

Module5: Other RNA viruses

Lecture 29: Double stranded RNA (dsRNA) viruses

Double stranded RNA (dsRNA) genome containing viruses infect wide range of animal as well as plants. The majority of the viruses in this group contain icosahedral capsid and similar strategies for the replication of genomic RNA. Despite similarities in the replication strategy, structure, and cognate proteins, the amino acid and nucleotide sequence identity between different genera is generally low. The diversity of proteins and genomic sequence might be the reason for the wide host spectrum of these viruses.

Table 29.1 Important members of viruses containing dsRNA genome:

Family	Host	Genome/ Segments	Genera	Structure
<i>Reoviridae</i>	Mammals, Plants, Fungi, Fish, Insects	10-12	<i>Rotavirus</i> , <i>Coltivirus</i> , <i>Orbivirus</i> <i>Aquareovirus</i> <i>Seadornavirus</i>	Icosahedral around 100 nm in diameter
<i>Birnaviridae</i>	Birds, Fish, insects	2	<i>Avibirnavirus</i> <i>Aquabirnavirus</i> <i>Entomobirnavirus</i>	Icosahedral, 75nm in diameter
<i>Chrysoviridae</i>	Fungi	4	<i>Chrysovirus</i>	Icosahedral, 30-50 nm in diameter
<i>Hypoviridae</i>	Fungi	1	<i>Hypovirus</i>	Pleomorphic, 60-100 nm in diameter
<i>Totiviridae</i>	Plants	2	<i>Varicosavirus</i>	Rod Shaped

Total of 8 families of dsRNA viruses are currently recognized by ICTV. Two most important family members are Reoviruses and Birnaviruses. These two groups contain viruses of medical as well as of veterinary importance. Rotavirus is a major cause of infant diarrhea while blue tongue virus is a big issue for cattle and sheep. Infectious bursal disease virus is another important member of family *Birnaviridae* which causes an

immunosuppressive disease in poultry and are of great economic importance. Another member of family *Birnaviridae*, Infectious pancreatic necrosis virus causes significant losses to the fisheries industry around the world.

29.1 Expression of viral proteins

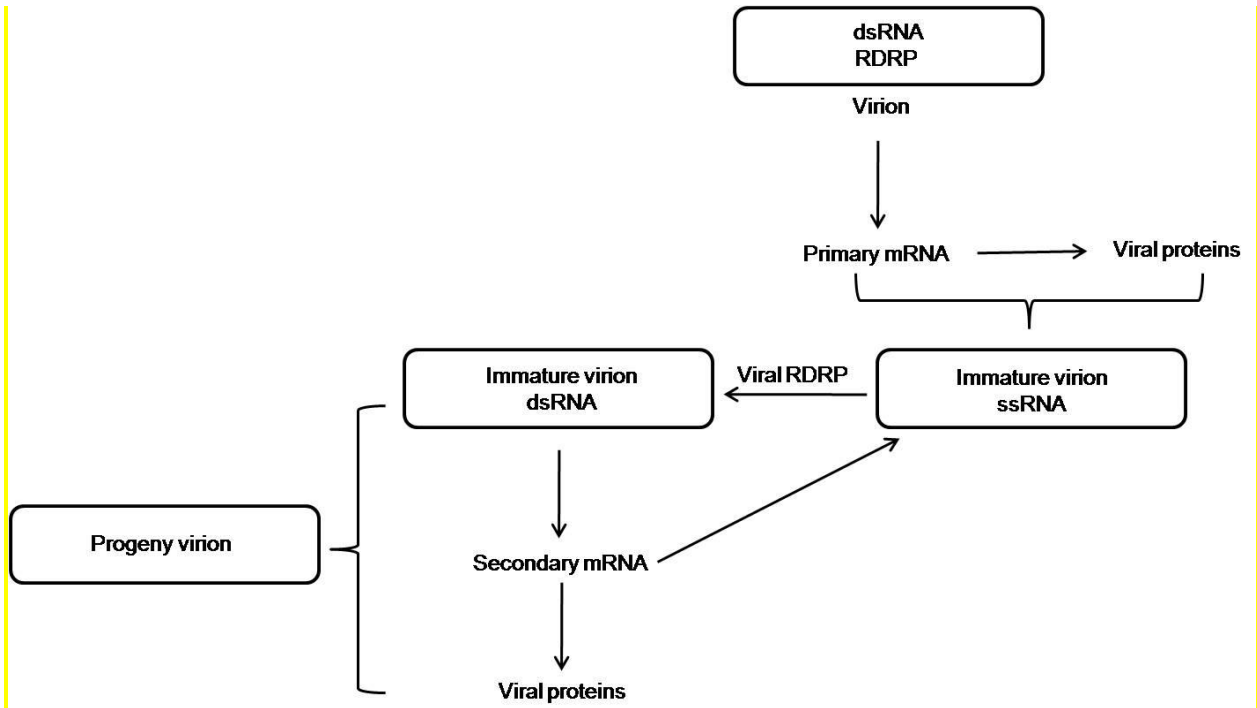
The size of dsRNA genome of the viruses is limited by the icosahedral capsid which can accommodate the segmented genome and at the same time actively allow the transcription of the viral messages. The viruses evolve in such a way to conveniently dissociate the total translation products from the genome into several distinct proteins. Sometimes individual genome segments can also be exchanged in the presence of a suitable donor or acceptor which increases the genetic diversity in virus population. The dsRNA transcribes separately to full-length +ve sense RNA. The full-length +ve sense RNA acts as mRNA for viral protein synthesis as well as template for the synthesis of genomic RNA for the progeny virions.

29.2 Replication of the genome

The replication of the genome is divided into several steps as follows

1. In the first step primary transcription of the viral genome takes place inside viral core in the cytoplasm using viral RNA dependent RNA polymerase (RDRP)
2. Positive sense RNA is then transported into the cytoplasm
3. Positive sense RNA is translated to form viral proteins
4. Positive sense RNA and viral proteins are assembled to form immature virions
5. Positive sense RNA is then transcribed to form dsRNA in virions by viral RDRP
6. dsRNA undergoes secondary transcription
7. Final assembly and maturation of virions

Figure 29.1 Replication strategy of dsRNA virus:



Lecture 30: Reoviruses

The family Reoviridae contains viruses which are most complex in nature. The term Reo stands for “respiratory enteric orphan,” which was named because the first member was identified in the respiratory and the enteric tract of animals and humans and was not associated with any type of disease. They are generally spherical in shape and have icosahedral symmetry. They do not contain any envelope. The different viruses belonging to this family have their names which are indicative of their unique morphological features. For example in case of rotaviruses, rota stands for wheel like capsid with spikes, similarly in orbiviruses, orbi stands for ring shaped capsid. Some of the reoviruses are transmitted through the bite of female culicoides (blue tongue virus) or through tick bite (Colorado Tick fever).

The family *Reoviridae* contains six genera based on group specific antigen present on VP6 capsid protein

- 1) Orthoreovirus
- 2) Orbivirus
- 3) Coltivirus
- 4) Rotavirus
- 5) Seadornavirus
- 6) Aquareovirus

Table 30.1 Diseases caused by Reoviruses:

VIRUS	DISEASE
Orthoreovirus	Respiratory and enteric diseases
Orbivirus	Blue tongue fever in cattle and Sheep African Horse Sickness disease
Coltivirus	Colorado Tick fever
Rotavirus	Gastroenteritis and Diarrhoea
Aquareovirus	Diseases of Fish

30.1 Virion Property

Reovirus particles are non-enveloped, spherical having a diameter of approximately 85 nm. Genome contains a linear dsRNA divided into 10-12 segments. The overall genome size varies from 16 to 29 Kbp. They contain a cap at their 5' end while poly A tails are absent from 3' end.

30.2 Virus Replication

Virus replication takes place in the cytoplasm of the cell. Because of the segmented RNA genome chances of reassortment of genomic segments between different strains is very high. This results in genetic drift and shift leading to diversity among viruses which is reflected by numerous serotypes within each genus.

Virus enters the cell by receptor mediated endocytosis. The coated vesicle uncoats and fuses to the lysosomes under low pH condition. Virions are disrupted and viral inner core is released into the cytoplasm. Virus associated RNA polymerase utilizes the negative strand of each dsRNA segment as template to transcribe viral mRNA. These viral mRNA's are translated to form viral structural proteins that eventually assemble to form the infectious virion. Progeny viruses remain cell associated but are often released by lysis of the infected cell. Genomic RNA replication takes place within sub-viral particles in the cytoplasm of infected cells.

30.3 Important Reoviruses

30.3.1 Rotavirus

Rotaviruses are the most important human pathogens which lead to life threatening diarrhoea in young ones. It was first isolated from a children hospital in Australia. Rotavirus infection leads to destruction of intestinal villi. Transmission of the virus mainly takes place by fecal-oral route. Destruction of enterocyte (intestinal cells) causes mal-digestion and poor absorption of food. Intestinal epithelium of young ones has a slow turnover rate and that is the reason that rotavirus infection is more severe in case of infants. Rotavirus infection is characterized by severe diarrhoea, dehydration, weight loss

and fatigue. Virus can be isolated in faeces in high amount. Enzyme immunoassay is commonly used to detect viral infection in infected individuals. Genetic reassortment vaccines are available for rotavirus infection.

30.3.2 African Horse Sickness

African horse sickness is caused by a member of genus orbivirus. It is a pantropic and fatal disease of horses, predominantly infecting endothelial cells and myocardium. Acute form of the disease is characterized by pneumonia, interlobular pulmonary oedema, pericarditis, haemorrhages and oedema of the visceral organs. The death can occur within 5 days in highly acute form of the disease. A more prolonged form of the sickness involves the **cardiac vascular system**. Mortality can be more than 80% depending on the immune status of the animal and virulence of the isolate. **Subclinical disease** can occur in donkeys and vaccinated horses. Diagnosis is usually carried out by using complement fixation tests and haemagglutination inhibition tests.

30.3.3 Bluetongue Virus

It is another important disease of livestock caused by a member of genus orbivirus. It causes high mortality in sheep and decrease in productivity of other farm animals. The virus is transmitted to the animals by the bite of *Culicoides* mosquitoes. The chances of outbreaks are more during the breeding season of *Culicoides*. Major signs of the disease include high fever, swelling of the face, hyperemia around the coronary band and **cyanosis of the tongue** (blue colour of the tongue). The disease can be restricted by controlling mosquitoes while polyvalent live vaccines are available for the animals.

30.3.4 Colorado Tick fever

Colorado Tick fever is a disease caused by bite of tick (*Dermacentor andersoni*) infected with Coltivirus. Transmission of this disease is reported to be through blood transfusion from an infected individual. The virus usually infects and replicates in the erythrocyte. The symptoms of the disease include fever, headache, vomiting, abdominal pain, and encephalitis.

Lecture 31: Retroviruses: structure, classification, life cycle

Retroviruses are single stranded RNA viruses that undergo DNA phase during their replication. All the retroviruses contain reverse transcriptase enzyme and are capable of integrating with the host genome. Currently retroviruses are divided into 7 classes based on their biological and molecular properties. In general they are classified into three categories –

- I. Oncoviruses
- II. Lentiviruses
- III. Spumaviruses

I. Oncoviruses

These viruses are associated with one or other form of cancer in different animal species. Some viruses carry the oncogenes as part of their genome e.g Rous sarcoma virus, Human T lymphotropic virus (HTLV).

II. Lentiviruses

These viruses have long incubation period and are associated with immune deficiencies. Occasionally encephalopathy and arthritis may also be evident eg human immuno deficiency virus (HIV), feline immuno deficiency virus (FIV), and simian immuno deficiency virus (SIV).

III. Spumaviruses

Spuma stands for foamy. The virus causes vacuolation and froth like appearance in infected cells.eg simian foamy virus (SFV).

31.1 General Concept

- a. Out of three subfamilies of Retroviruses two infect humans namely oncornaviruses (Human T Cell Lymphotropic Viruses (HTLV) I, II, and V) and lentiviruses (HIV 1, HIV 2).

HTLV-I causes cutaneous T-cell lymphomas,

HTLV-II causes hairy T-cell leukemias,

HTLV-V causes T-cell lymphomas and leukemias.

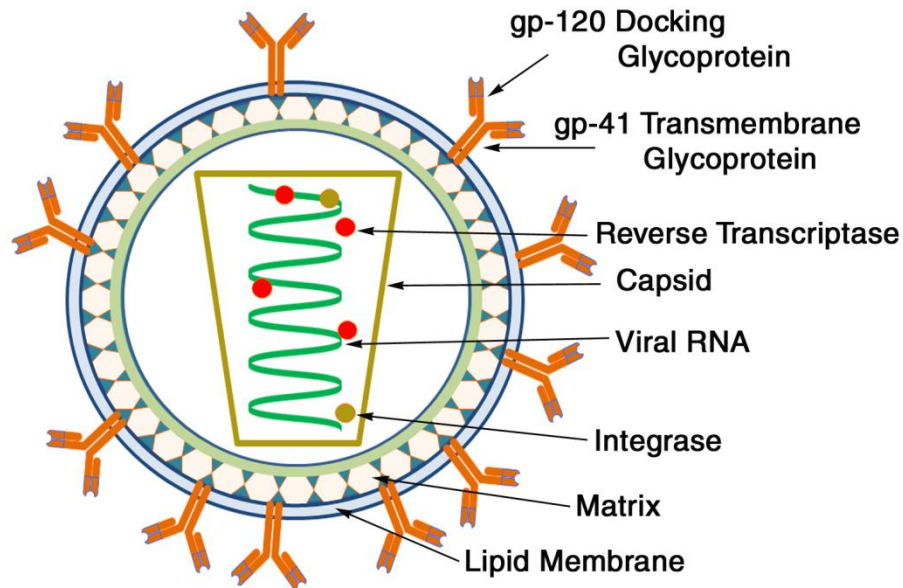
HIV-1 causes Acquired Immunodeficiency Syndrome (AIDS),

- HIV-2 causes AIDS related syndrome in Africa.
- b. Retroviruses contain RNA genome and an enzyme called reverse transcriptase, which makes circular DNA by using RNA as a template. The viral DNA integrates with the host cell chromosomes.
 - c. HIV is an enveloped virus which contains glycoprotein 120 (gp120) which binds to CD4 receptor present over the T helper cell.
 - d. Genome of HIV composed of two positive strands RNA which are capped at 5' end and polyadenylated at 3' end.
 - e. The tRNA acts as a primer for the synthesis of DNA using RNA as a template.
 - f. Long terminal repeats (LTR) present towards both the ends help in the integration of viral genome to host genome. It also serves as promoter and enhancer for the viral genome.
 - g. Group specific antigen (**gag**) helps in the formation of capsid protein. Polymerase (**pol**) encodes message for the formation of reverse transcriptase. Envelope (**env**) gene is associated with the formation of gp120 and gp41. **tax/rex** are the regions which encodes the factors involved in transactivation and other regulatory functions.

31.2 Morphology

Retroviruses are spherical and approximately 100 nm in size. The virion contains cone shaped nucleocapsid that encapsidates two copies of single stranded RNA about 10 Kb in size. In addition, capsid also contains three enzymes namely reverse transcriptase, proteases, and integrase. The nucleocapsid is covered by matrix protein which in turn is covered by an envelope containing two transmembrane glycoprotein called gp120 and gp 41.

Figure 31.1 Schematic representation of human immuno deficiency virus (HIV):



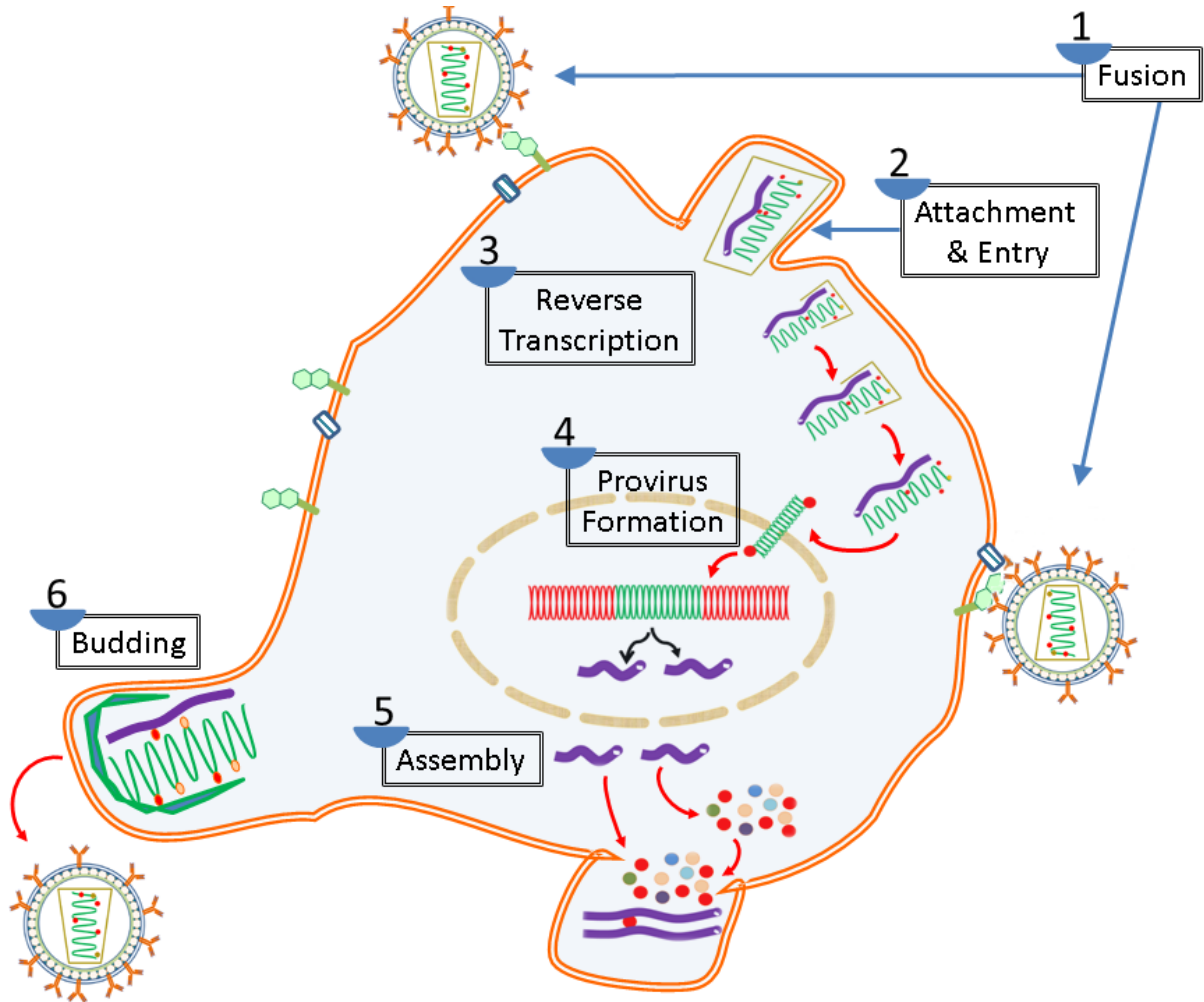
31.3 Virus entry

HIV enters the host cell by infecting the T- helper cells and macrophages which contain CD4 receptor. The viral gp120 binds to CD4 receptor to initiate the entry process. Coreceptors such as chemokine receptors (CCR5 and CXCR4) also assist in entry process. Different HIV serotypes use different coreceptors. Following the binding, the virus and cell membrane fuses releasing the nucleocapsid inside the cell.

31.4 Virus replication

Many of our present days understanding about the retrovirus comes from the work of HIV. Upon entry to the cells, viral RNA produces a polyprotein which is cleaved by virus encoded proteases to form individual protein. Virus uses the tRNA as a primer to synthesize DNA using reverse transcriptase enzyme. RNA from the hetroduplex form of RNA and DNA is then cleaved by RNase H. The remaining part of DNA is synthesized as double stranded and integrates into the host cell genome with the help of enzyme integrase. The viral genome remains integrated into the host genome and keeps on transcribing the viral RNA for the progeny virions. The progeny virions are assembled in the cytoplasm and exit by the process of budding taking the covering of the host cell membrane.

Figure 31.1 Schematic representation of HIV replication:



31.5 Endogenous Retroviruses

Genome of many vertebrate animals contains retroviral sequences. These sequences are mostly inactive or defective. On an average a normal human cell contains approximately 1, 00, 000 retroviral sequences. It is likely that the retroviruses can infect the germ lines early in the life, giving rise to these sequences in the human genome.

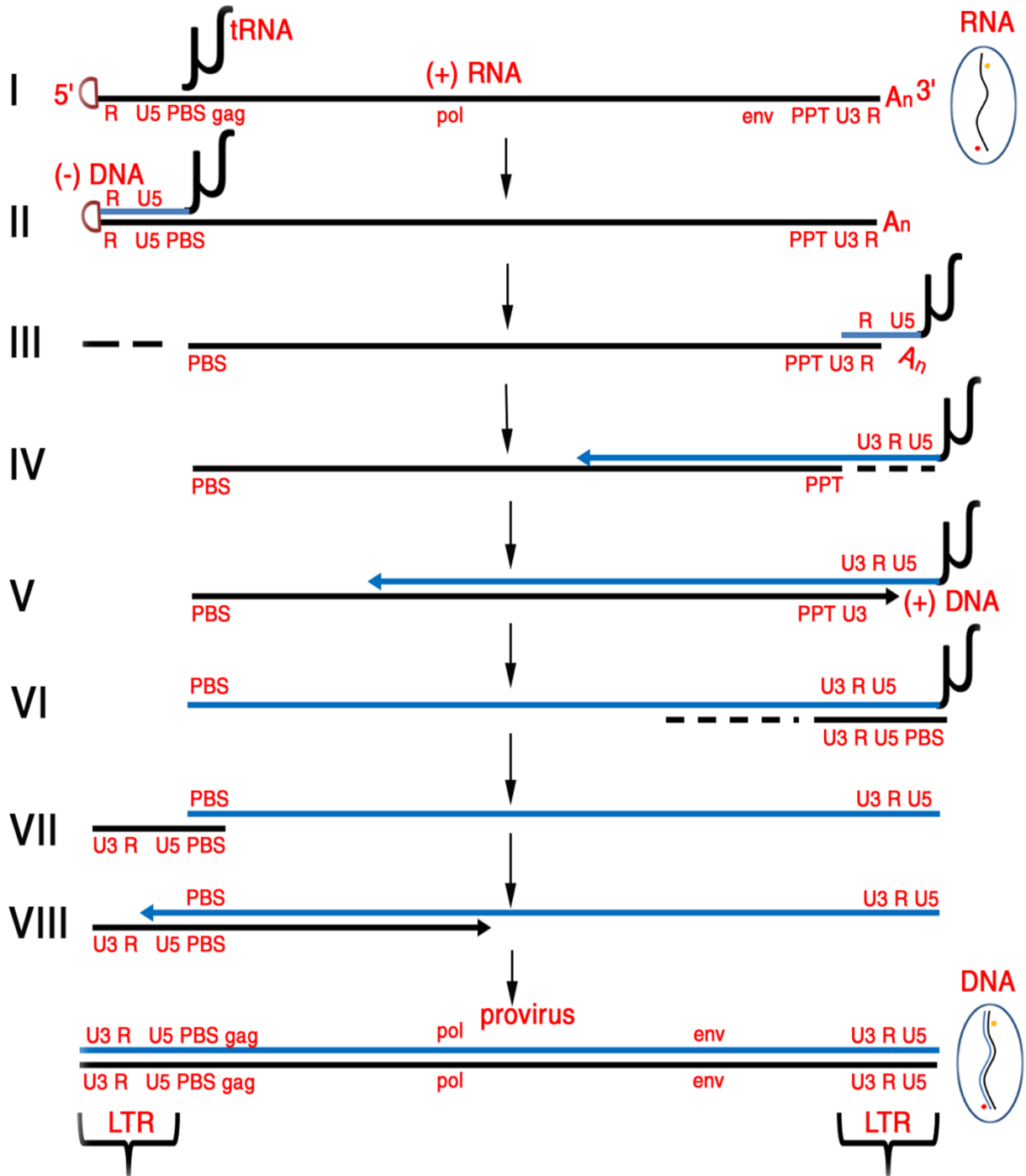
Lecture 32: Reverse transcription

The virus binds to the cell surface with the help of surface glycoproteins present over the envelope. This interaction leads to change in the configuration of the proteins which allows the fusion of virions to the host cells. The virus releases its content into the cytoplasm and a reverse transcription complex is formed in order to complete the genome replication.

32.1 Reverse transcription

The phenomenon of reverse transcription takes place in the reverse transcription complex. **Cellular tRNA** acts as a primer which binds to the 5' end of the RNA to form negative sense DNA. **Polypurine tract (PPT)** present on the RNA acts as a primer for the synthesis of positive sense DNA. **RNase H** enzymatic property of the reverse transcriptase helps in the cleavage of RNA from a RNA-DNA heteroduplex. The DNA formed because of the reverse transcription is called as **PROVIRUS**. The DNA of the provirus is longer than the parental genomic RNA. The 3' end of the genomic RNA contains unique region called as U3, similarly, 5' end also contains U5 as unique region. The provirus contains an extra U5 along with U3 and similarly extra U3 with U5 (reason for longer length of provirus). The provirus contains U3- Repeat (R)-U5 sequence at both the ends which is also called as long terminal repeats (LTR).

Figure 32.1 Schematic representation of the complete reverse transcription process:



Step1- A cellular tRNA binds to the primer binding site (PBS) in the RNA genome.

Step2- Negative sense DNA is synthesized towards 3' end with the help of virus reverse transcriptase.

Step3- RNase H digests the RNA from RNA-DNA hetroduplex. First jumps of the negative strand DNA occurs towards 5' end.

Step4- Negative strand DNA continues to elongate and RNase H digest the RNA- DNA hetroduplex till PPT.

Step5- Synthesis of positive strand DNA begins from PPT towards 5' direction.

Step6- All the remaining RNA degraded by RNase H.

Step7- Second jump occurs were positive sense DNA move from 5' end and binds to the 3' end of the negative sense DNA.

Step8- Synthesis of remaining strand of the DNA completed.

32.2 Integration of the provirus

Newly formed provirus associated with viral integrase is then migrated to the nucleus. The migration of the provirus usually occurs at the time of mitosis because of the fragility of the nuclear membrane at the time of cell division. The provirus with integrase is referred as pre-integration complex. Once inside nucleus, the viral integrase cuts the host chromosome and ligates the provirus into the gap. The provirus may express immediately or may be inactive in case of latent infection. When cell starts dividing the provirus is also copied into the daughter cells along with the host chromosome.

32.3 Transcription

The transcription of the provirus starts from the LTR sequence with the help of cellular RNA polymerase II. The transcription begins at U3-R junction and terminates at the R-U5 junction. All the transcripts are capped and polyadenylated. Some of these act as mRNA and some as a progeny genome (Fig 32.2). LTR present on the viral genome act as the promoter and other cellular transcription factors help RNA polymerase II to transcribe the RNA. Transcription produces mRNA's similar to that of genomic RNA that are spliced at different locations to form different mRNA subspecies. Without spliced mRNA forms gag and pol, one time spliced to env mRNA, and two times to TAT, REV and NEF mRNA's. During early phase of transcription only double spliced

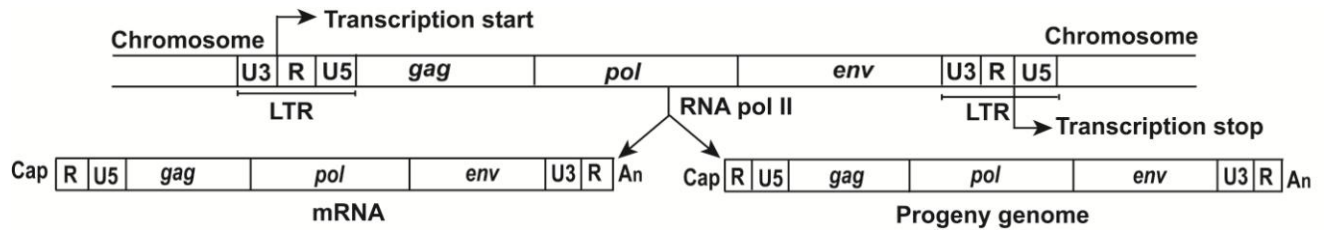
mRNA's are formed which increases the level of TAT, REV and NEF in the cell. TAT acts as transcription enhancer and REV helps in the export of spliced mRNA.

TAT stands for "Trans-Activator of transcription".

REV stands for "Regulator of Virion Expression".

NEF stands for "Negative Regulatory Factor".

Figure 32.2 Transcription process in the integrated provirus:



32.4 Translation, Assembly and Release

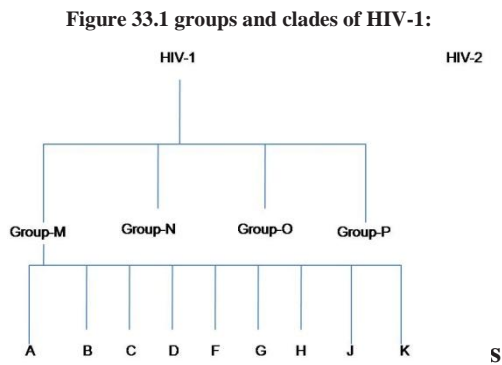
The envelope proteins are synthesized by the “env” gene following splicing. The proteins (gp 120 and gp41) become glycosylated in the endoplasmic reticulum and transported through Golgi apparatus to the surface of plasma membrane. Gag and Pol are produced as polyprotein which later on are cleaved by viral proteases to form individual protein. The virus usually needs more of Gag protein as compared to Pol, hence, the synthesis of Gag protein is always towards the higher side. The envelope proteins in retroviruses are glycosylated while the Gag and Pol proteins are myristylated. Virus assembly takes place at the plasma membrane. Viral protein also migrates to the membrane where genomic RNA interacts with viral protein and gets assembled. Many viral polyproteins undergo cleavage and rearrange after budding through plasma membrane.

Lecture 33: HIV- viral pathogenesis

Human immunodeficiency virus (HIV) -1 and -2 was thought to evolve from simian immunodeficiency virus (SIV). Both of these viruses emerged during 18th century and prevalence of HIV-1 infection is far greater than HIV-2. The virion has a general features of other retroviruses (Please refer lecture 31 and 32). HIV-1 contains on an average 14 surface glycoprotein (gp120 and gp41) over its virion. In contrast, the glycoproteins in HIV-2 are composed of gp130 and gp38 and other internal proteins similar to HIV-1. The genome of HIV is around 9.6kb in size and contains all the essential genes for the expression of viral proteins (Please refer lecture 31 and 32). HIV infects the CD4+ T helper cells and macrophages. Apart from binding with cell receptor (CD4), the HIV also requires co-receptors such as chemokine receptors **CXCR4** (α) and **CCR5** (β). HIV strains which uses CXCR4 are known as **X4** while those using CCR5 are called **R5**. The use of chemokine receptor by HIV largely determines the tropism of the HIV. T-cell tropic strain of HIV uses CXCR4 co-receptor while macrophage tropic strain of HIV uses CCR5 as a co-receptor.

33.1 HIV-1 and -2 clades or subtypes

HIV-1 is divided into four groups M, N, O and P. Group M is divided into nine different clades based on the sequence alignment of gag and env gene. Clades A, C, D, and E are responsible for high incidence rate of HIV.

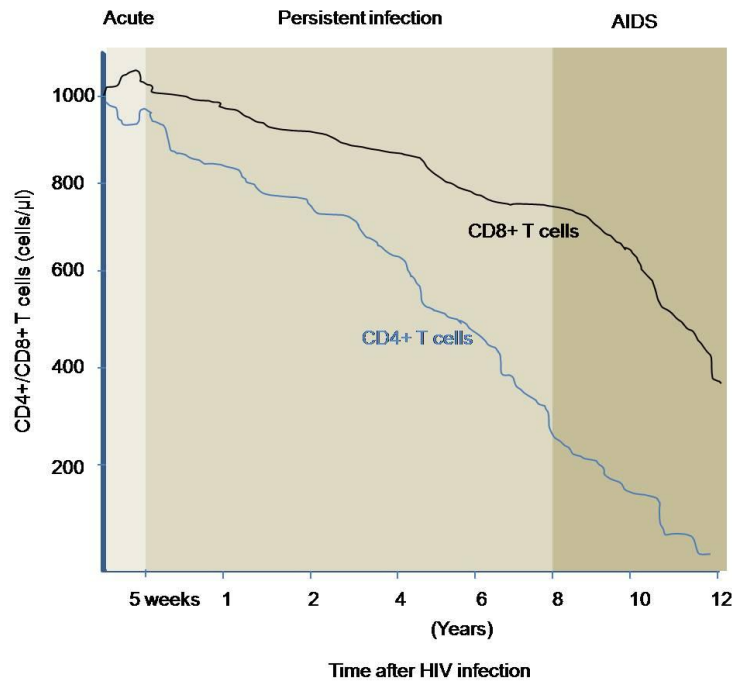


33.2 Primary HIV infection

Viruses infect the macrophages during the early phase of infection through binding with CCR5. The virus is then transferred to the dendritic cell with the help of DC-SIGN (dendritic cell specific ICAM-3 grabbing nonintegrins) receptor present over the surface of dendritic cells. Dendritic cells then deliver the virus to the draining lymph node where

it replicates and causes viremia after their dissemination into the blood circulation. As the disease progresses the body produces an immune response which reduces the viral load in the body. The virus set point is reached after 6 months of initial infection and persists till many years. 50-90% of the infections are symptomatic which occurs after 5-30 days post exposure.

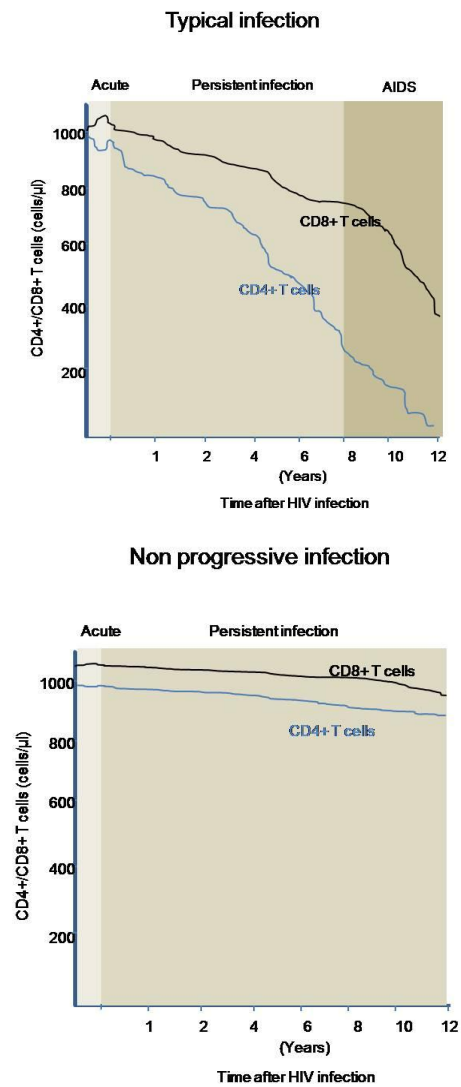
Figure 33.2 Graphical representation of HIV infection and its progression:

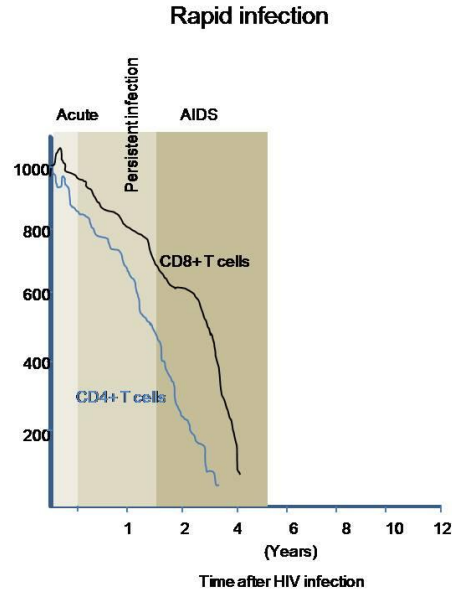


33.3 Established HIV infection

Viruses actively replicate throughout the course of the disease. The virus replicates outside the CD4+ cells in gut associated lymphoid tissues, central nervous system, and genital tracts. Around 10^{10} virus particles are made and destroyed everyday during the course of the disease. The half life of a mature HIV virion is around 30 min to 6 hrs in the plasma of infected patient.

Figure 33.3 Type of HIV infection towards host susceptibility:





33.4 HIV and Cancer

HIV-1 infection leads to a variety of cancers in the infected individuals. This may occur because of immune dysfunction and absence of proper immune cells for surveillance inside the body. Generally this causes the replication of many oncogenic viruses. In addition, unregulated level of cytokines can cause proliferation of the cells and angiogenesis.

Lecture 34: Acquired immuno deficiency syndrome (AIDS)

World AIDS day is celebrated every year on 1st of December.

Approximately 35 million people are affected with HIV worldwide. In India around 2.5 million people are suffering from HIV while in US more than one million people are suffering. There is decline in HIV infectivity in India as well as other parts of the world during the past 10 years. About 80 percent of the AIDS cases are reported in men between the age group of 20 to 44. In general males, percentage varies from 78 to 80 percent and the female around 20 to 22 percent. Nearly 1.8 percent of the total AIDS cases are children born to HIV infected mothers. In HIV infection an infectious dose means presence of ten thousand or more particles in the body. The level of HIV particle in different body fluids varies.

“AIDS is defined as HIV positive patient with CD4+ count less than 200cell/mm³ or CD4+ count less than 14% of total lymphocyte population”.

34.1 Transmission of HIV –

There are three major ways of HIV transmission

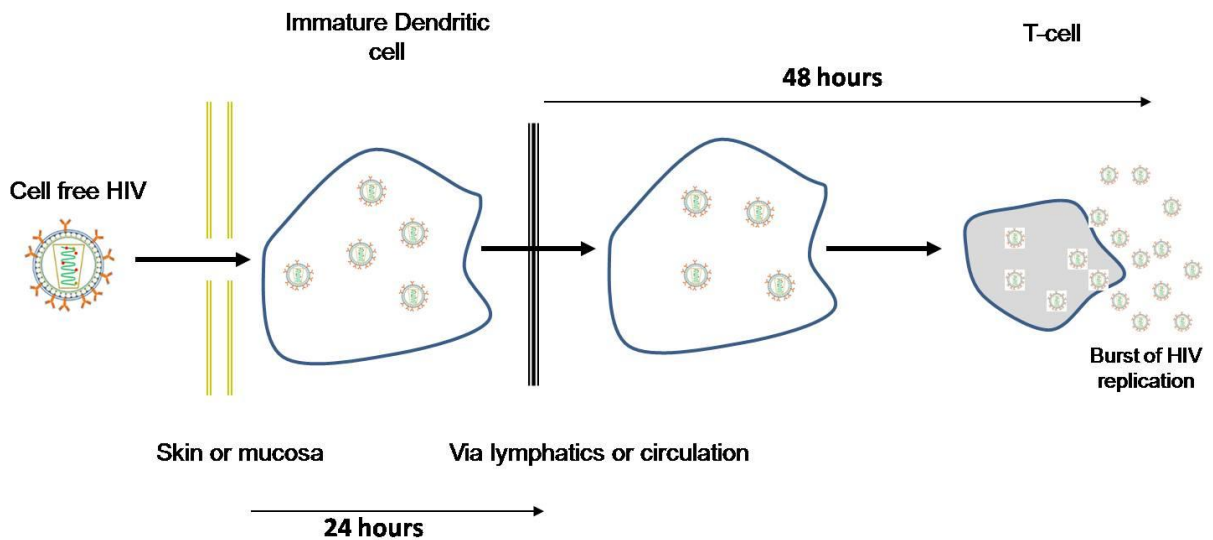
- 1) Sexual interaction
 - 2) Blood transfusion
 - 3) Perinatal infection
- 1) Sexual interaction - Risk of transmission of HIV is higher if person is infected with other sexually transmitted disease (STD) eg. herpes, syphilis, Gonorrhoea etc. Passive partner is always in the higher risk side. This is more established way of HIV transmission.
 - 2) Blood transfusion – HIV transmission is also possible by the way of infected blood, syringes, needles and other body fluids including saliva. Since now blood donors are screened first for HIV, the number of HIV cases decreased to a greater extent.

- 3) Perinatal infection – Young born children get infected if the mother is infected with HIV. There are several theories established regarding transmission of HIV through placenta or during delivery. Many studies have suggested involvement of breast milk for the transmission of HIV.

34.2 Early Phase of HIV Infection

Decrease in the number of circulating CD4+ lymphocyte is the hallmark of AIDS. CD4+ T helper cell and the macrophages are the major reservoir of HIV. Replication of HIV in macrophages results in budding of progeny virions through the membranes of endoplasmic reticulum (ER) which means that it acquires its envelope from ER similar to that of Coronaviruses. After entry into the body the virus travels to circulating lymph nodes and starts its replication. Initial infection of about 1 to 3 weeks results in fever, high virus titer in blood and high depletion of CD4+ T helper cell. After one month of infection the virus titer gets reduced in the blood circulation because of cytotoxic T cells, natural killer cells and antibody dependent cellular cytotoxicity

Figure 34.1 Mucosal entry of HIV and its path of circulation:



34.3 HIV-infected person during the latent period?

Cells are called latently infected if they are infected by HIV and are not active in producing virus but can be activated by various signals during the course of infection. Since they are not producing the virus they would not be detected by the immune system and acts as a perfect reservoir of the virus in the body. Only a small amount of cells are latently infected with HIV (around 1%). During the asymptomatic stage, the virus appears in low titers in the blood and it is counter balanced by the host immune system.

34.4 AIDS associated disease conditions

Several specific syndromes are associated with infection by HIV. These include:

1. **Fever and Lymphadenopathy** It is characterized by loss of weight and malaise.
2. **Opportunistic infections:** HIV infected patients have lower immune response because of depletion of CD4+ cells. Several diseases that rarely affect normal individuals may occur in HIV positive patient. The organisms are: *Pneumocystis carinii*, a causative agent of pneumonia, tuberculosis, fungal infection such as candidiasis, herpesvirus, *Salmonella*, *Shigella* and *Campylobacter*.
3. **Cancer:** Kaposi's sarcoma is a rare type of cancer that occurs in HIV-infected persons. These normally benign lesions become malignant and disseminate to involve visceral organs.
4. **Wasting disease:** Disease is characterized by hide bound condition.
5. **AIDS dementia:** Sometime HIV infection of the brain leads to condition that mimics Alzheimer's disease.

34.5 Antiviral drug against AIDS and some facts

- a) Around 95% reduction of virus within 14 days after treatment with essentially any one of the following drug (nucleoside RT inhibitors, nonnucleoside RT inhibitors, protease inhibitors).
- b) Average half life of an HIV infected cell is about 24-48 hrs.
- c) Approximately 5% of total CD4+ T lymphocytes are productively infected in an infected individual at any given time during the latent period.

- d) The CD4+ T lymphocytes that are dying each day are being replaced nearly as fast as they die. This means that bone marrow, spleen and other reservoirs of T lymphocytes must be producing the cells at an exponential rate.
- e) Nearly within 7 -25 days after treatment with any one drug there is emergence of resistant virus.
- f) The rapid emergence of mutant virus suggests that the resistant virus was already present in the population at the time drug treatment was started.
- g) Resistant viruses do not grow quite as well as the wild-type viruses.
- h) Upon removal of the anti HIV drug, the wild type virus once again becomes predominant over the course of infection.
- i) One potential factor in the development of AIDS may be excessive stress on the immune system due to rapid turnover of T lymphocytes.

34.6 Control

- I. The use of condoms during sexual intercourse can reduce the chance of infection.
- II. Avoiding intravenous drugs (*Needle sharing*).
- III. Monitoring the blood for HIV before transfusion.
- IV. Educating people regarding the cause, severity, and preventive measure of HIV.
- V. Anti HIV drugs: These drugs are often given in combination of two or three. Nucleoside and Non-nucleoside analogues are called as reverse transcriptase inhibitors.
- VI. Protease Inhibitors- saquinavir
- VII. Nucleoside analogues- Zidovudine and AZT
- VIII. Fusion inhibitors- Enfuvirtide
- IX. Non- nucleoside analogues- Nevirapine