virion and complete its maturation to infectious virus.

Obstacles for the treatment of HIV

1-HIV infection takes different forms within different cells, infected lymphocytes are found in the T-cell areas of the lymphoid tissue, latently memory CD4 cells that are activated in response to antigen presentation also produce virus that can spread by rounds of replication in other activated Cd4 T cells.

2-Infected memory CD4 T cells were found to have an extremely long mean half life of 44 months. This findings means that drug therapy may never be able to eliminate HIV infection and will need to be administrated throughout life. In addition to these cells ,there is a further large population of cells infected by efective provirus; such cells are not a source of infectious virus.

3-Macrophages and dendritic cells seem to be able to harbor replicating virus without necessarily being killed by it , and therefore believed to e an important reservoir of infections ;they also serve as a means of spreading virus to other tissues as a brain.

4-Although the function of the microphage is as the antigen-presenting cells does not seem to be compromised by HIV infection.

5-The virus causes abnormal patterns of cytokine secretion that could account for the wasting that commonly occurs in AIDS patients late in their disease.

From all the previous we can say that:

An immune response controls but does not eliminate HIV

HIV Vaccination

-The main problem is the nature of the infection itself, featuring a virus that proliferates extremely rapidly and causes sustained infection in the face of strong cytotoxic T-cell and antibody response.

-The development of the vaccine is extremely difficult as the virus is capable to persist in latent form as a trancriptionally silent provirus.

-There are many approaches in vaccination of HIV as DNA vaccination, envelope Vaccination……etc

Examples:

- Monkeys were vaccinated with a DNA vaccine given with IL-2 fusion protein and then challenged with a pathogenic hybrid Simian-human immunodeficiency virus. One of the monkeys, 6 months after the challenger, developed an AIDS-like illness that was associated with the emergence of a mutant virus carrying a point mutation in an immuno-dominant gag epitope recognized by cytotoxic T-cells.

-Vaccine has been made from the envelope protein gp120 and has been tested on chimpanzees, this vaccine proved to be specific to the precise strain of virus used to make it and was therefore useless in protection against infection.

Our Recommendations

In my point of view:

-The therapy is to find a drug or a compound which have the ability to conjugate the human cytokines with HIV enzymes or proteins to be finally a unified compound in which the T-lymphocytes can recognize these new structures as cytokines.

-These method if succeeded will direct the immune system to the virus even the latent Viruses, patient must be found in a closed sterilized place and must be provided with a good nutrition.

I hope for this suggestion to come over the latent virus stored in Memory B lymphocytes (latent reservoir of the virus).

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