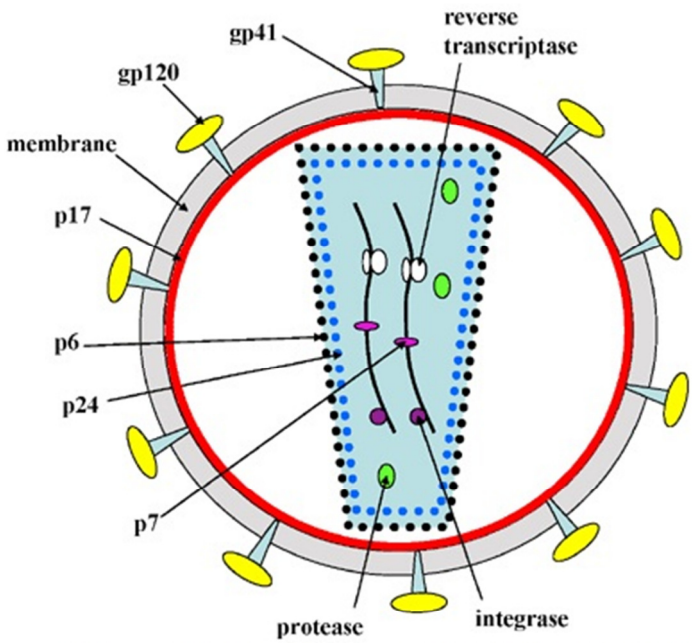
HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is a virus that is well known for its paralyzing effect on immune system of the host causing acquired immunodeficiency syndrome (AIDS). Originally, it has zoonotic origin (descended from animals particularly form primates), but now it is able to infect one human to another through contact. It is originated from the simian immunodeficiency viruses (SIVs) of primates ("Simian" relates to apes). It has two subtypes HIV-1 and HIV-2 that represents two different types of epidemics. HIV-1 is first isolated in 1983 and HIV-2 in 1986. It has been understand that the SIV of **c**him**p**an**z**ee (SIV_{cpz}) gave rise to HIV-1, and the SIV of the **S**ooty **M**angabey Monkey (SIV_{sm}) gave rise to HIV-2 in humans, however it is still not clear how the transmission of these SIVs to humans occurred (It is thought that it may have descended from primates during the hunting and preparation of these primates for food by the indigenous people of Central and Western Africa)[1].

Classification of HIV

The human immunodeficiency viruses, HIV-1 and HIV-2, are members of the family of Retroviruses, in the genus of Lentiviruses. Retroviruses have been found in various vertebrate species, associated with a wide variety of diseases, in both animals and humans. In particular, retroviruses have been found to be associated with malignancies, autoimmune diseases, immunodeficiency syndromes, aplastic and hemolytic anemia, bone and joint disease and diseases of the nervous system.

Many different strains of HIV-1 have been separated into major (M), new (N) and outlier (O) groups, which may represent three separate zoonotic transfers from chimpanzees. Groups N and O are mainly confined to West and Central Africa (Gabon and Cameroon), though cases of Group O have been found world-wide due to international travel, after contact with infected individuals from these areas. The HIV strains in Group M are the ones mainly responsible for the HIV/AIDS pandemic, and they are so diverse that they have been sub-classified into subtypes (or clades) A-K and CRF (circular recombinant forms) where E and I are later classified as CRF01_AE and CRF04_cpx. These subtypes of HIV1 have distinct geographical distribution such as C subtype of HIV-1 (Group M) is distributed in



Cleghorn et al., Principles and Practice of Infectious Diseases. 6th ed. 2005.

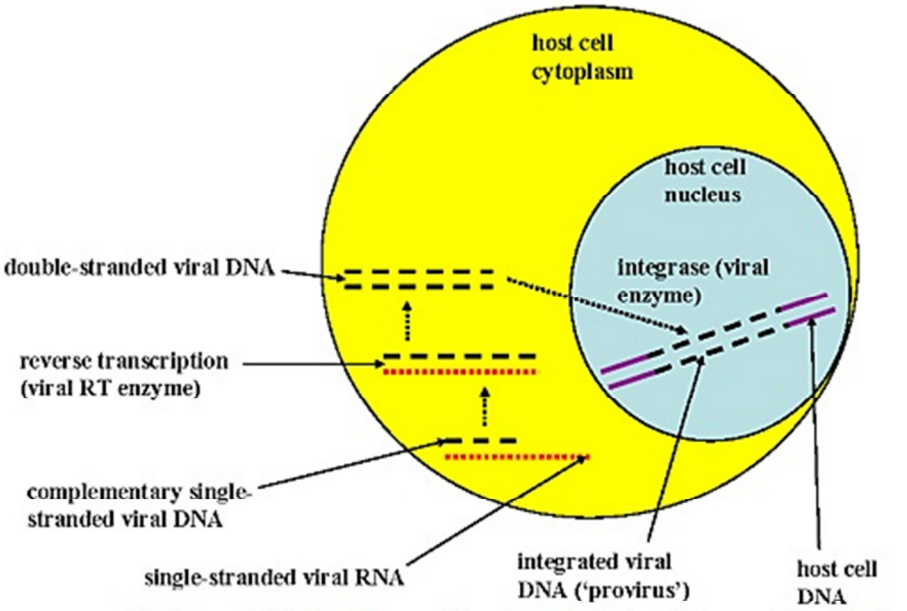
Structure of HIV

[1] Studies using molecular clock evolutionary assumptions suggest that the ancestor virus for HIV-1 appeared in around 1931 and that of HIV-2 in around 1940. After this initial transmission, it is likely that infected individuals with these primate SIVs then transmitted the human form HIV-1 and HIV-2 to other people.

Indian subcontinent, A subtype in western and eastern Africa, Russia and Ukaraine, B subtype in Europe, America, Japan, Australia, Korea, India and Singapore, etc.. This huge diversity of HIV-1 is important when diagnostic testing, treatment and monitoring are applied as the results may differ between different subtypes or clades. The diversity of HIV-2 is much less, but subtypes A-H have been proposed.

Structure of HIV

The human immunodeficiency viruses are approximately 100 nm in diameter. It has a lipid envelope, in which the trimeric trans-membrane glycoprotein-41 (gp41) are embedded to which the surface glycoprotein gp120 is attached. These two glycoproteins are responsible of attachment of HIV to the host cell and are encoded by the *env* gene of the HIV RNA genome. Beneath the envelope, there is the matrix protein p17, the core proteins p24 and p6, and **the nucleocapsid protein p7 (bound to the RNA)**, all encoded by the HIV *gag* gene. Within the HIV core, 2 (two) copies of the *~10* kilobase (kb) positive sense, viral RNA genome (i.e. dsRNA genome) together with the protease, integrase and reverse transcriptase enzymes lie. These enzymes associated with HIV RNA i.e. protease, integrase and reverse transcriptase enzymes are encoded by the HIV *pol* gene. There are several other proteins with various regulatory or immuno-modulatory functions, including *vif* (viral infectivity protein), *vpr* (viral protein R), *tat* (transactivator of transcription), *rev* (regulator of viral protein expression) and *nef* (negative regulatory factor). An additional protein found in HIV-1 but not HIV-2 is *vpu* (viral protein U). Similarly, *vpx* (viral protein X) is found in HIV-2 and not HIV-1. These viral proteins are characteristics of HIV subtypes.



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Integration of HIV viral RNA into host cell DNA

Replication of HIV

HIV invades various immune cells such as $CD4^+$ T cells, monocytes, macrophages, and microglial cells. Firstly, the HIV gp120 binds to CD4 molecules present on $CD4^+$ T cells leading to a conformational changes in the host cell membrane that allows binding of the co-receptor either CCR5 or CXCR4 (chemokine receptor) which is required for fusion of HIV envelope to the host cell membrane. The lymphocytes carry CXCR4 receptor hence those HIV strains that binds CXCR4, are called as **'lymphotropic'**, whereas macrophages carry CCR5 co-receptor, hence those HIV strains that binds to CCR5, are called as **'macrophage-tropic'**. Macrophage-

tropic HIV strains are also capable of infecting lymphocytes as well. These HIV strains are also known, phenotypically, as R5 or non-syncytium inducing (NSI) strains as they do not form syncytia (cell-fusion) when cultured with CD4 lymphocytes *in vitro*. Primary HIV-1 infections tend to involve this R5 NSI macrophage-tropic phenotype. Uncommonly, individuals may have a homozygous deletion mutation in the CCR5 gene (CCR5Δ32) resulting in the absence of the CCR5 molecule on their macrophages. Therefore, these individuals cannot be infected by this R5 phenotype. On the other hand, lymphotrophic HIV strains use CXCR4 as the co-receptor, and therefore these viruses are also known as X4 viruses which do produce syncytia (i.e. are phenotypically syncytium-inducing, SI) when cultured *in vitro* with CD4 lymphocytes. X4 viruses tend to appear later in about 50% of HIV-1 subtype B-infected individuals, but seldom with other subtypes, as they progress to AIDS. So far, CXCR4 deficient individuals have not been found. This attachment and fusion process allows the HIV viral core to enter the host-cell.

Like other retroviruses, HIV also encode an reverse transcriptase enzyme that transcribes its viral RNA into double-stranded DNA (dsDNA), which is then integrated, via the action of the integrase enzyme into the host-cell genome. The viral integrated dsDNA or 'provirus' then acts as a template for viral genomic and messenger RNA transcription by the host cell's nucleic acid replicating machinery. *Recombination between these two RNA strands during viral replication, coupled with the extremely error-prone action of the RT enzyme, give rise to the extreme genetic diversity of HIV (RNA replication error rate of about 1:10^4 bases).* Integration of this linear provirus dsDNA into the genome of the host-cell finally results in viral replication along with cellular replication that is enhanced by various factors, including co-infection with other organisms, the presence of inflammatory cytokines and cellular activation.

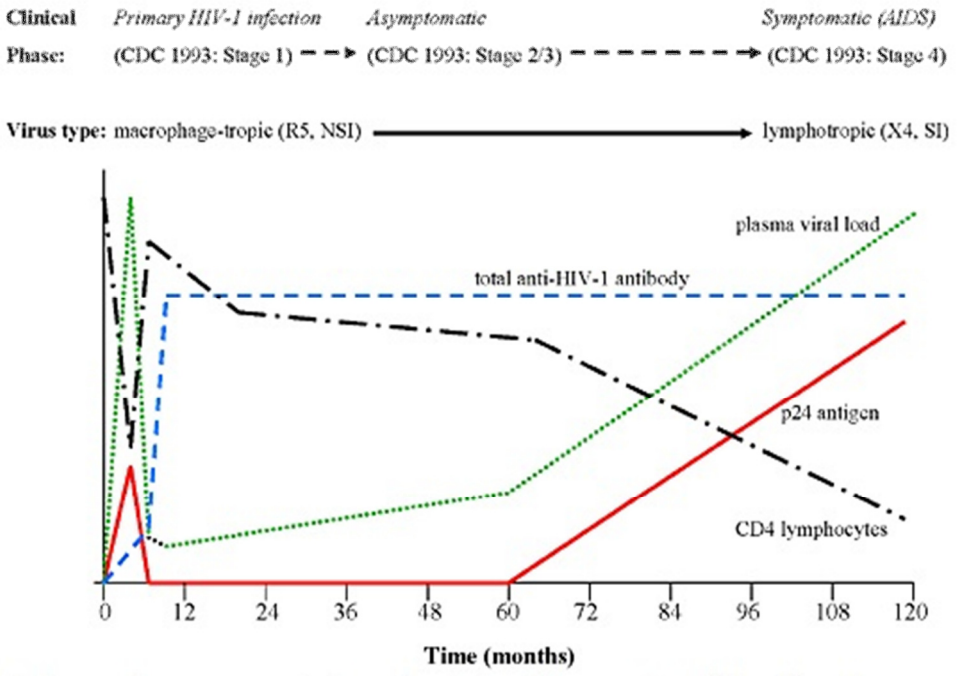
During cellular replication, the provirus is transcribed by the host-cell RNA polymerase II enzyme, and the viral messenger RNA (vmRNA) and genomic RNA, are carried with the cellular mRNAs, to be translated into proteins. This vmRNA codes for a *gag-pol* precursor polypeptide that is ultimately cleaved by the viral-encoded protease enzyme to produce the *gag* and *pol* viral proteins. In addition, the vmRNA is also spliced to produce other vmRNAs coding for the viral proteins *tat, rev, vif, vpr, vpu* (for HIV-1), as well as the *env* precursor polypeptide. Ultimately, the *env* precursor polypeptide is cleaved by cellular (not viral) proteases, producing the envelope glycoproteins gp41 and gp120. These viral proteins, together with the replicated diploid viral genomic RNA, are assembled and enveloped by budding through the host-cell membrane, producing complete HIV virions.

Pathogenesis of HIV

The natural course of the disease is generally starts with primary HIV infection (Centers for Disease Control, CDC stage 1; about 50% of infected individuals are symptomatic with fever and lymphadenopathy) during which the virus is easy to isolate, and is mainly a homogenous population of macrophage-tropic R5 viruses. Meanwhile, the CD4 lymphocyte count drops rapidly for a short period, before recovering to almost normal levels. After this, comes the asymptomatic phase (CDC stages 2/3) when the viruses evolve into a more heterogenous population, and are less easy to isolate. Over this period, which may last between 2-15 years, there is a steady decline in the CD4 count[2]. As the patient becomes more symptomatic, and the

[2] During period of 2-15 years of primary infection, viral replication is continuing at high rate of up to 10^{10} infectious virions/day, leading to approximately 10^8-10^9 lymphocytes/day being infected, which is replaced almost as quickly. It can be observed that the rate of CD4 lymphocyte depeletion is not more rapid than said or observed.

lymphotropic X4 viruses begin to predominate, at least in subtype B HIV-1 infection, the CD4 count drops even more quickly as the patient approaches end-stage disease. The degree of immune activation increases in a reciprocal relationship to the drop in CD4 count. The viral load and p24 antigen (Ag) levels initially peak during primary HIV infection, then decline to a 'set-point' during the early asymptomatic phase, the actual level depending on the degree of the immune response. There is then a gradual rise in viral load and p24 Ag, after about 2 years from initial infection, which becomes accelerated during the symptomatic phase. This rapid turnover of HIV and its enormous diversity underlie the difficulty in producing antiretroviral drugs with long-term efficacy, and is one of many problems facing the development of an effective vaccine against HIV. Elimination of HIV or elimination of HIV-infected lymphocytes occurs through several mechanisms namely (a) direct cytopathic effect of HIV, including the formation of syncytia by SI X4 HIV lymphotropic strains, (b) immune destruction of HIV-infected cells by cytotoxic CD8 T lymphocytes that recognize HIV antigens presented on major histocompatibility complex (MHC) molecules, (c) apoptosis due to lymphocyte activation in the presence of specific cytokines. HIV antibodies (Ab) rise to maximum levels within 3-6 months after initial infection, remaining detectable for the duration of the disease. However, there are antibody subsets (anti-p24 antibodies), that do decline and may be indicative of disease progression. Markers of immune system activation, such as tumour necrosis factor (TNF) can be as useful as viral load at the time of infection, in predicting disease prognosis. With the use of antiretroviral therapy, and the suppression of viral loads to undetectable level, the antigenic stimulus has been removed in such patients, resulting in a decline in the level of anti-HIV antibodies.



Schematic representation of relative time-scale of the clincal, virological and immunological events during HIV infection

Weiss et. al. Principles and Practice of Clinical Virology. 5th ed. 2004.

There are various genes which expressed in HIV viruses that contribute its pathogenesis. These genes are also targets for anti-HIV drug. These are as follows:

- ***gag*** (group-specific antigen): makes the cone-shaped viral capsid.
- ***pol*** (**pol**ymerase): codes for viral enzymes e.g. reverse transcriptase, integrase, and viral protease.
- ***env*** (**env**elope): makes surface protein gp120 (glycoprotein120) and trans-membrane

gp41 enabling HIV to fuse to CD4 cells.

- ***tat*** (**t**rans-**a**ctivator of **t**ranscription): eliminates the hairpin structure in RNA by phosphorylating Cdk9 (cyclin dependent kinase 9)/CycT (cytochrome c testis specific). This increases transcription and creates a positive feedback loop.
- ***vpr*** (**v**iral **p**rotein **R**): involved in getting the viral RNA into the nucleus of the T cell. It causes the cell to stop growing, stimulating an immune dysfunction.
- ***rev*** (**r**egulator of **e**xpression of **v**irion proteins): creates a rev Response Unit that exports the HIVrev RNA into the cytoplasm before it is spliced. It is also a positive feedback loop.
- ***nef*** (**ne**gative regulatory **f**actor or negative factor): down regulates CD4 on the T cell, inhibiting response to infectious agents.
- ***vif*** (**v**iral **i**nfectivity **f**actor): helps to infect other cells, though it is still unclear how. It is thought that it interferes with the T cells proteins that modify RNA.
- ***vpu*** (**v**iral **p**rotein **U**): enhances HIV release from the cell.

Genetic makeup and immune system response to HIV infection can affect disease progression and outcome. Indeed, in some individuals with certain human lymphocyte antigen (HLA) types, disease progression has been found to be more rapid (e.g. patients with HLA-1 B8 DR3), or slower (e.g. Caucasians with HLA B27). In addition, the HIV virion incorporates many host-cell molecules into its envelope including HLA and MHC peptides, which may act to enhance the host immune system activation, thereby producing more activated lymphocytes and enhancing the infectivity of HIV. In the absence of antiretroviral therapy,

A summary of HIV-1 subtypes and CRFs, and their Geographical distribution

Geographical distribution of HIV-1 Group M subtypes

HIV-1 (Group M) subtype	Geographical location
A	Western and eastern Africa, Russia, Ukraine
B	Europe, the Americas, Japan, Australia, Korea, India, Singapore
C	Mainly southern (Botswana, Zimbabwe, Malawi, Zambia, Namibia, south Africa) and eastern Africa, India, Nepal, Malaysia, China, Scotland.
D	East and central Africa
F	Central Africa, south America, eastern Europe
G	Western and eastern Africa, central Europe
H, J, K	Widespread distribution in Africa (Burkina Faso, Mali, Nigeria, Ivory Coast, Gabon, Democratic Republic of Congo), southern Europe, Asia

Geographical distribution of HIV-1 Group M CRFs

HIV-1 (Group M) subtype	Geographical location
CRF03_AB	Kaliningrad, Russia
CRF02_AG	Ibadan, Nigeria
CRF07_BC, CRF08_BC	China
CRF01_AE	Southeast Asia (Thailand, Vietnam, Cambodia, Myanmar, China, Taiwan), central Africa (Central African Republic, Cameroon and Democratic Republic of Congo).
CRF12_BF	South America (Argentina, Uruguay, Brazil)
CRF06_cpx	Burkina Faso, Mali
CRF09_cpx	Senegal
CFR11_cpx	Cameroon, Central African Republic
CRF13_cpx	Cameroon
CRF18_cpx, CRF19_cpx	Africa
CRF01_AE, CRF14_BG, CRF03_AB, CRF05_DF, CRF06_cpx, CRF11_cpx, CRF02_AG.	Have all been found in the UK

IDUs – injection drug users

Treatment of HIV

HIV infection is treated with mainly antiretroviral drugs that affect its entry to the host cells and its replication. Though, anti-HIV vaccine has not been developed so far, monoclonal antibody therapy by targeting various antigenic motif have been under clinical trial.

Anti-HIV drugs: As described, there are many kinds of antiviral drugs under clinical practices. Some of them target the entry of HIV into the host cells such as **Enfuvirtide** and **Maravirac**, some acts as protease inhibitor that prevent the activation of viral enzymes such as **Saquinavir**, **Indinavir**, **Ritonavir**, **Nelfinavir**, **Amprenavir**, **Lopanivir + Ritonavir**, **Tipranavir**, etc., some are non-nucleotide reverse transcriptase inhibitors such as **Nevirapine**, **Efavirenz**, and **Delavirdine**, while some are nucleotide reverse transcriptase inhibitors such as **Zidovudine**, **Didanosine**, **Zalcitabine**, **Lamivudine**, **Abacavir**, etc. A comparative new class of drug Bevirimat (a maturation inhibitor that prevents late stage gag polyprotein processing) and other targeting CCR5 co-receptor agonists, and novel fusion inhibitors are under development.

Anti-HIV monoclonal antibody: A recombinant humanized monoclonal antibody **Ibalizumab** is a, perhaps, first monoclonal antibody approved for the treatment of HIV infection. Other approved mAbs are **Maraviroc** (a CCR5 co-receptor antagonist), and **Raltegravir** (HIV-1 integrase inhibitor) till now, while a newly developed monoclonal antibody **PRO140** (a mAb against CCR5) is under development for antiretroviral therapy.

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