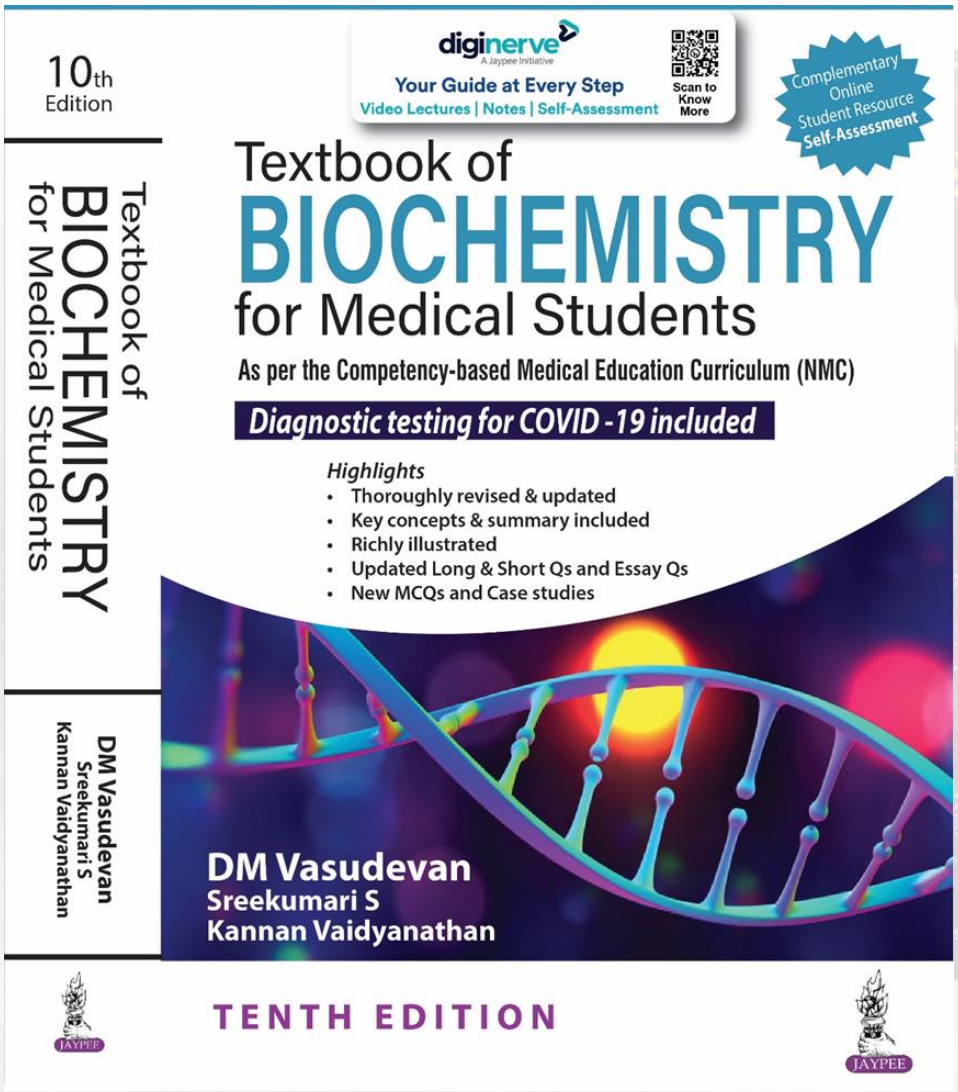


Chapter 41:

Biochemistry of AIDS and HIV

Textbook of
BIOCHEMISTRY
for Medical Students

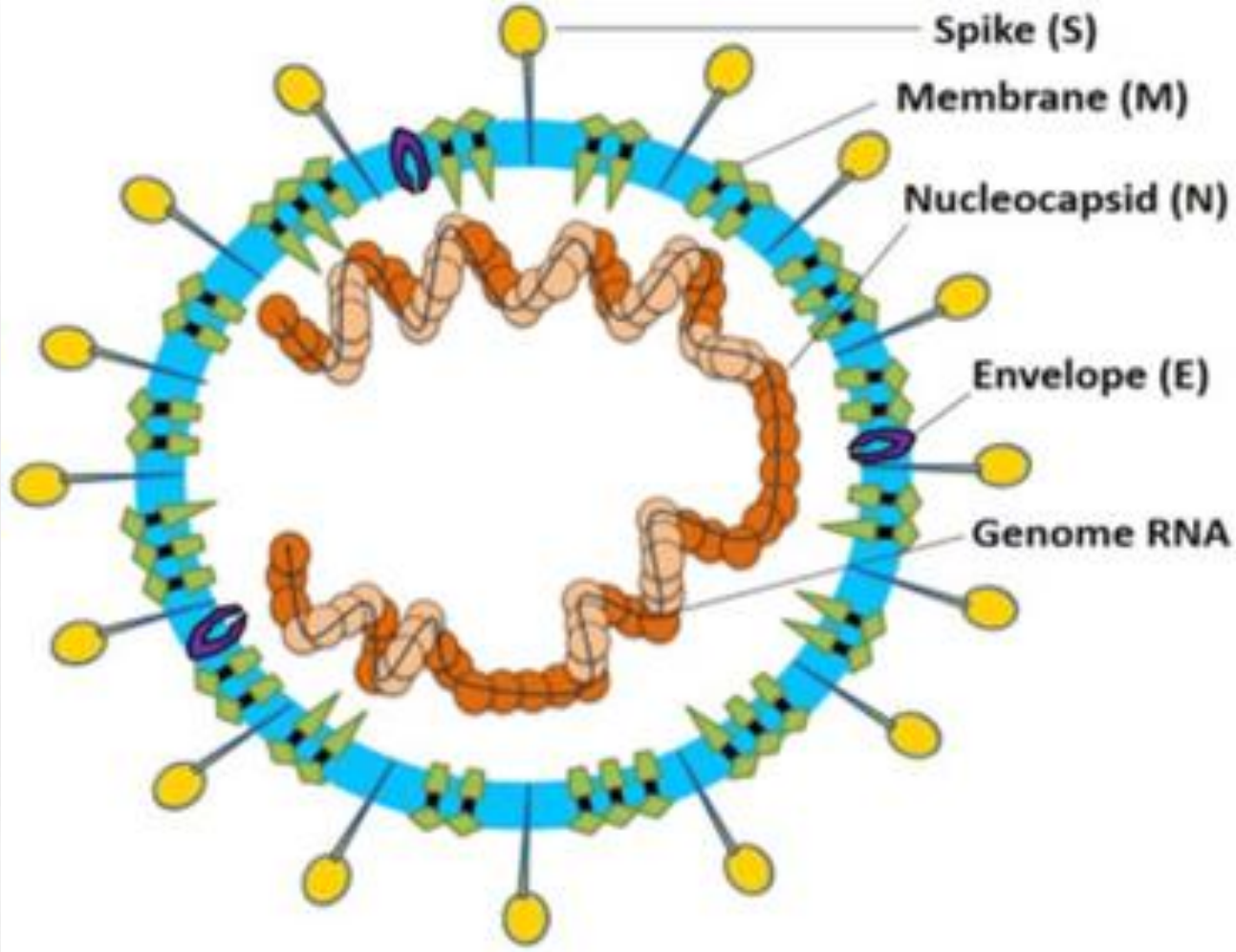
By DM Vasudevan, *et al.*

TENTH EDITION

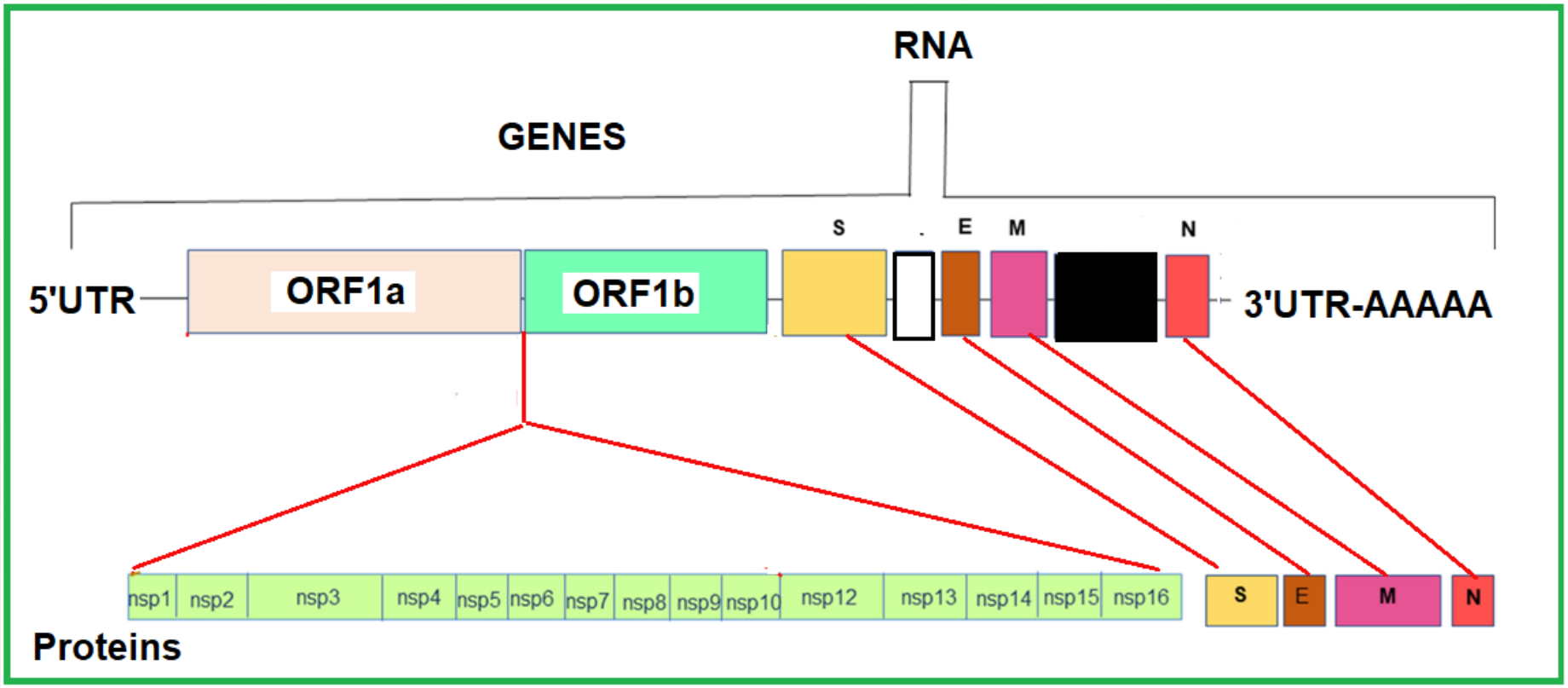
Coronavirus Disease

COVID-19 is the third coronavirus outbreak in the last 20 years, after Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). In December 2019, a new disease, named as COVID-19 (abbreviation of Coronavirus Disease 2019) emerged from Wuhan, China. It has been recognized as a pandemic by WHO, affecting almost all the countries of the world. As of May 2022, a total of 525 million cases have been reported worldwide, with 6.3 million deaths. India reported a total of 43 million cases, with 0.5 million deaths.

Structure of Coronavirus

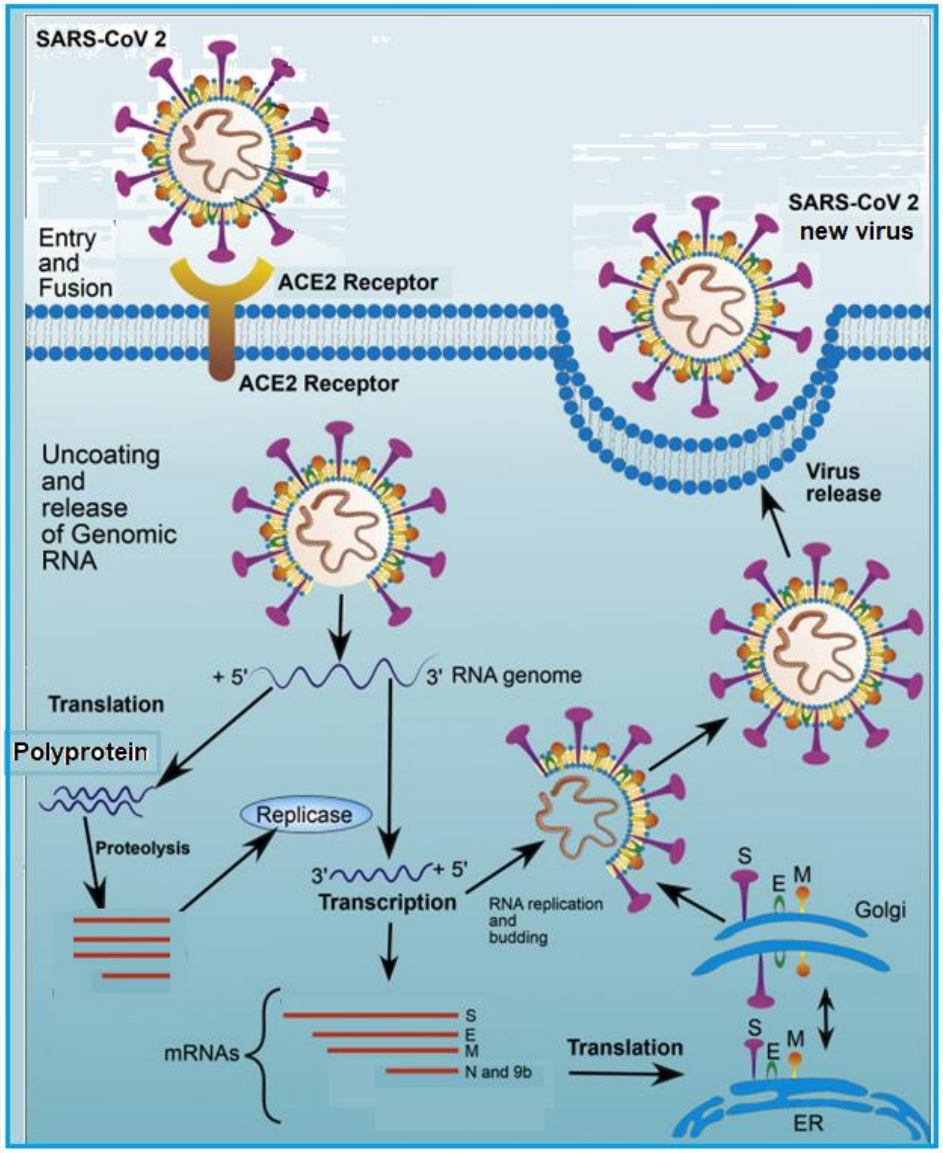


SARS-CoV-2 genes



ORF= open reading frame; UTR = untranslated region; nsp = nonstructural proteins; S = spike protein; E = envelope protein; M = membrane protein; N = nucleocapsid protein.

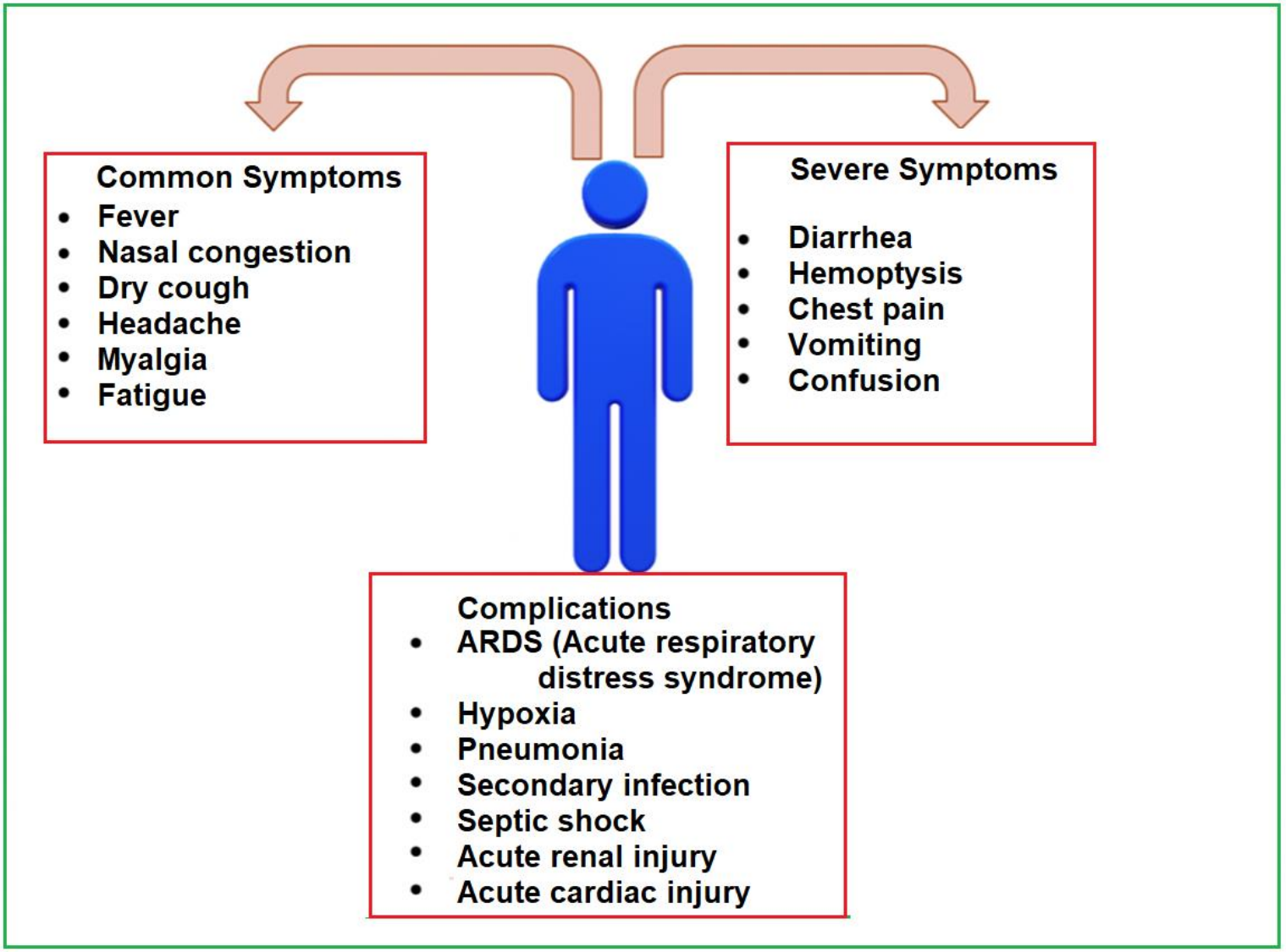
Life cycle of virus inside the host cell



ACE = angiotension converting enzyme;
ER = endoplasmic reticulum;
S = spike protein;
E = envelope protein;
M = membrane protein;
N = nucleocapsid protein.

The virus binds to the Angiotensin-Converting Enzyme 2 (**ACE2) receptor**. The ACE2 is expressed in high amounts in the upper and lower respiratory tract, in the alveolar epithelial cells. The virus uses a special surface glycoprotein, the "**spike protein**" (S), to attach to the ACE2 receptor and enter the host cell.

Clinical manifestations of COVID-19



Pathophysiology of COVID-19

In severe cases, there is an over-reaction of immune system (**cytokine storm**) which leads to eventual death. Such patients have substantially lower lymphocyte counts and higher plasma concentrations of inflammatory cytokines such as IL-6, IL-10 and tumor necrosis factor (TNF). A **low oxygen saturation** (83%) is common in many patients. Denaturation of hemoglobin leads to iron overload tissues as evidenced by hyperferritinemia, the release of free circulating heme, systemic hypoxia, reduction in nitric oxide, and finally activation of coagulation causing thromboembolic episodes.

Pulmonary lesions contain extensive focal hemorrhage, necrosis, alveolitis, and exudation. **Immune organ damage** consists of necrosis of splenic lymphoid tissue, focal necrosis of lymph nodes, and suppression of bone marrow.

Laboratory diagnosis of COVID-19

A nasopharyngeal swab specimen is preferred. The swab should be placed in the transport medium and kept at 2-8°C. Diagnostic laboratory tests to detect Covid infection are

1. RTPCR (Reverse Transcriptase Polymerase Chain Reaction)
2. RT-LAMP (Reverse Transcriptase Loop Mediated Isotheramal Amplification) test.
3. Rapid antigen test is less sensitive.
4. Serological tests: antibodies usually develop days to weeks after the initial infection. The presence of IgM antibody indicates recent exposure to the virus, while the presence of IgG antibody indicates later-stage infection.

Vaccines against COVID-19

India produced and used mainly two types of vaccines,

1. Recombinant DNA vaccine carried by a genetically modified adenovirus (Covishield)
2. Killed virus vaccine (Covaxin).

The major types COVID-19 vaccines currently available in large-scale clinical trials all over the world include:

1. Messenger RNA (mRNA) vaccine
2. Vector vaccine
3. Protein subunit vaccine

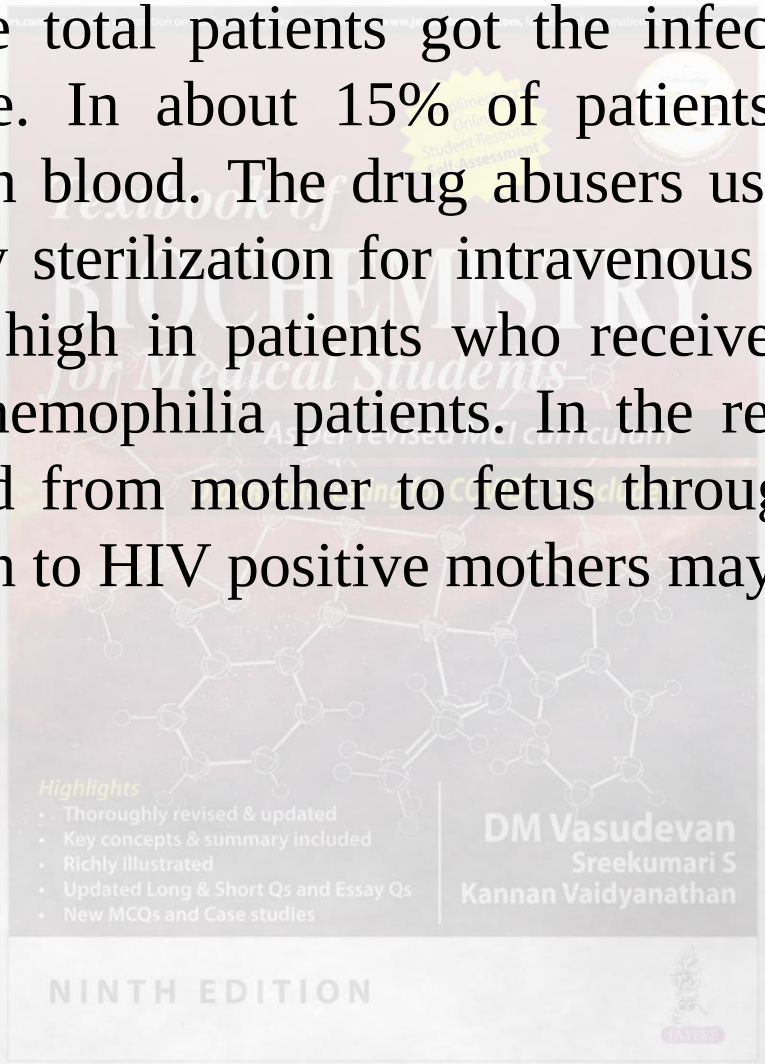
HUMAN IMMUNODEFICIENCY VIRUS

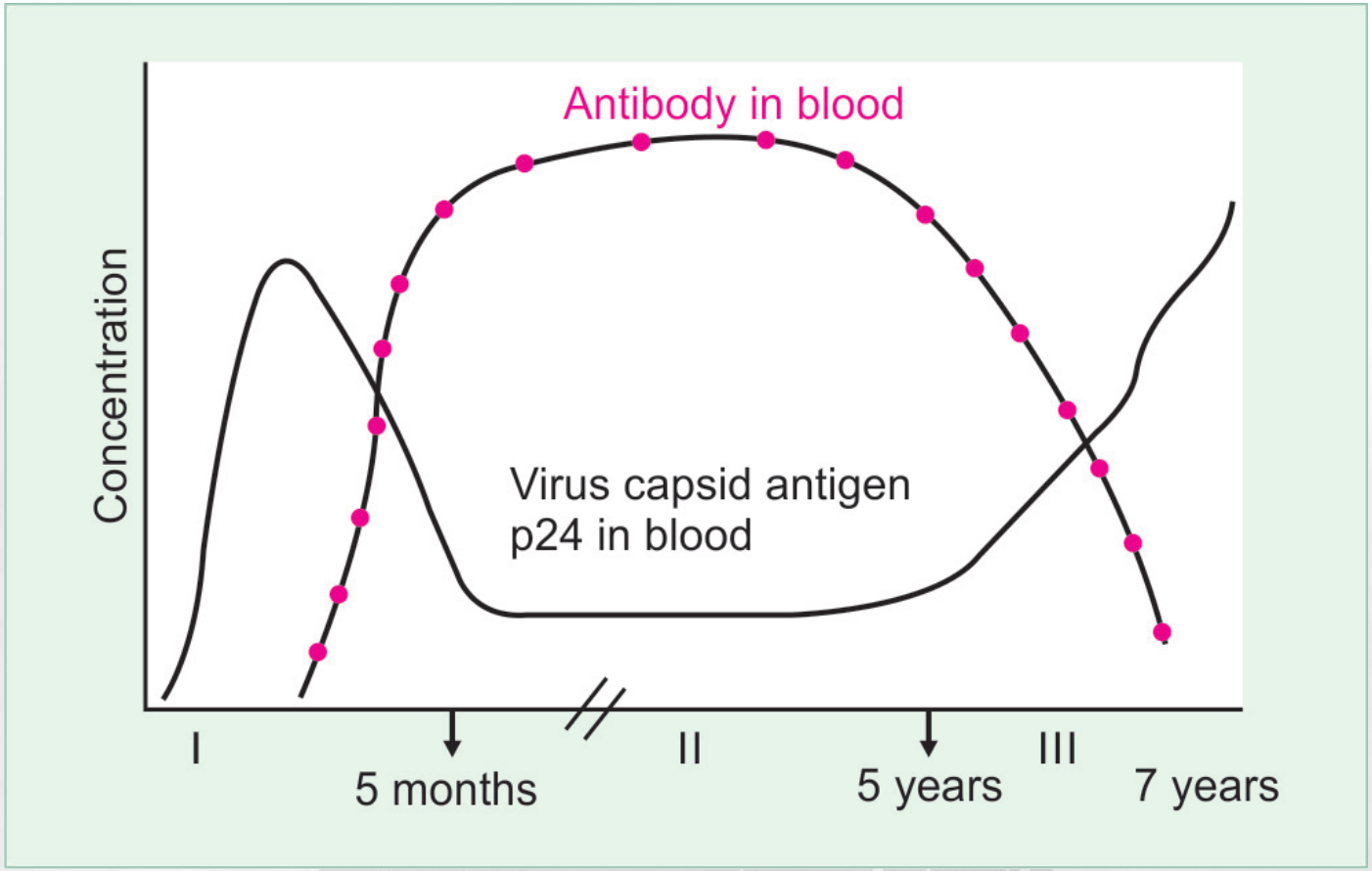
In 1981, a cluster of 5 cases of *Pneumocystis carinii* pneumonia were reported in USA. These protozoa can produce pneumonia only in immunodeficient individuals. Based on the clinical manifestations, the disease was named as Acquired Immuno Deficiency Syndrome with acronym of AIDS.

In 1983, Francoise Barre-Sinoussi showed that the disease is due to a retrovirus (virus having reverse transcriptase enzyme).
The isolation of a virus from the lymphocytes of the AIDS patients in 1984 independently by Robert Gallo at the NIH, USA and Luc Montagnier at the Pasteur Institute, Paris.

Transmission

About 80% of the total patients got the infection as a sexually transmitted disease. In about 15% of patients, the disease was transmitted through blood. The drug abusers usually use the same needle without any sterilization for intravenous injection. The risk of getting HIV is high in patients who receive blood transfusion many times, e.g. hemophilia patients. In the rest 5% cases, virus may be transmitted from mother to fetus through placenta. About 30% of infants born to HIV positive mothers may get the infection.





Course of HIV infection. I = window period; II = seropositive period; III = AIDS disease. Black line is antigen in blood. Red dots indicate antibody response.

Natural Course of the Disease

Window Period

When the virus enters the body, it is multiplied in the body cells, but it cannot be detected easily. This is called the window period. The viral capsid antigen p24 can be detected in the blood during this time.

Seropositive Stage

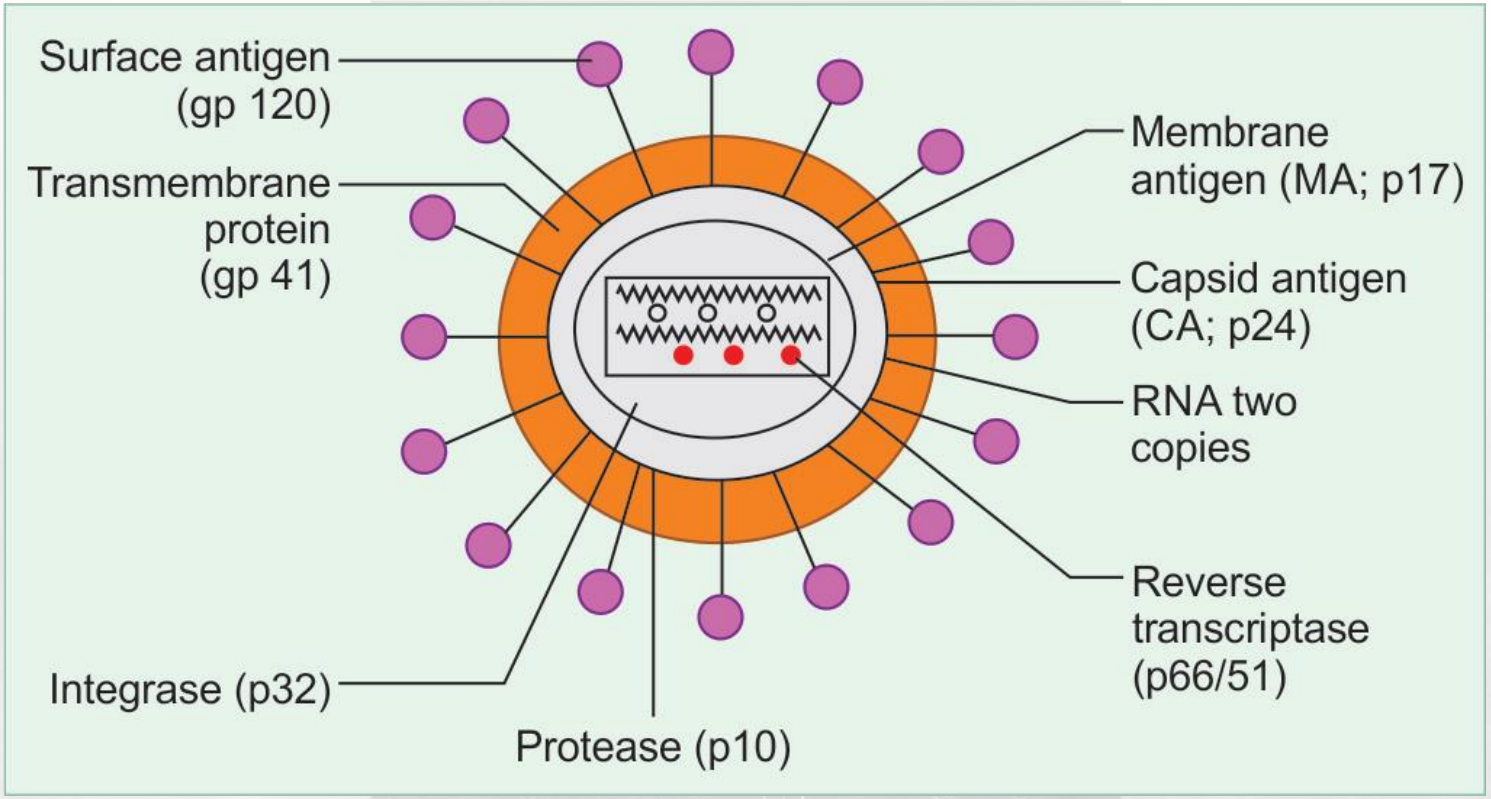
After a few months, antibodies are seen in circulation. This is called **seropositivity. T**his person is in **carrier** of the disease, and can transmit the disease to others. About 10% of seropositive individuals progress to the third stage of AIDS disease within 5 years, another 50% will manifest the disease within 10 years and about 90% will have apparent disease within state within 15 years.

Third Stage

The third stage is when the clinical manifestations start. By this time, the immune status of the individual is depressed. Therefore, commensal microbes will start multiplication inside the body. The patient usually succumbs to death within about 2 years after entering this stage.

Clinical Presentations

Lymphadenopathy and fever may be seen by the end of the second stage. The AIDS-related symptoms (ARS) are lesions in skin, gastrointestinal tract, lungs, urinary tract, and brain. Gastroenteritis and **tuberculosis** are the predominant pattern in India. In all the cases, there will be weight reduction.

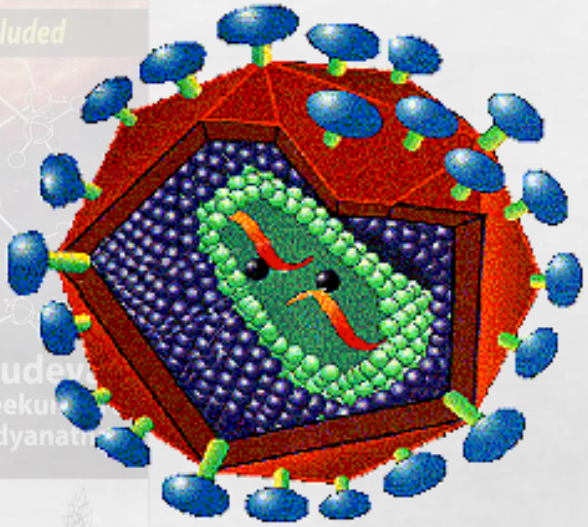


Structure of human immunodeficiency virus. (Gp: glycoprotein with molecular weight in kD; gp120: glycoprotein with mol.wt 120,000.)

HIV Genes

There are 3 structural genes (gag, pol and env), 3 regulatory genes (tat, rev and nef) and 5 accessory genes (vif, vpr, vpu, vpt and tev / tnv).

Gag → Capsid antigen (p24)
Pol → Reverse Transcriptase (p66)
Env → Surface antigen (gp120)



Virus Entry

The binding of the HIV with target cell is through a receptor. The **gp 120** of the virus envelope will specifically bind with the CD4 molecule on the surface of target cells. Thus the **CD4 acts as a receptor** for the virus.

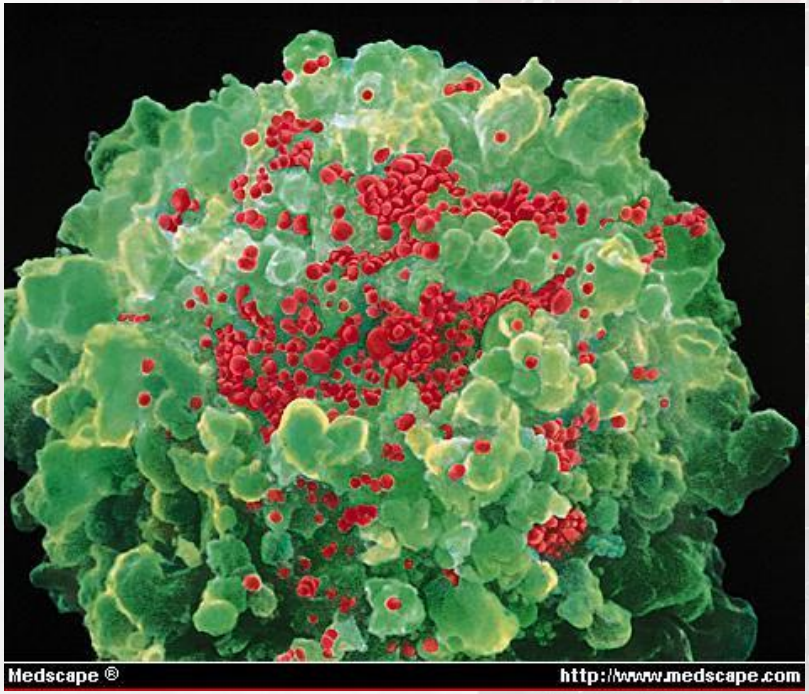
The CD4 molecules are present on the surface of the **T helper cells** and therefore helper cells are preferentially attacked by the HIV. Macrophages, monocytes, Langerhans' cells, follicular dendritic cells and glial cells are also susceptible to HIV entry and propagation. Macrophages act as the reservoir of the virus.

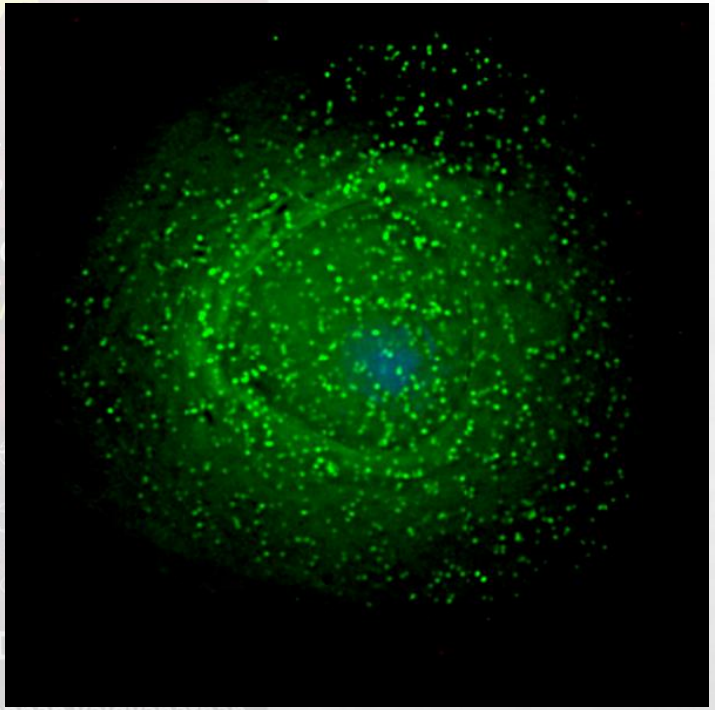
Replication of HIV

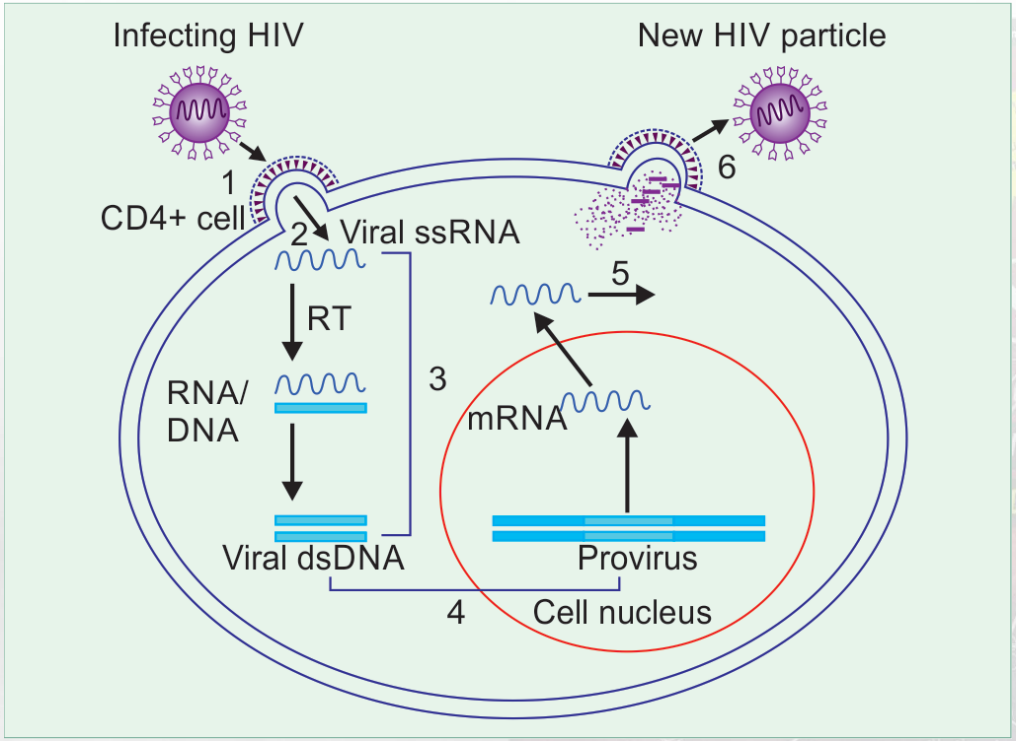
After entry, the viral RNA is acted upon by the **reverse transcriptase** (RT). Based on the RNA, a DNA strand with complementary sequence (minus strand) is produced. Then a complementary (plus strand) of DNA is synthesized. This DNA double strand migrates into the nucleus of the host cell, integrated into the host cell DNA by the action of the viral integrase (p32). HIV requires activation and division of T cells for the viral particle synthesis. The viral genes are transcribed and translated by the host cell mechanisms. The products of *gag* and *pol* genes, proteins p55, and p160, the precursor big proteins are incorporated into the virus assembly. This is then cleaved by host cell proteases into gp120 and gp41. These are then inserted to host cell membrane. Then the virions are evaginated out. Part of the host membrane, along with gp120 and gp 41 are added.

Virus Replication

The virus has an extra-ordinary rate of replication; over 10 billion particles are produced per day inside the host.







Human immunodeficiency virus replication cycle. 1 = binding of HIV through CD4, fusion, and entry; 2 = uncoating of virus; 3 = transcription; 4 = integration of viral DNA into host chromosome; 5 = protein synthesis and assembly; 6 = budding of new HIV particle. (RT = reverse transcriptase)

Replication of HIV

The gp 120 of the virus will bind with CD4 molecule.

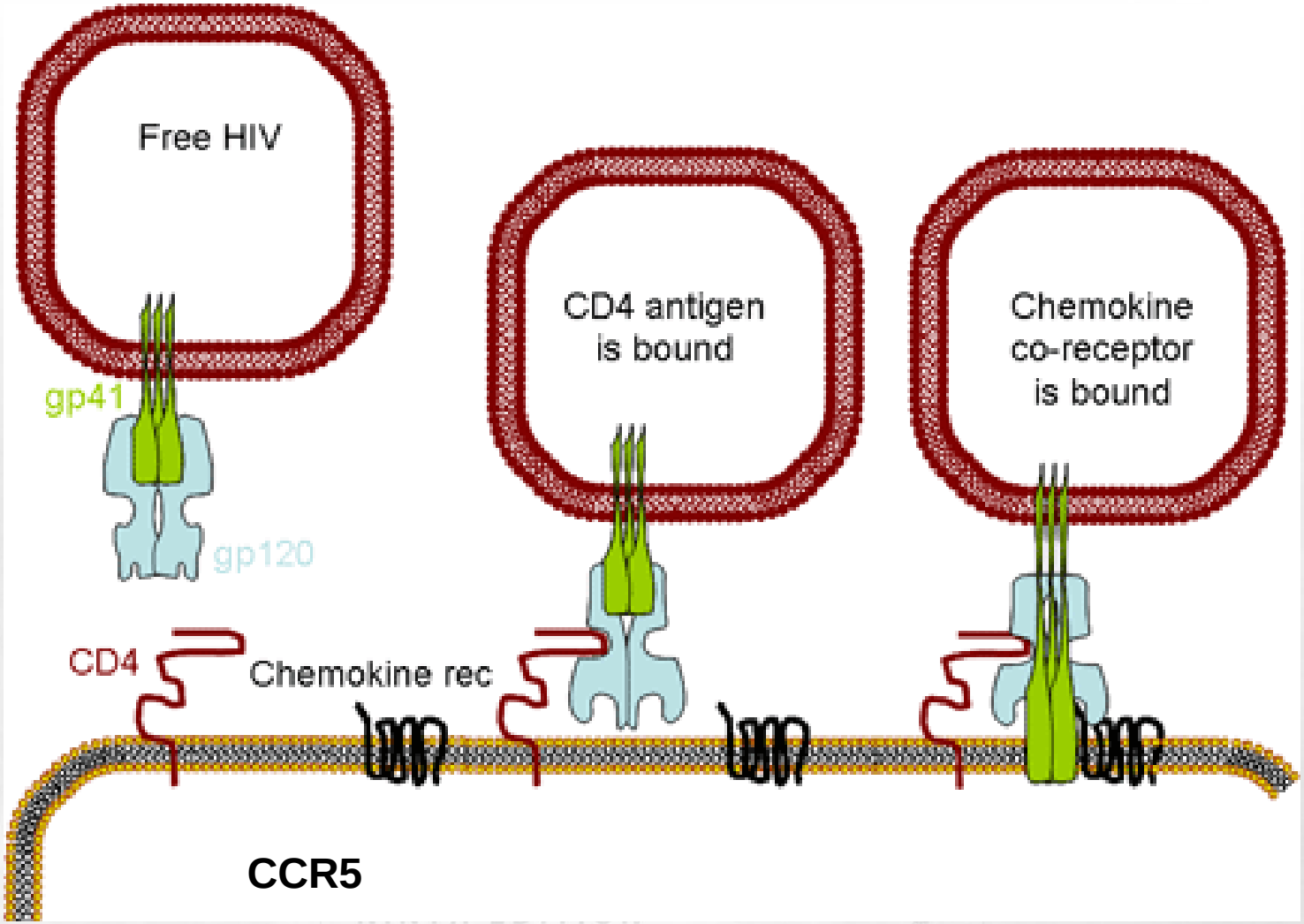
Based on the RNA, a complementary DNA is synthesised. This DNA double strand (proviral DNA) migrates into the nucleus of the host cell.

The viral DNA is then integrated into the host cell DNA. The viral genes are transcribed and translated by the host cell mechanisms.

Assembly and Maturation of HIV. Then the virions are evaginated out

Immunology of AIDS

The CD4 (T-helper) lymphocytes are decreased in number, leading to immunodeficiency. The gp120 surface unit could specifically attach with CD4 molecule present on the surface of T-helper cells. Therefore, HIV preferentially enters into the T-helper cells and they are lysed. Macrophages and monocytes act as the reservoir of HIV infection, which disseminate the virus to various organs, including central nervous system (CNS). In turn, macrophage activity is also reduced. Since T-helper cells play a pivotal role in the immunological system, their deficiency will lead to suppression of almost all the immunological effectors. The T-helper (CD4) count is < 400/cumm of blood. Antibody response is poor. Lymphokines such as interferon, interleukin-2, etc. are lowered. When all the effector mechanisms of immunity are thus paralyzed, opportunistic pathogens get entry into the body.



Cellular receptor is composed of CD4 and CCR5.

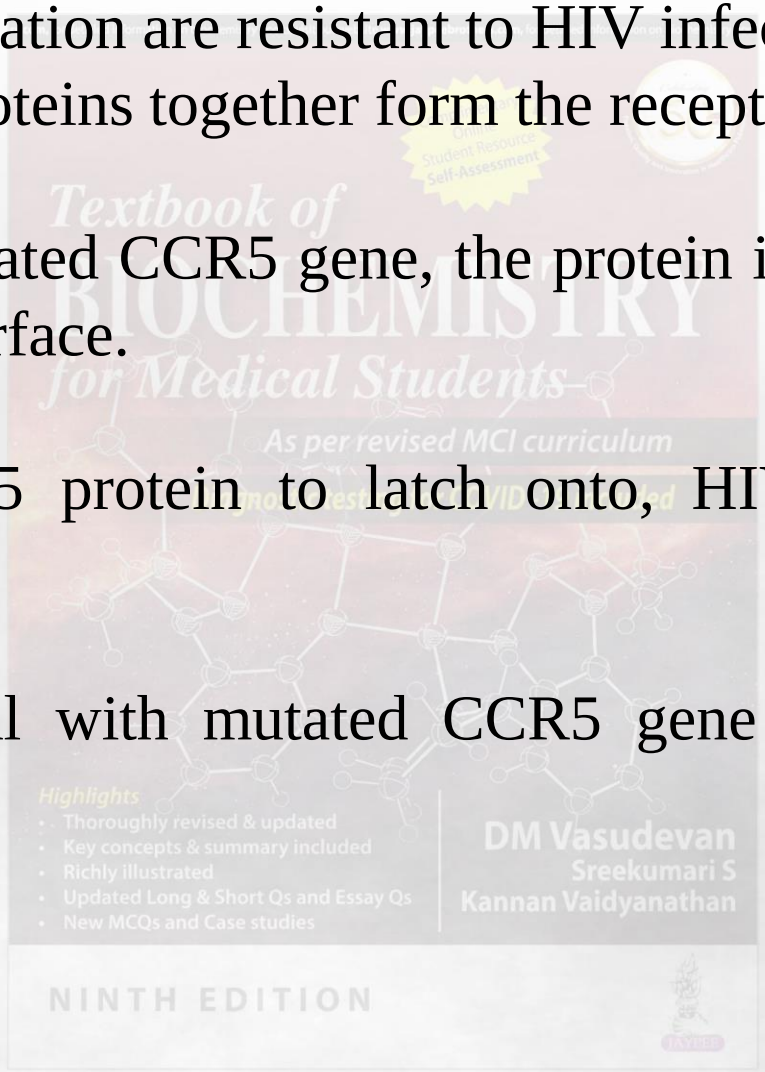
Genetic Resistance

About 1% of population are resistant to HIV infection.
CCR5 and CD4 proteins together form the receptors for HIV.

People having mutated CCR5 gene, the protein is not displayed on the macrophage surface.

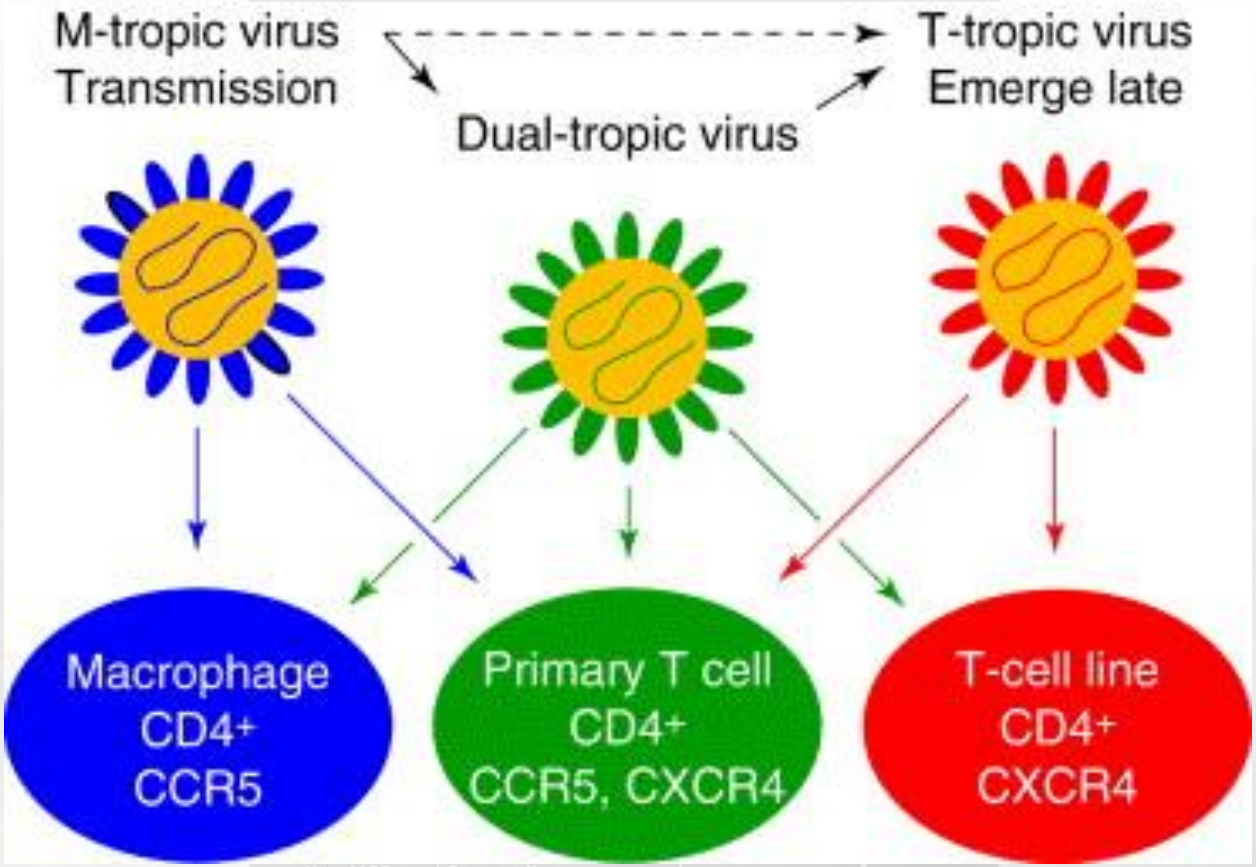
Without the CCR5 protein to latch onto, HIV fails to invade macrophages.

Thus an individual with mutated CCR5 gene escapes the HIV infection.



Immunoselection of Virus

In the early phase, the HIV enters macrophages through a **receptor** jointly made by **CD4 and CCR5** proteins on the surface of macrophages. The HIV surface antigen (gp120) has a perfect fit only for this type of receptors on the macrophage (M-tropic HIV). So in the early period, lymphocytes are spared. After a few years, the gp120 is mutated, so that dual tropic viruses are produced. In the late phases of the diseases, T-tropic HIV is generated. By this time, all the gp120 molecules are mutated, such that they can fit only with T lymphocyte receptors made up of **CD4 and CXCR4** proteins. So, the T helper lymphocytes are preferentially killed leading to the disease manifestations.

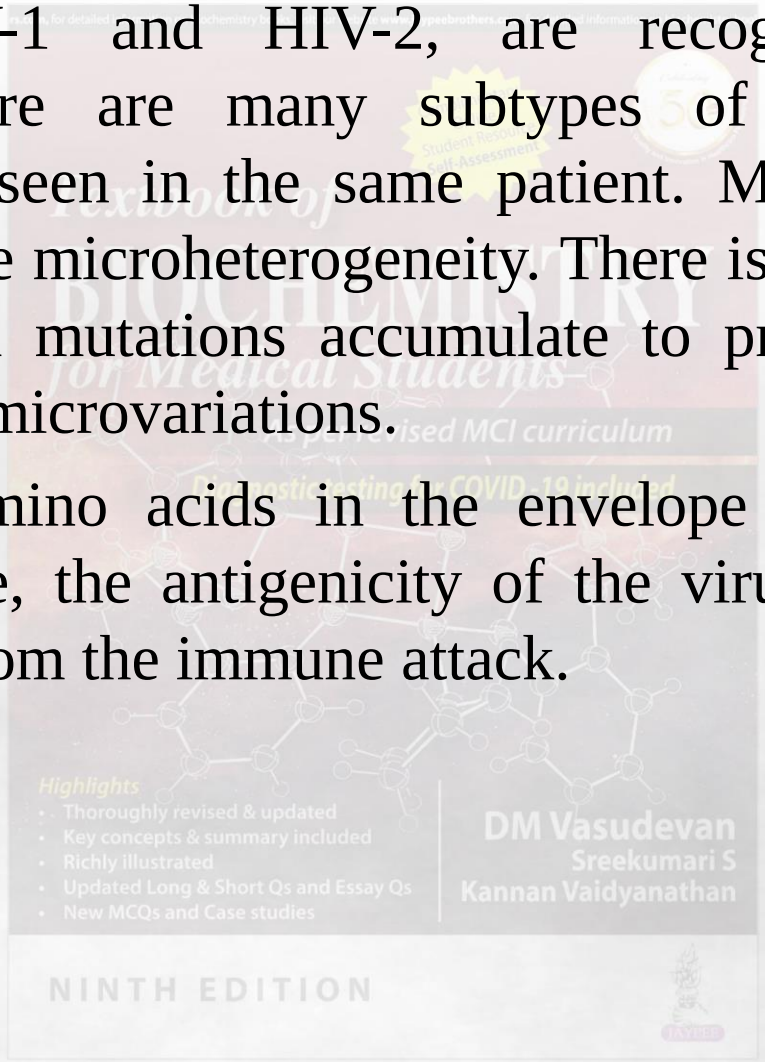


CD4 + CCR5 **CD4 + CXCR4**

Genetic Heterogeneity of Virus

Two types, HIV-1 and HIV-2, are recognized; HIV-1 is predominant. There are many subtypes of HIV-1. Different subtypes may be seen in the same patient. Moreover each type exhibits remarkable microheterogeneity. There is high mutation rate in the virus. Such mutations accumulate to produce the various types, strains, and microvariations.

About 15% of amino acids in the envelope gp120 are highly variable. Therefore, the antigenicity of the virus also varies, and virus can escape from the immune attack.



Genetic Heterogeneity of Virus

Species	Virulence	Infectivity	Prevalence
HIV-1	High	High	Global
HIV-2	Lower	Low	West Africa

Immunology of AIDS

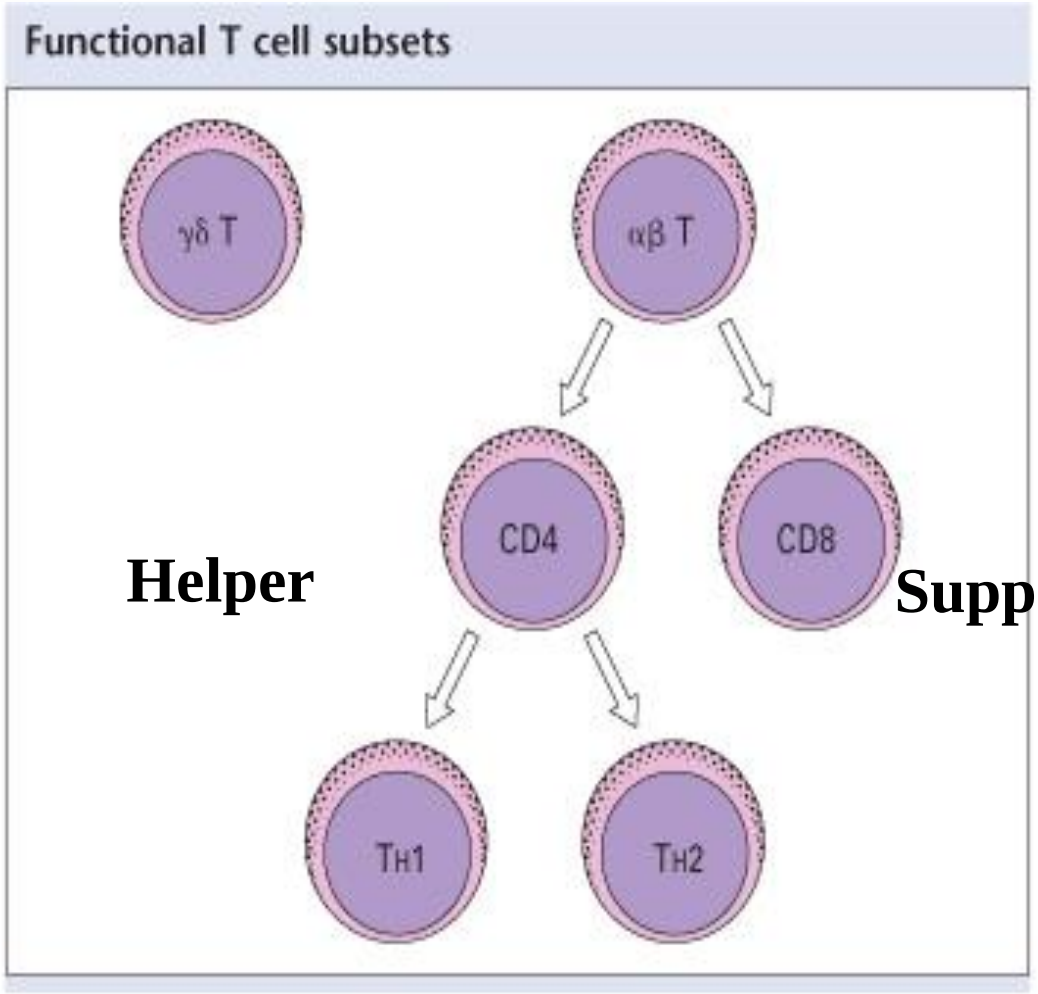
i) The CD4 (T-helper) lymphocytes are decreased in number, leading to immunodeficiency.

ii) Since T-helper cells play a pivotal role in the immunological system, their deficiency will lead to suppression of almost all the immunological effectors.

iii) T-helper (CD4) count is less than 400/cu.mm. of blood.

iv) When all the effector mechanisms of immunity are thus paralysed, opportunistic pathogens get entry into the body.

v) Macrophages and monocytes act as the reservoir of HIV infection.

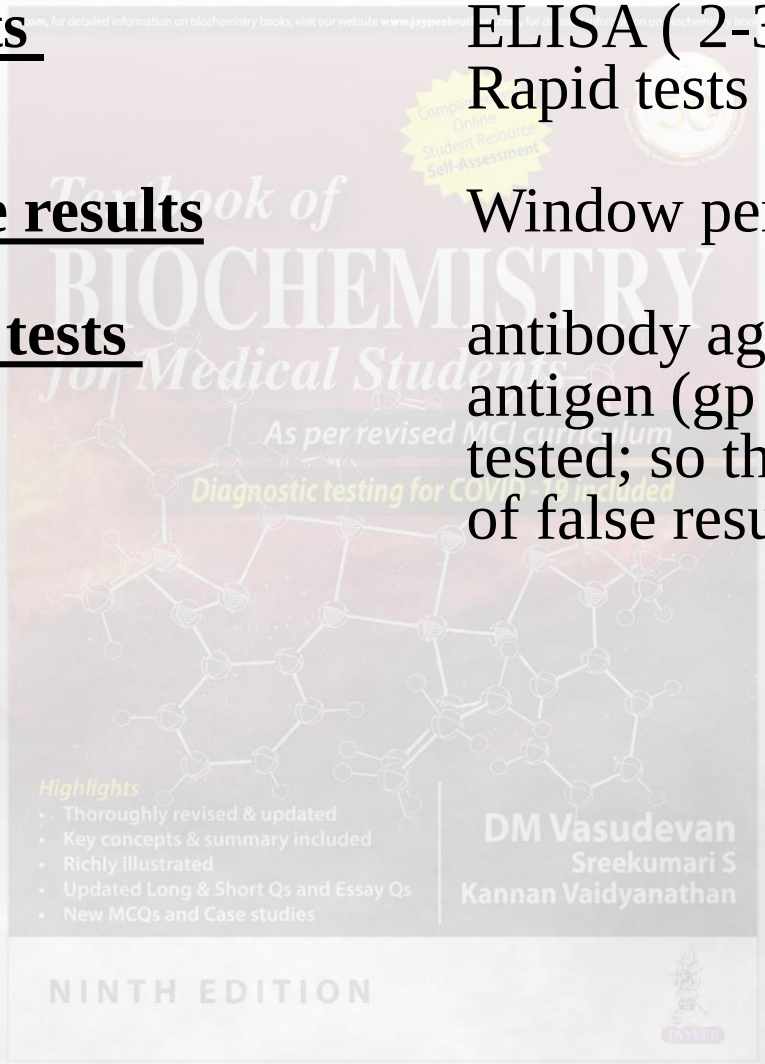


Helper

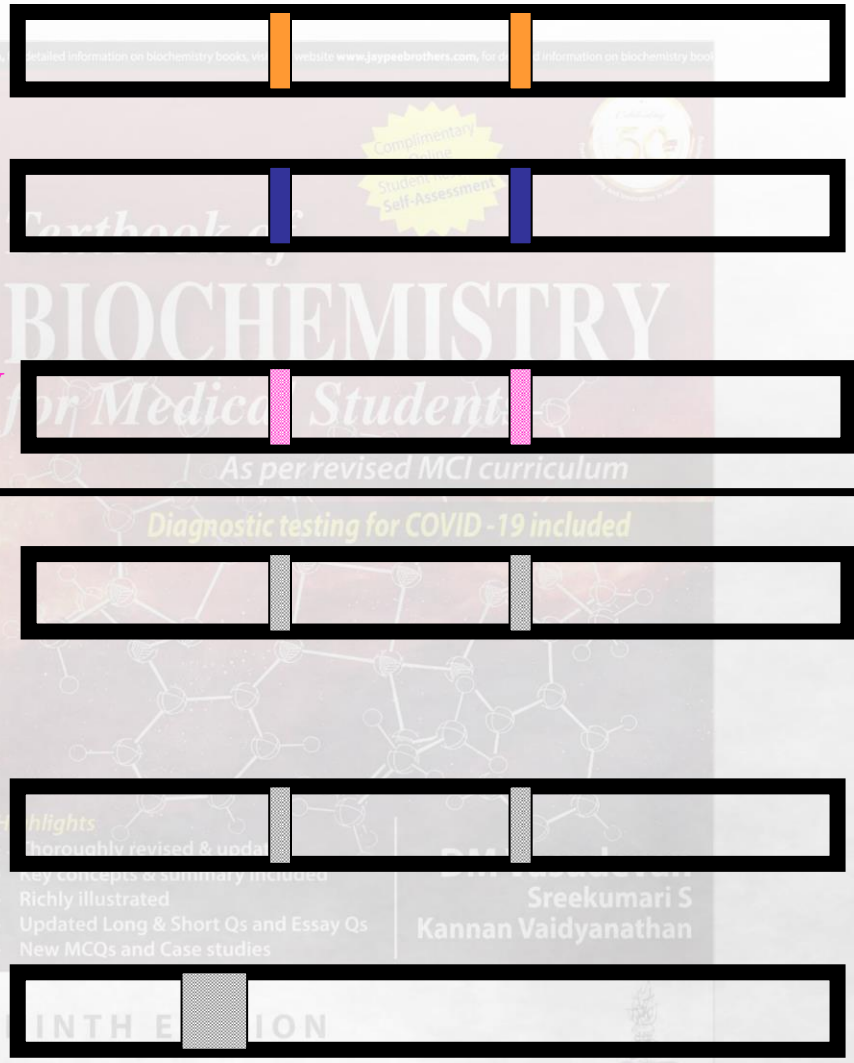
Suppressor

Lab diagnosis of HIV infection

- **Screening tests** — ELISA (2-3 hrs)
  Rapid tests (min)
- **False negative results** — Window period
- **False positive tests** — antibody against only one antigen (gp 120) is being tested; so there is probability of false results



2. Immunoblot



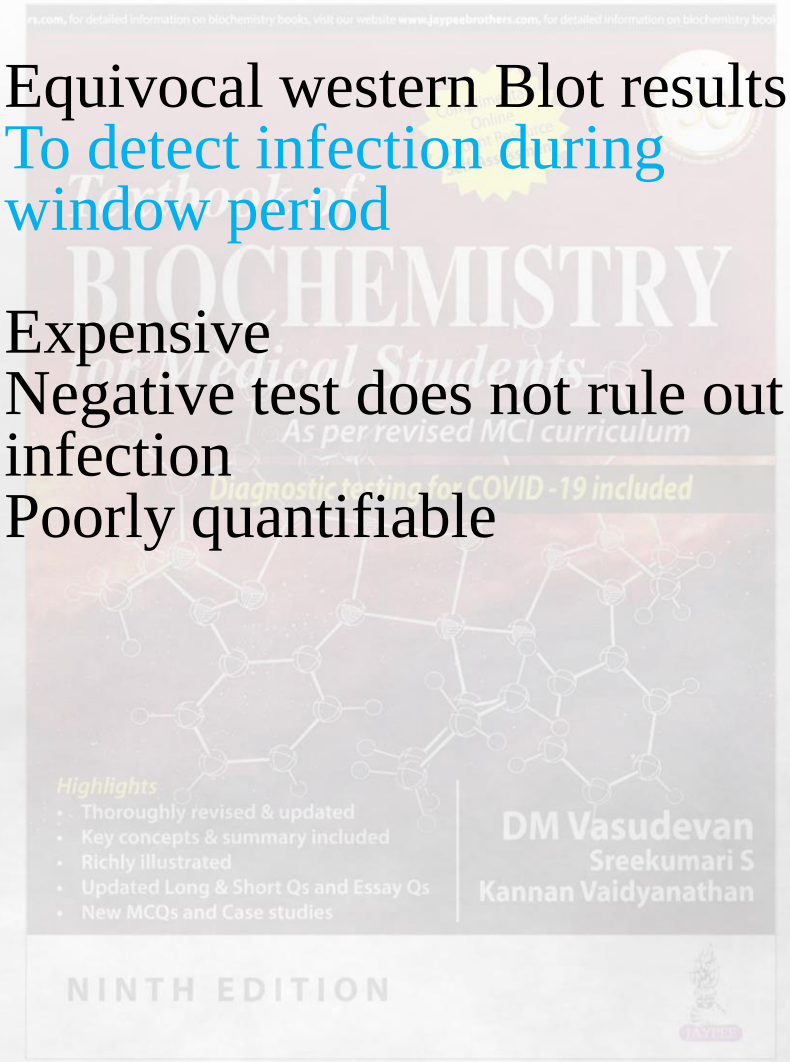
3. Detection of P24 Antigen

Useful in

1. Equivocal western Blot results
2. To detect infection during window period

Disadvantage

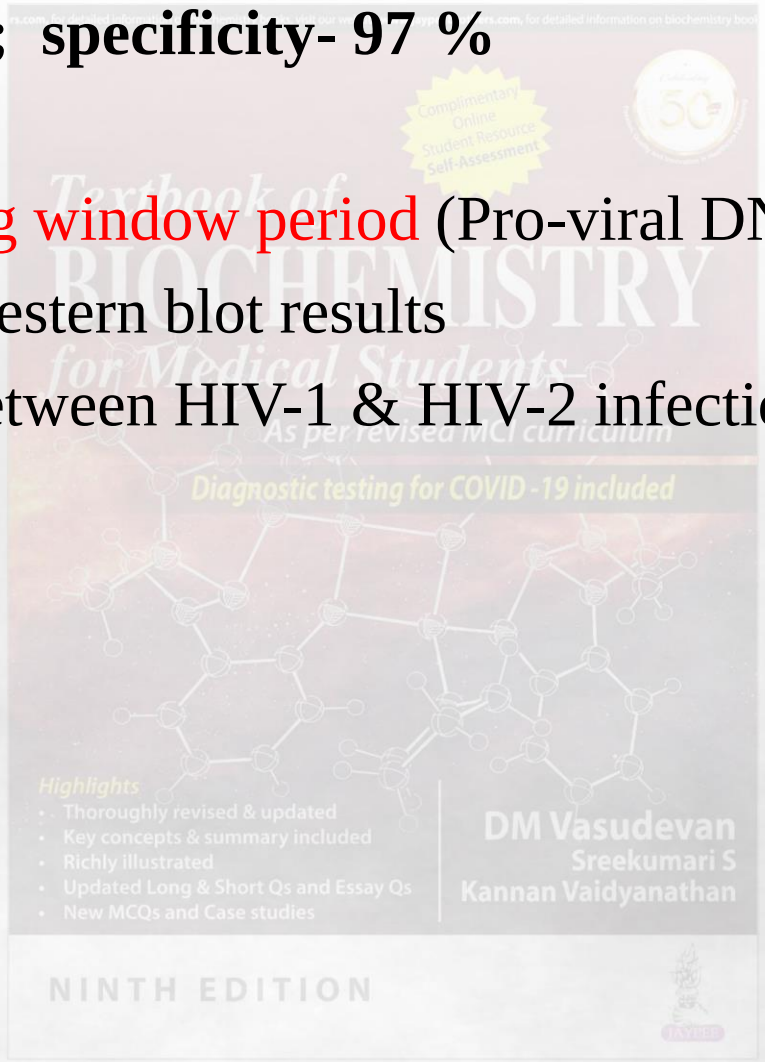
1. Expensive
2. Negative test does not rule out infection
3. Poorly quantifiable



4. Proviral DNA : HIV-DNA PCR

Sensitivity -95 % ; specificity- 97 %

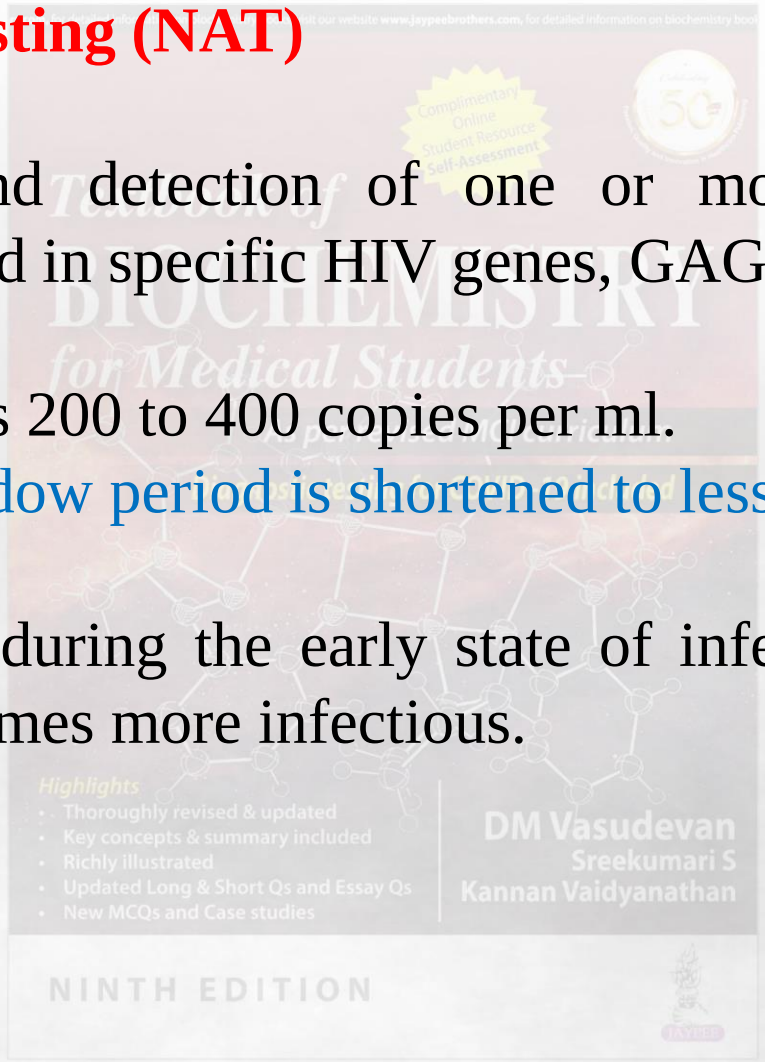
- Diagnosis during window period (Pro-viral DNA)
- Indeterminate western blot results
- Differentiates between HIV-1 & HIV-2 infections



Lab Diagnosis of HIV Infection

4. Nucleic acid testing (NAT)

- amplification and detection of one or more specific target sequences located in specific HIV genes, GAG, POL and ENV.
- Detection limit is 200 to 400 copies per ml.
  By this test, window period is shortened to less than 2 weeks.
- critical because during the early state of infection, people with HIV can be 30 times more infectious.



5. Real time PCR

Number of HIV particles in blood

Quantitation of HIV RNA in plasma (free viral load assay) helpful in monitoring progression of disease & efficacy of antiretroviral therapy

A value of less than 5000 copies per ml of blood has good prognosis, while a count more than 1 lakh per ml means very bad prognosis.

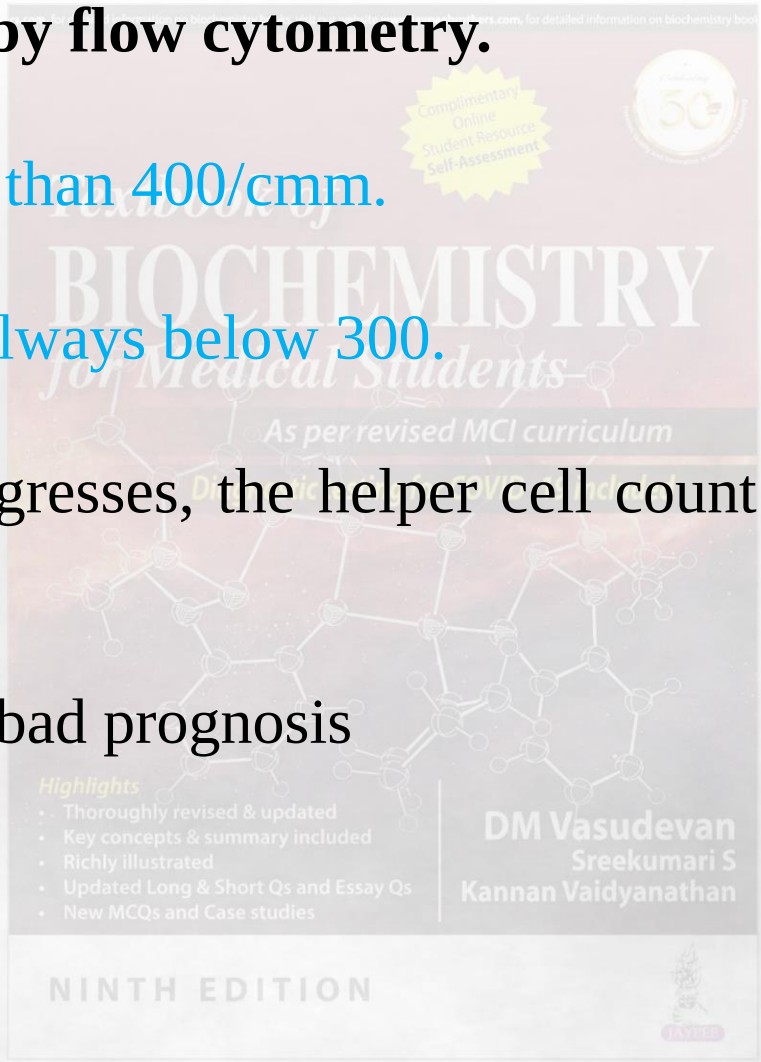
6. T-helper count by flow cytometry.

Normal level more than 400/cmm.

In AIDS patients, always below 300.

As the disease progresses, the helper cell count is correspondingly lowered.

Below 200/cumm, bad prognosis



Anti-HIV Drugs

Reverse transcriptase (RT) inhibitors; nucleoside analogs:

Dideoxynucleosides are nucleosides, where oxygen is absent from both 2' and 3' positions of the ribose group. So, they inhibit RT of the HIV. The drugs licensed for clinical use are AZT (dideoxy-thymidine, azidothymidine, Zidovudine); ddI (dideoxy-inosine, didanosine, Zidanosine); ddC (dideoxy cytidine, Zalcitabine); d4T (Stavudine), 3TC (Lamivudine), and Abacavir.

RT inhibitors; non-nucleoside analogue:

Examples are delavirdine, nevirapine, loviride and efavirenz.

Protease inhibitors:

They block final assembly and package of HIV particles. Examples include saquinavir, ritonavir, indinavir, and nelfinavir.

A combination of drugs [highly active antiretroviral therapy **(HAART)**] will reduce the virus load and the life of the patient can be extended indefinitely.

Prevention

- Since, the major method of transmission is through sexual contact, avoidance of extramarital relationships will stop the spread.
- All the blood samples should be tested for the presence of HIV antibodies before transfusion.
- All surgical instruments should be properly sterilized.
- Disposable syringes and needles are to be used and destroyed immediately after use.
- Boiling for 10 minutes will inactivate the virus.
- Ordinary autoclaving at 120°C for 20 minutes is effective to sterilize instruments and gloves.
- Blood spills can be decontaminated by washing with 1% sodium hypochlorite solution, containing 10,000 ppm chlorine.