

Clinical Tract

Module on

The anatomy and pathophysiology of HIV

LEARNING OUTCOMES FOR SOCIAL WORKERS, LABORATORY TECHNICIANS, PHARMACISTS, NURSING STAFF AND DOCTORS

After completion of this module the learner should be able to:

- Give a basic description of the anatomy of the virus.
- Describe the important steps in the life cycle of the virus and the different enzymes involved.
- Describe the organs making up the human immune system and understand how the immune system is damaged.
- Understand the basic disease processes.

This module is technically the most difficult module in the manual. It is not meant to be studied by counsellors and data typists. Even other members of the team should only have a working knowledge of HIV and the immune system and are not required to give a detailed description.

1. THE ANATOMY OF THE VIRUS

HIV belongs to the family of human retroviruses (*Retroviridae*) and the subfamily *Lentiviruses*. It is an RNA virus and its hallmark is the reverse transcription of viral RNA to DNA by reverse transcriptase enzyme, a unique characteristic of retroviruses. All the other non-retroviral viruses lead to the transcription of new RNA from a DNA strand. There are two types of HIV, HIV-1 and HIV-2 with the former being the predominant type in most parts of the world.

HIV virion morphology

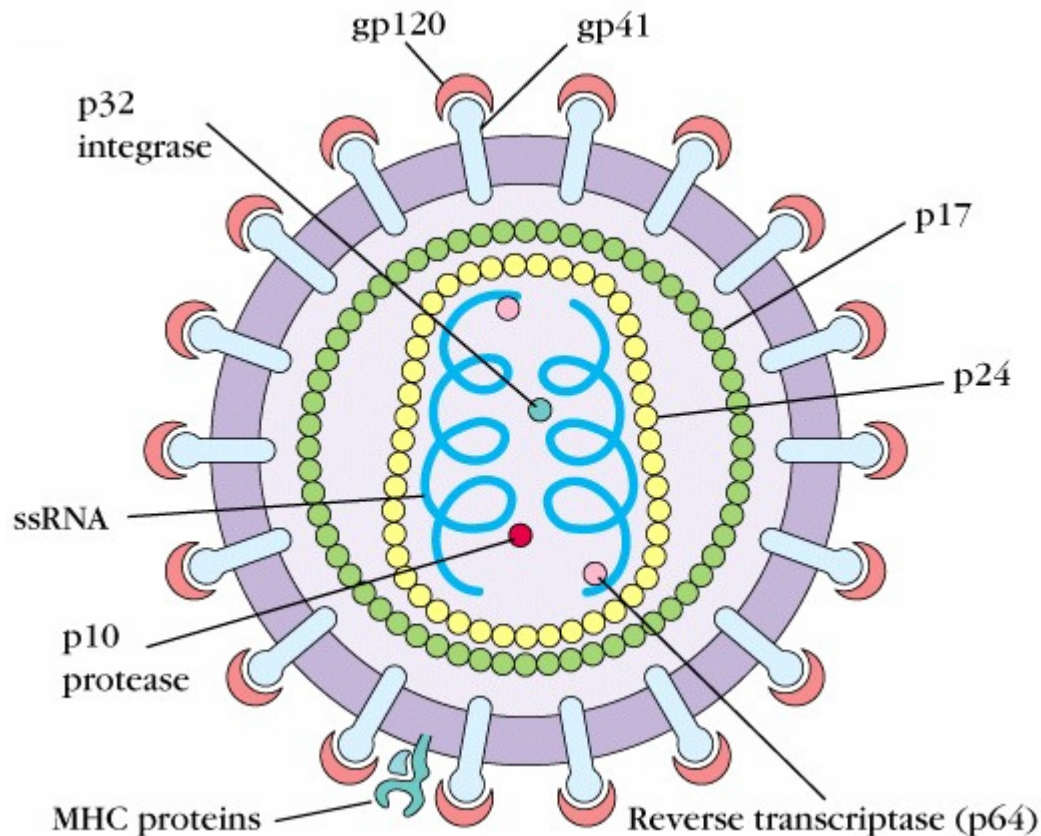


Figure 1. Schematic structure of the HIV virion

The virus detaches from the surface of the host cell membrane by a process called budding. Part of the host cell membrane therefore forms the envelope of the new virion with some of the host proteins becoming incorporated into the envelope in the process. Of most significance is the major histocompatibility complex (MHC) class I and II host proteins. The envelope is also studded by specific glycoproteins that facilitate attachment and subsequent entry of the virus into the host cell. These envelope glycoproteins are, the outer membrane glycoprotein (gp120) and the transmembrane glycoprotein (gp41).

On the inside, the envelope is lined by a structural protein called the matrix protein (p17) while another structural protein, capsid (p24) forms a cone-shaped casing around the core of the virion. The core consists of two single-stranded RNA molecules which form the RNA genome of the virus, and enzymatic proteins, reverse transcriptase, integrase and proteases.

Glycoproteins

The surface glycoprotein, gp120 is attached to the viral membrane via the transmembrane glycoprotein gp41. It recognizes specific receptors called the CD4 receptors on the surface of the target cells. gp41 the transmembrane glycoprotein traverses the virion membrane and mediates fusion of the host and viral membrane thereby allowing HIV to enter the host cell.

HIV genome

Common to most retroviruses are genes that encode the structural proteins of the virus,

Gag-Pol-Env genes. Gag encodes the proteins that form the core of the virion, Env encodes the envelope glycoproteins and Pol encodes the viral enzymes, reverse transcriptase, proteases and integrase.

HIV-1 is however more complex than other retroviruses and contains other genes, HIV-regulatory genes which code for proteins involved in the regulation of gene expression (tat, rev, nef, vif, vpr, and vpu). Some of these regulatory genes play a role in pathogenesis of HIV disease.

Target cells of HIV

Any cell that expresses the CD4 receptor together with the co-receptor molecules (chemokine receptors) can be a target. The principal targets are the CD4 T-lymphocyte cells (T helper cells), monocytes and macrophage lineage cells. Others with few CD4 receptors are follicular dendritic cells (FDC) and the epidermal Langerhans cells present in the skin.

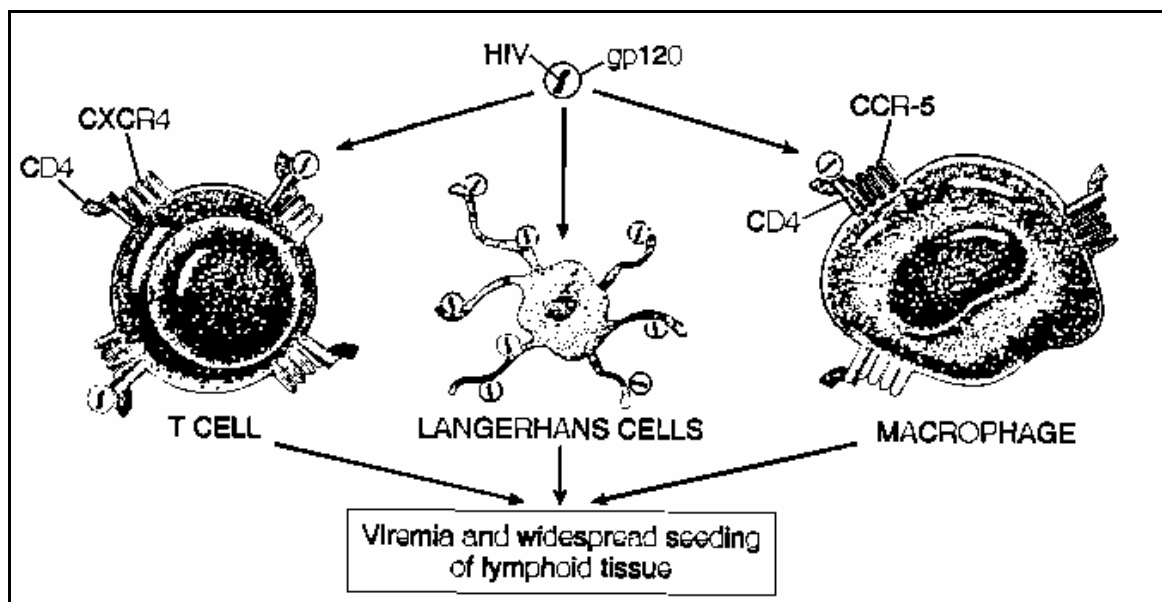


Figure 2. Target cells of HIV

(From; *Robin's Pathologic Basis of Disease*, 6th Edition; pg 241)

Normally, two surface receptors are required for the virus to gain entry into the host cells. Chemokine receptors (co-receptors for HIV) belong to the large group of seven-transmembrane domain G protein-coupled cellular receptors. The two most

important co-receptors for HIV-1 are CCR5 (R5) and CXCR4 (X4). These co-receptors also help in determining the cellular tropism of the HIV-1. The T-tropic HIV-1 strains bind to X4 co-receptor cells, mainly the T-cells while the M-tropic strains bind to the R5 co-receptor cells like the macrophages.

2. REPLICATION CYCLE OF HIV

1. The replication cycle begins with binding of the viral gp120 to the target host cell CD4 receptor.
2. gp41-mediated fusion of the host and viral membrane then follows leading to internalization of the HIV genomic RNA and its associated proteins into the target cell. The co-receptor is vital for this stage.
3. Reverse transcriptase enzyme then catalyses the reverse transcription of the viral RNA to produce a double-stranded viral DNA.
4. Viral DNA translocates to the nucleus where it is integrated randomly into the host cell genome with the help of yet another viral enzyme, integrase.
5. Integrated proviral DNA may remain inactive (latent) in the nucleus or it may lead to gene expression and transcription forming genomic viral RNA or mRNA if the infected cell is activated.
6. HIV mRNA is translated into viral proteins
7. Viral particle is formed by the assembly of the HIV proteins, enzymes and genomic RNA at the cell plasma membrane.
8. Budding of the immature virus occurs at the host cell membrane and the core acquires its external envelope.
9. During or just after budding, the third viral enzyme protease, cleaves the Gag-Pol precursor proteins yielding a mature virion that is infectious.

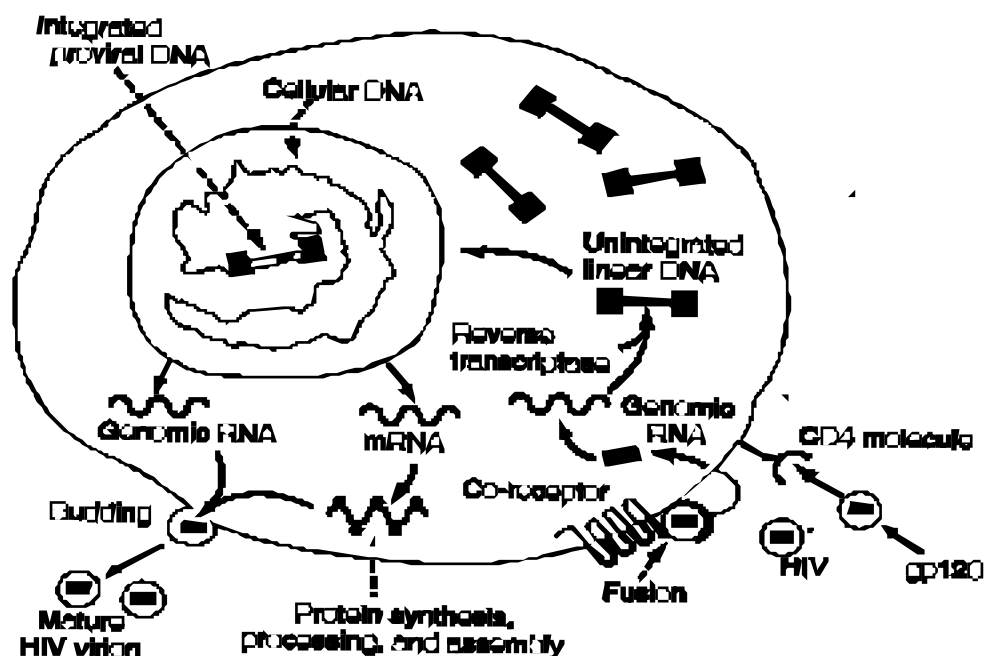


Figure 3. The replication cycle of HIV
(From; *Principles of Internal Medicine*, Harrison's 15th Edition; pg 1855)

Looking at the different stages of the life cycle, there is a potential for therapeutic intervention at each one of them.

3. IMMUNOPATHOGENESIS OF HIV

Normal immune system

Consists of two arms;

- Innate (ancient) immune system
- Adaptive (acquired) immune system

Innate immunity – It is non-specific and includes natural killer cell lymphocytes that kill the target cells directly or indirectly by antibody-dependent cellular cytotoxicity (ADCC), and dendritic cells that localize and present antigens to responsive T and B cells. Other participating cells are, monocytes, macrophages, neutrophils, basophils, eosinophils, tissue mast cells and epithelial cells.

Adaptive immune system – Is characterized by antigen-specific responses to a foreign antigen. It consists of cellular and humoral immunity. T cells are the main mediators of cellular immunity. The CD8 cytotoxic T cells target the virus-infected cells or foreign cells while the CD4 T cells are the primary regulatory cells of both cellular and humoral specific immunity.

The CD4 T cells activity can be either by direct cell contact or indirectly by release of regulatory chemical messengers called cytokines. The latter then activates the CD8 T cells, antibody-producing B cells or monocytes and macrophages.

The B cells are the principal effectors of humoral immunity and leads to specific antibody production once activated.

Together, the innate and the adaptive immunity protect the host from infections by multiple organisms.

Immune deficiency in HIV infection

Entry of HIV initiates a complex chain of immunopathogenic mechanisms that lead to a vicious cycle of pathogenesis and finally to profound immunosuppression characteristic of advanced HIV infection.

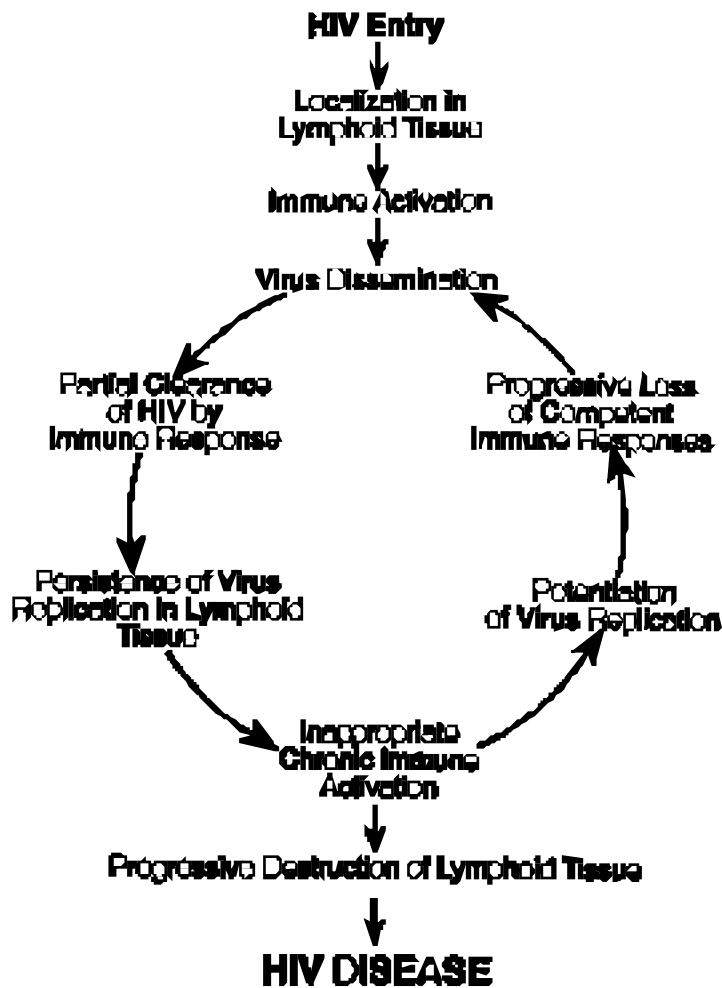


Figure 4 – The vicious cycle of HIV pathogenesis

(From; Pantaleo G, Fauci A S.1995. *Annu Rev Immunol*;13:487-512)

Primary infection is accompanied by widespread dissemination of the virus. Rapid replication of the virus in this early stage of infection triggers an appropriate immune response that is HIV-specific. This leads to a marked reduction in the initial viremia. However, this immune response is inadequate and only partially eliminates the virus from the body. A stage of clinical 'latency' that is seemingly dormant and asymptomatic develops. During this 'latent' stage, viral replication continues especially in the lymph nodes. A chronic and persistent state of viral replication ensues and gradual deterioration of the immune system occurs. This finally leads to clinically apparent disease.

Factors affecting the pathogenesis of HIV

- Host factors
 - Cell-mediated immunity
 - Humoral immunity
 - Genetic factors
- Viral factors

- Other co-factors

Cell-mediated immune response

1) CD4 T lymphocyte

The depletion and dysfunction of the CD4 T cells with persistent HIV infection is central to the pathogenesis of HIV disease. This occurs via direct and indirect mechanisms.

a) Direct HIV-cytopathic effects

This causes single-cell killing and cell fusion with syncytia formation. Many uninfected CD4 cells fuse with a single HIV-infected cell via CD4-gp120 interaction forming giant multinucleated cells (syncytium) that may be short lived.

Several mechanisms are thought to contribute to the single-cell cytotoxicity of HIV infection;

- Accumulation of unintegrated viral DNA
- Inhibition of cellular protein synthesis by impairing cellular RNA processing
- Excessive production and accumulation of gp120 also has a direct cell-killing (cytopathic) effect apart from accelerating the virus-cell and cell-cell fusion
- Viral budding from the cell plasma membrane causes loss of membrane integrity

b) Inhibited lymphopoiesis

Lack of the ability to completely regenerate or repopulate the peripheral blood CD4 T lymphocytes that are persistently diminished in HIV infection. This is due to destruction and depletion of lymphoid precursor cells in the lymphoid organs and in the bone marrow. The thymic progenitor cells in the thymus gland are also affected.

This leads to depressed formation of new blood cells, and other hematological abnormalities.

2) CD8 T lymphocytes

a) Cytotoxic CD8 T lymphocytes (CTL)

HIV-specific immune response has both humoral and cellular immune responses. The HIV-specific cytotoxic CD8 T lymphocytes are predominantly involved in the cell-mediated immune response. When activated, they are cytotoxic to HIV-infected CD4 cells and other target cells expressing identical MHC class I molecules associated with HIV antigen.

The CTL are also cytotoxic to uninfected CD4 cells (innocent bystanders) with surface-bound envelope proteins like gp120.

The HIV-specific CD8 CTLs appear early in primary HIV infection and play a major role in reduction of the viremia in the initial partial immune response. There is a significant inverse correlation between the CTLs and the viral load. As the disease progresses, their specific

functional capability decreases and in late stages of the disease, the numbers of CTLs may be significantly reduced. Their functional integrity is partly dependent on adequate inductive signals from CD4 T cells, the main regulatory cells of adaptive immunity. Therefore, the defect in advanced disease might be compounded by the marked CD4 cell reduction.

- b) **Suppressor CD8 T lymphocytes** – Another group of CD8 T cells that cause suppression of HIV replication without killing the invaded host cell. Suppression is mediated via chemical messengers in form of antiviral cytokines and chemokines.
- 3) **B cells** – Many functional abnormalities of B cells occur in patients with HIV infection like, polyclonal activation, hypergammaglobulinaemia, autoantibody production and also impaired primary and secondary (memory) antibody responses to microorganisms.
- 4) **Natural killer lymphocyte cells (NK cells)** – Involved in the killing of HIV-infected cells either directly or indirectly via the ADCC where the NK cells with specific antibodies against HIV antigens are directed towards HIV-expressing cells.
- 5) **Monocytes and macrophages**

HIV infection usually leads to death of the invaded target host cell. It however has a low cell-killing effect on monocyte-lineage cells like monocytes and macrophages that are also targets of HIV. These cells therefore act as reservoirs of HIV infection, a function that may be an obstacle in eradication of HIV infection with antiretroviral treatment.

- 6) **Dendritic and Langerhans cells**

They are the first cells to appear at inflamed mucous membrane sites and are vital in initiating T cell response. On entering an inflamed site, HIV is bound by dendritic cells and carried to the lymphoid organs rich in CD4 T cells. They are also thought to initiate the partial HIV-specific cell mediated immune response by interacting with CTLs in the lymphoid organs.

Humoral immune response

HIV-specific humoral immune response is important in the initial downregulation of HIV replication. HIV-binding antibodies produced by the B cells within six to twelve weeks are directed to multiple HIV antigens. The antibodies, which can be detected by ELISA or western blot do not have a clear definite function. Some have a protective function while others enhance the pathogenesis of HIV infection.

Protective function

- By participating in ADCC after binding to the NK cells. Leads to the killing of the HIV-infected cells.
- Neutralizing antibodies neutralize the HIV directly therefore preventing the spread of infection. The envelope glycoproteins that induce production of these neutralizing antibodies can be considered as a component for a potential HIV vaccine.

Pathogenic role

HIV-specific antibodies may also contribute to disease progression by:

- Antibody enhancement mechanism – production of antibodies that facilitate infection of other cells like macrophages and NK cells.
- Bystander killing – Anti-gp120 antibodies involved in ADCC might also kill uninfected CD4 T cells expressing free gp120.

Other mechanisms in pathogenesis of HIV

Autoimmune mechanisms

Several auto-antibodies directed against individual's own normal antigens in the body, have been detected in HIV-infected patients. The possible explanations of the autoimmune processes are:

- B cell polyclonal proliferation and antibody production due to lack of T cell regulation in HIV infection after depletion of the CD4 T cells.
- Molecular mimicry – some degree of structural resemblance is shared between the HIV proteins and the major histocompatibility complex (MHC) class II molecules. There could therefore be a cross-reaction between the antibodies to the HIV protein and the human leucocyte antigens (HLA) class II molecules. Several auto-antibodies have been detected in HIV infection.

Apoptosis (Programmed cell death)

This is a normal mechanism of cell death where the body eliminates autoreactive clones of T cells. HIV infection of CD4 T cells may lead to activation-induced cell death sometimes without direct infection of the CD4 T cells by HIV. It is thought that cross-linking of the CD4-gp120 or gp120-anti gp120 immune complexes prepares the cells for apoptosis.

Lymphoid organs in HIV infection

When HIV enters the body, it is widely disseminated especially to the lymphoid tissues. The latter plays a major role as a reservoir of HIV where the virus particles are trapped within the follicular dendritic cells (FDC) in the germinal center and persistent viral replication occurs even during the clinical latency period. The FDCs facilitate infection of CD4 T helper cells and memory T cells within the germinal centers of the lymph nodes. Persistent activation of the immune cells in the lymphoid organs further facilitates their infection by trapped virus and its replication once inside the cells. The expanding network of FDCs and the chronic, persistent viral replication leads to follicular hyperplasia clinically presenting as enlarged lymph nodes.

In advanced HIV disease, degeneration of the follicular dendritic cell network and involution of the lymph node germinal center occurs releasing the trapped HIV virions into circulation with resultant massive increase in plasma viremia in late disease.

Genetic factors in HIV pathogenesis

Some host genetic factors have been described that may influence the pathogenesis and course of HIV disease;

- MHC class I and II alleles – specific alleles of the HLA loci are associated with different rates of progression and varying susceptibility to HIV infection.

- Chemokine receptor (co-receptor) mutations and mutations of the chemokines, chemical messengers specific for these receptors will interfere with viral binding to the host cell membrane and subsequent progression of the infection.
- Certain immunodominant HIV T helper or cytotoxic T lymphocyte epitopes lead to a relatively protective immune response against the HIV.

These varying genetic factors are associated with different clinical conditions in HIV-infected persons and also with different clinical courses of the disease.

- Long-term nonprogressors or slow progressors
CCR5 mutations (homozygous defect) are associated with decreased susceptibility to infection while the heterozygous defect causes delayed progression of the disease. Approximately 1% of Caucasians of western European origin have the homozygous mutation while up to 20% are heterozygotes of the CCR5 mutation. These mutations are extremely rare in Africans.
CCR2 heterozygous mutations are also associated with slow progression.
- Rapid progression – HIV-infected patients with homozygous mutation of CX₃CR1 co-receptor showed rapid progression to AIDS. HLA B35 is also associated with rapid progression.
- Exposed yet uninfected individuals – they remain negative despite repetitive sexual exposure to HIV in high risk environment. In vitro tests done on these individuals specimens revealed that they had homozygous defects for the CCR5 mutation, the co-receptor for R5 strains of HIV-1 but were rapidly infected with X4 strains.

Viral (HIV) factors that affect pathogenesis of disease

- Viral escape from immune response by mutations.
- Viral attenuation – attenuated HIV can slow the rate of disease progression, like a deletion in the nef gene (a regulator gene) slows progression.
- Viral tropism – progression from M-tropic to T-tropic viruses is associated with increased pathogenicity and progression of disease.
- Viral subtypes – different subtypes (M,N,O) have different virulence and transmissibility.

Co-factors contributing to HIV pathogenesis

Co-factors can be endogenous or exogenous factors. They alter the pathogenesis by several mechanisms like induction of HIV expression.

- Cytokines – Pro-inflammatory cytokines like TNF α and β , IL-1 and IL-6 produced in response to most infections or inflammations are also increased in HIV-infected persons. They enhance HIV replication while interferon α and β and IL-16 suppress HIV replication.
- Co-infection – Co-infection or simultaneous cotransfection of cells with HIV and other viruses like cytomegalovirus, human herpes virus type 6, herpes simplex virus type 1, Epstein-Barr virus, human T lymphotropic virus type 1 and others can up-regulate the expression of HIV. Other microbes like *Mycobacterium tuberculosis* and *Mycoplasma* also have a similar effect.

4. CONCLUSION

While appreciating all the research efforts in the last two decades that have provided much insight into the pathogenesis of HIV infection, these complex immunopathogenic mechanisms are still not completely understood. A better understanding of these mechanisms would help a great deal in the development of new therapeutic agents and hopefully the development of an effective vaccine.

5. FURTHER READING

- Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection, *N Engl J Med*, 1993;328:327-335
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- Braunwald E, Fauci AS, Kasper DL, et al. HIV:AIDS and related diseases, *Principles of Internal Medicine*, Harrison's 15th Edition;2001:chapter 309
- Hogan CM, Hammer SM. Host determinants in HIV infection and diseases. *Ann Intern Med*, 2001;134:761-776 and 978-996