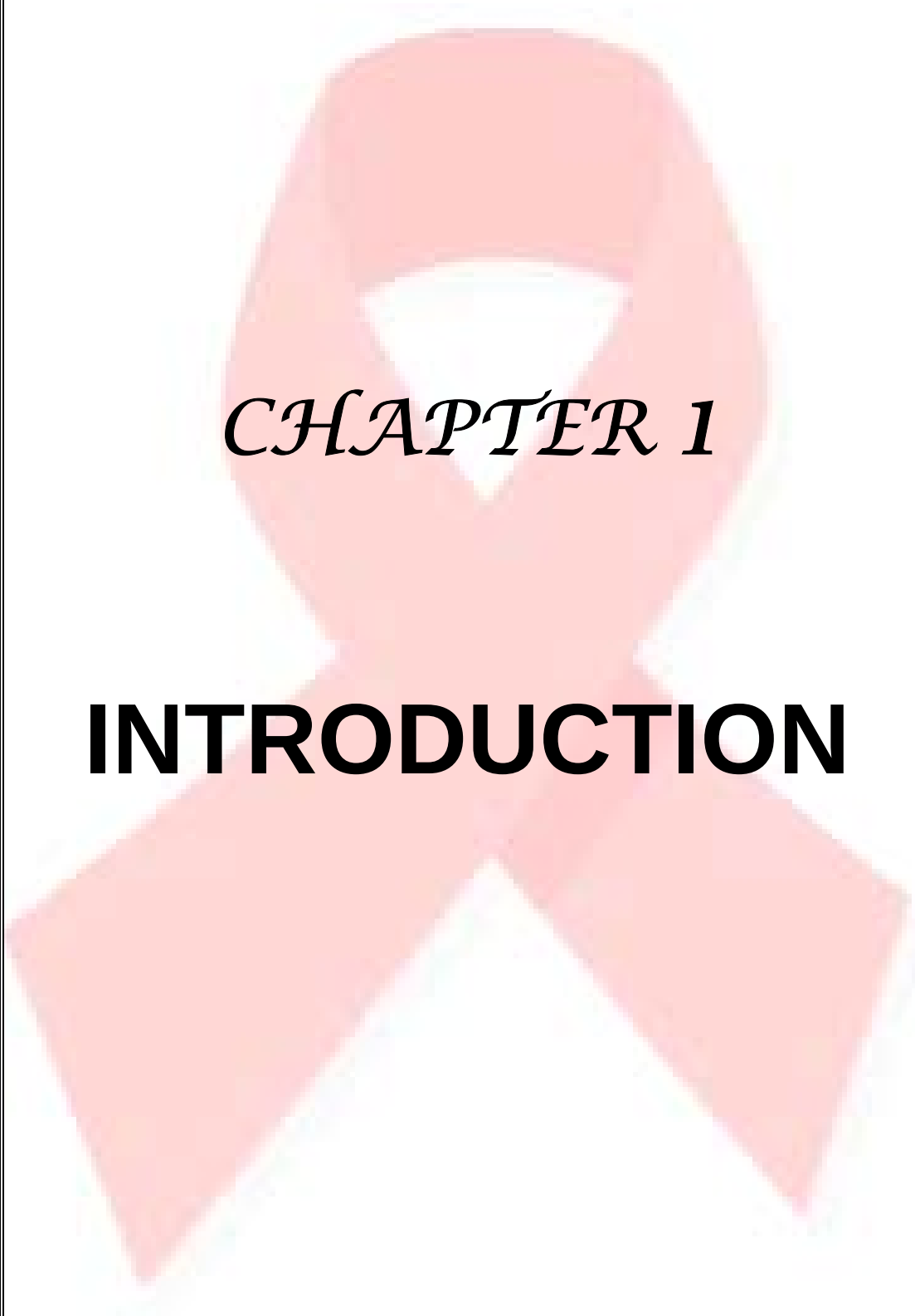

CHAPTER 1

INTRODUCTION

Major goals of the study

The continued spread of HIV virus responsible for global AIDS pandemic remains an unavoidable issue. Much has been done regarding the science of HIV, such as, its disproportionate global distribution, virological properties, evolution of virus, role of biological and cellular factors in viral spread and pathogenesis. Lot of data about the virus and its properties has been accumulated over a decade, still we are unable to stop the continued progression of this virus and till now there is no effective vaccine against this virus. More studies are needed to understand the mechanism of interaction between viral proteins and between cellular factors and viral proteins. Hence, this study focused on identifying specific motifs in HIV-1 Gag that interacts with envelope and how it affects the function of envelope in presence and absence of Vpu, a unique accessory protein to HIV-1.

HIV-1 encodes a structural protein known as p55Gag that acts as an orchestrator of viral assembly. It forms the core of virus particle and it itself is capable of forming non-infectious virus-like particles. For making infectious virus particles it requires interaction with HIV-1 envelope (Env) protein. Several studies have suggested an interaction between these two proteins, however, whether the interaction is direct or indirect and what are the cellular and viral factors involved during the process is still under investigation and intense scrutiny. Specific mutations in HIV-1 Gag have previously been shown to alter the infectivity of virus by affecting Env incorporation on virus particles. The HIV-1 envelope is known to be enriched in cholesterol and both the Gag and Env proteins have been isolated in detergent resistant membrane (DRM) fractions **(Aloia *et al.*, 1993; Brugger *et al.*, 2006; Nguyen and Hildreth, 2000)**. This was later shown that Gag point mutation resulted in defective association of Env with DRM fraction of plasma membrane **(Bhattacharya *et al.*, 2006)**. However, previous studies have been performed in lab-adapted cell lines but more studies in biologically relevant cell types are needed to come to a concrete conclusion. Therefore, I have studied the effect of Gag matrix mutation on HIV-1 envelope infectivity and association with DRM in primary $CD4^+$ T cells.

Another important fact about HIV-1 virus is that both Env and Viral protein U (Vpu) are synthesized from the same bicistronic mRNA and Vpu is only present in HIV-1 and several simian immunodeficiency virus (SIV). Vpu has been shown to affect the trafficking of HIV-1 Gag and thereby the release of virus particles among different cell-

types. Previous studies have shown that Vpu-mediated virus release is cell-type specific, suggesting a role of restriction factor that inhibits virus release in absence of Vpu. It has been recently shown that bone marrow stromal antigen-2 (BST-2/ tetherin/ CD317/ HM1.24) is the restriction factor whose function to keep virus particles tethered to plasma membrane is antagonized by Vpu **(Neil *et al.*, 2008; Van Damme and Guatelli, 2008)**. There are HIV-1 strains (AD8, YU-2) that naturally lack Vpu or contain stop codon in *vpu* gene. Previous study had shown that AD8 Env has the ability to replicate with efficiency similar to wild-type and can compensate the loss of Vpu **(Schubert *et.al.,* 1999)**. While another study reported that viruses lacking Vpu are consistently released at lower level and cannot compensate the loss of Vpu in macrophages **(Richards and Clapham, 2007)**. However, the variations in results could be attributed to difference in cell-types, viral strains and methodology used for the study. These conflicting results prompted us to re-examine the role of Vpu on virus release among different cell-types including primary cells.

In addition, I have studied the effect of Gag matrix mutation on envelope function in presence and absence of Vpu, as Gag has been shown to interact with both Env and Vpu. Later I examined the effect of Vpu deletion and Gag mutation on biological properties of unrelated primary envelopes isolated from HIV-1 infected patients.

General background of HIV-1

A clinical syndrome, which was later called Acquired Immunodeficiency Syndrome (AIDS), was identified in year 1981 at United States. This was followed by reports of around 1300 similar cases in next two years. These patients were either men having sex with men or injecting drug users (IDUs). They showed rare diseases like Kaposi's sarcoma and *Pneumocystis Jirovici* (earlier known as *Pneumocystis carinii*) pneumonia and showed reversal of $CD4^+$: $CD8^+$ T cell ratio in peripheral blood. These patients appeared to have lost their immune competence rendering them vulnerable to opportunistic infections as well as to lymphoid and other malignancies. Isolation of etiological agent from these patients was first reported in 1983 by Barre-Sinoussi *et al.*. at the Pasteur Institute **(Barre-Sinoussi *et al.*, 1983)** as a reverse transcriptase containing virus (retrovirus) from lymph node of a man with persistent lymphadenopathy. They termed the virus as Lymphadenopathy Associated Virus (LAV). In 1984, Gallo *et al.*. from NIH, USA reported isolation of retrovirus from AIDS patient called 'Human T cell Lymphotropic Virus III' (HTLV-III) **(Gallo *et al.*, 1984)**. Few other investigators also reported isolation of AIDS associated Retroviruses (ARV). Subsequently the three prototype viruses (LAV, HTLV-III and ARV) were recognized as members of same group of retroviruses. In 1986, the International Committee on Taxonomy of Viruses (ICTV) recommended giving the AIDS virus a separate name, the Human Immunodeficiency Virus (HIV) **(Coffin *et al.*, 1986)**. Subsequently two types of HIV (type 1 and type 2) were identified.

Although AIDS was first detected in 1981, earlier there was phase of silent spread of HIV-1. Few cases of AIDS were identified retrospectively between year 1972 and 1976 in USA and Haiti **(Korber *et al.*, 2000)**. HIV-specific antibodies were seen in a serum sample from Kinshasa (Democratic Republic of Congo) that was stored in 1959 **(Zhu *et al.*, 1998)**. This is the earliest evidence of HIV infection. On the basis of HIV-1 Envelope gene from 1959 strain and 159 strains obtained at different time points, it has been estimated that HIV-1 was probably transmitted to humans in early thirties **(Korber *et al.*, 2000).** The evidences such as similarities in genomic organization, phylogenetic relatedness, geographic coincidence, prevalence in the natural host and plausible routes of transmission suggest that HIV-1 was transmitted from chimpanzee (*Pan troglodytes*) to humans in central Africa **(Stebbing *et al.*, 2004)**. It is estimated that humans were first infected with HIV-2 about 60 years ago **(Lemey *et al.*, 2003)** and has been speculated to

have originated from SIVsm a lentivirus from the Sooty Mangabey (*Cercocebus atys*) of West Africa **(Hahn *et al.*, 2000)**. The HIV-2 show distinctly slower rate of disease progression compared to HIV-1 and has largely remained restricted to West Africa.

According to UNAIDS global estimates, 39.5 million (range: 34.1 to 47.1 million) individuals were living with HIV and 2.9 million (2.5–3.5 million) died due to HIV/AIDS in year 2006. Out of these deaths, 380,000 were children below 15 years of age. In some of the African countries (e.g. Kenya, Malawi, Zimbabwe, Swaziland) prevalence of HIV is >25%, which has significantly reduced the life expectancy. Since majority of individuals infected with HIV fall in the age group of 15 to 50, which is prime time of working life, HIV/AIDS has devastated several millions of families.

HIV Classification:

HIV is a member of genus Lentivirus, belonging to the family *Retroviridae*. There are two types of retrovirus: 1) *oncogenic* or transforming retroviruses, which lead to neoplasms; and 2) *cytopathic* or *lentiviruses*, of which HIV is an example. Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase (RT) that is transported along with the viral genome in the virus particle. Two types of HIV have been characterized: HIV-1 and HIV-2, on the basis of serologic properties and sequence analysis of molecularly cloned viral genomes. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective, and is the cause of the majority of HIV infections globally.

HIV types, groups and subtypes:

The strains of HIV-1 can be classified into four groups: the "major" group M, the "outlier" group O and two new groups, N and P. The M group is most prevalent and consists of nine subtypes (A-D, F-H, J and K). These group M viruses frequently recombine and these intersubtype recombinants are classified as either circulating recombinant forms (CRF) or unique recombinant forms (URF) (Figure 1.1).

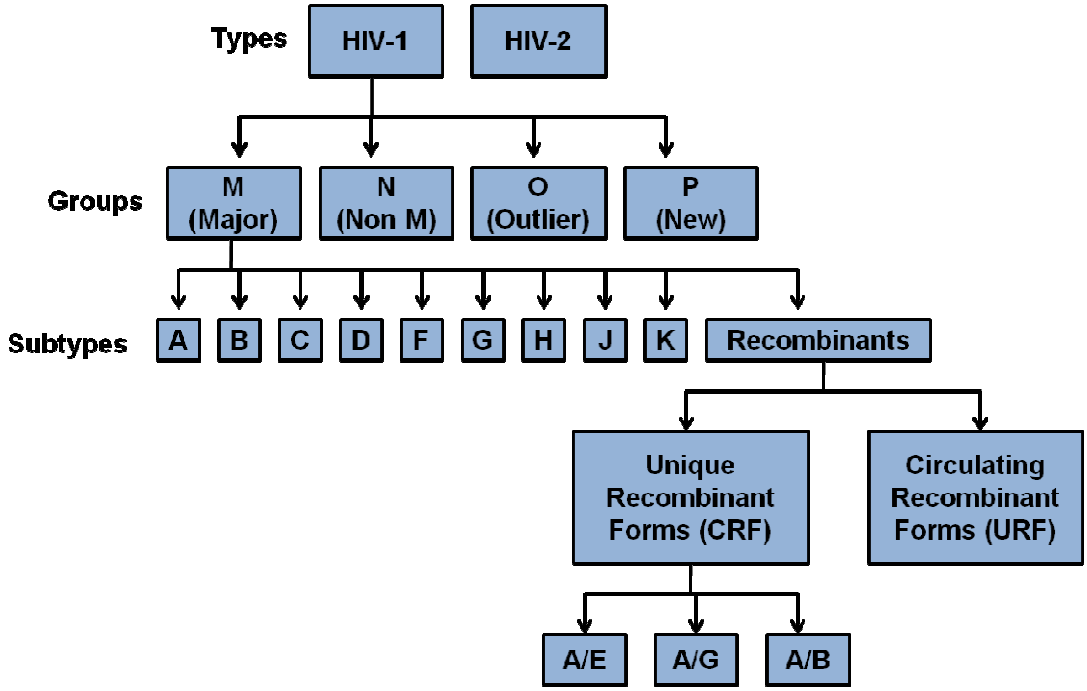

Figure 1.1 Schematic representation of circulating HIV-1 groups and subtypes.

Different HIV-1 subtypes predominate in different geographic regions; subtype A in Africa and Eastern Europe, subtype B in America, Europe and Australia; subtype C in India, China and South Africa. Apart from these subtypes there are HIV-1 strains that have mosaic genomes. They have been reported in geographic areas where more than one HIV-1 subtypes are circulating. These strains are product of recombination between two different HIV-1 subtypes that infect a single cell. The recombination occurs due to "template switching" during reverse transcription by reverse transcriptase enzyme. Recombination between different groups **(Peeters *et al.*, 2003)** different subtypes **(Lal *et al.*, 2005)**, within subtype **(Rousseau *et al.*, 2007)** as well as among recombinant strains of HIV-1 **(Yang *et al.*, 2005)** has been reported. To date, 19 CRFs have been identified and several have played a significant role in the establishment of certain regional epidemics, including CRF01_AE and CRF02_AG **(Casado *et al.*, 2005)**. Approximately 8% of HIV-1 genome sequences available in Los Alamos HIV sequence database (http://www.hiv.lanl.gov) display mosaic genome structures **(Kothe *et al.*, 2006)**. When HIV-1 strains with similar mosaic structure are obtained from three or more epidemiologically unlinked individuals, they are referred as Circulating Recombinant Forms (CRFs).

Origin of HIV:

HIV is thought to have originated in non-human primates in sub-Saharan Africa and was transferred to humans late in the 19th or early in the 20th century. The first paper recognizing a pattern of opportunistic infections characteristic of AIDS was published in 1981. Both HIV-1 and HIV-2 are believed to have originated in West-Central Africa and to have jumped species (a process known as zoonosis) from non-human primates to humans. HIV-1 appears to have originated in southern Cameroon through the evolution of SIVcpz, a simian immunodeficiency virus (SIV) that infects wild chimpanzees (HIV-1 descends from the SIVcpz endemic in the chimpanzee subspecies *Pan troglodytes troglodytes*). The closest relative of HIV-2 is SIV (smm), a virus of the sooty mangabey (*Cercocebus atys atys*), an Old World monkey living in litoral West Africa (from southern Senegal to western Ivory Coast). New World monkeys such as the owl monkey are resistant to HIV-1 infection, possibly because of a genomic fusion of two viral resistance genes. HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N, and O.

Discovery of AIDS:

AIDS was first clinically observed between late 1980 and early 1981. Injection drug users and gay men with no known cause of impaired immunity showed symptoms of *Pneumocystis carinii* pneumonia (PCP), a rare opportunistic infection that was known to present itself in people with very compromised immune systems. Soon thereafter, additional gay men developed a previously-rare skin cancer called Kaposi's sarcoma (KS). Many more cases of PCP and KS quickly emerged, alerting U.S. Centers for Disease Control and Prevention (CDC). A CDC task force was formed to monitor the outbreak. After recognizing a pattern of anomalous symptoms presenting themselves in patients, the task force named the condition acquired immune deficiency syndrome (AIDS).

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients, and published their findings in the same issue of the journal *Science*. Gallo claimed that a virus his group had isolated from an AIDS patient was strikingly similar in shape to other human T-lymphotropic viruses (HTLVs) his group had been the first to isolate. Gallo's group called their newly isolated virus HTLV-III. At the same time, Montagnier's group isolated a virus from a patient presenting Lymphadenopathy (swelling of the lymph nodes)

of the neck and physical weakness, two classic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of HTLV-I. Montagnier's group named their isolated virus Lymphadenopathy-associated virus (LAV). HIV was chosen as a compromise between the two claims (LAV and HTLV-III). Together with his colleague Françoise Barré-Sinoussi, Montagnier was awarded one half of the 2008 Nobel Prize in Physiology or Medicine for his "discovery of human immunodeficiency virus".

Epidemiology: Current Global Distribution of HIV:

According to the latest report by UNAID,, new HIV infections are declining. Following the discovery of HIV in 1983, HIV-1 has caused a worldwide pandemic resulting in more than 25 million deaths and the current estimate of 33 million people living with HIV-1 infections (Global distribution of HIV-1 infections shown in Figure 1.2; Statistics provided by the Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO)). According to the 2009 UNAIDS/WHO report on the global AIDS epidemic, there were an estimated 2.6 million [2.3 million–2.8 million] people who became newly infected with HIV. This is nearly one fifth (19%) fewer than the 3.1 million [2.9 million–3.4 million] people newly infected in 1999, and more than one fifth (21%) fewer than the estimated 3.2 million [3.0 million–3.5 million] in 1997, the year in which annual new infections peaked.

In 33 countries, the HIV incidence has fallen by more than 25% between 2001and 2009; 22 of these countries are in sub-Saharan Africa. In sub-Saharan Africa, where the majority of new HIV infections continue to occur, an estimated 1.8 million [1.6 million–2.0 million] people became infected in 2009; considerably lower than the estimated 2.2 million [1.9 million–2.4 million] people in sub-Saharan Africa newly infected with HIV in 2001. This trend reflects a combination of factors, including the impact of HIV prevention efforts and the natural course of HIV epidemics. In Western, Central, and Eastern Europe, Central Asia, and North America, the rates of annual new HIV infections have been stable for at least the past five years. However, evidence is increasing of a resurgence of HIV in several high income countries among men who have sex with men. In Eastern Europe and Central Asia, high rates of HIV transmission continue to occur in networks of people who inject drugs and their sexual partners.

The number of annual AIDS-related deaths worldwide is steadily decreasing from the peak of 2.1 million [1.9 million–2.3 million] in 2004 to an estimated 1.8 million [1.6 million–2.1 million] in 2009. The decline reflects the increased availability of antiretroviral therapy, as well as care and support, to people living with HIV, particularly in middle- and low-income countries; it is also a result of decreasing incidence starting in the late 1990s.

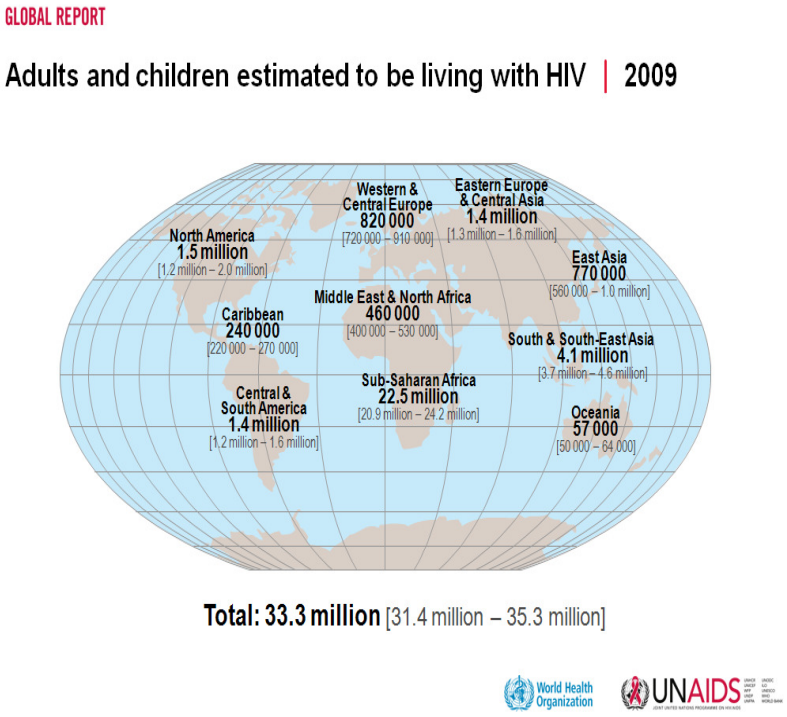

Figure 1.2: Global distribution of HIV-1 infections according to the UNAIDS/WHO 2009 Report on the Global AIDS Epidemic

HIV-1 Transmission:

Major routes of HIV-1 transmission are summarized below:

Sexual

The majority of HIV infections are acquired through unprotected sexual relations. Sexual transmission can occur when infected sexual secretions of one partner come into contact with the genital, oral, or rectal mucous membranes of another.

Blood products

In general, if infected blood comes into contact with any open wound, HIV may be transmitted. This transmission route can account for infections in intravenous drug users,

haemophiliacs, and recipients of blood transfusions (though most transfusions are checked for HIV in the developed world) and blood products. Sharing of needles significantly contributes to HIV transmission among Intravenous Drug Users (IDUs).

Mother-to-child

The transmission of the virus from the mother to the child can occur *in utero* (during pregnancy), *intrapartum* (at childbirth), or via breast feeding. Mother-to-child transmission occurs in 15-30% cases in absence of any intervention.

Structure of HIV:

HIV is roughly spherical with a diameter of about 120 nm and consists of a lipid bilayer membrane or envelope that surrounds the cone shaped nucleocapsid (figure 1.3). The nucleocapsid is composed of two copies of positive single-stranded RNA, about 9.2 kb long that have positive polarity with respect to translation. The RNA codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase (RT), proteases, ribonuclease and integrase. A transfer RNA_{lys} molecule is positioned near the 5' end of each genomic RNA strand and serves as the primer for initiation of negative strand viral DNA synthesis by RT. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle. The capsid in turn is surrounded by the viral envelope that is composed of phospholipid bilayer derived from host cell. Within the viral envelope are encoded two glycoproteins, gp120 and gp41. This protein, known as Env, consists of a cap made of three molecules of gp120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope. Both

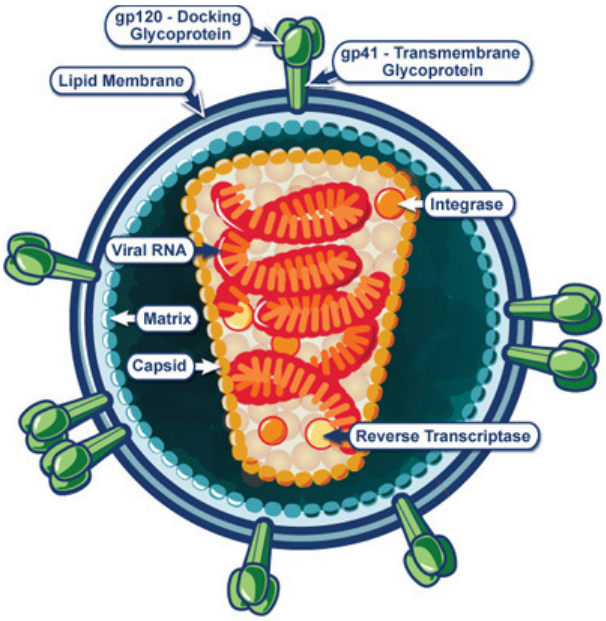

Figure 1.3 Structure of mature HIV-1 virion (*www.niaid.nih.gov*)

gp120 and gp41 are linked non-covalently. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle.

Genome organization of HIV-1:

The HIV-1 proviral RNA genome consists of about 9200 nucleotides, which encodes for nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat, env and rev), encoding 19 proteins (Figure.1.4).

Three important genes that contain information needed to make structural proteins for new virus particle are:

- *gag* (group-specific antigen): codes for the structural proteins of the nucleocapsid.
- *pol*: codes for three important enzymes, *reverse transcriptase* (RT), *protease* (PR), RNaseH and *integrase* (IN).
- *env*: codes for gp160, the precursor of gp120 and gp41.

The remaining six genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease. HIV-1 uses three reading frames for transcription of mRNAs and HIV-1 proteins are synthesized after differential splicing of primary mRNA transcripts.

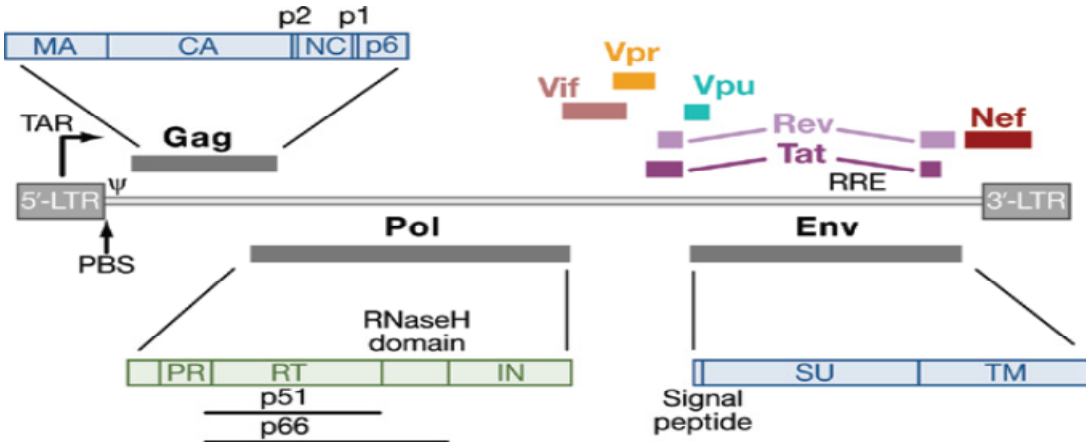

Figure 1.4 Genome Organization of HIV-1 (Swanson *et al., Cell* 2008)

Specific functions of HIV-1 genes in virus life cycle are summarized in Table 1:

Table 1:

S.No.	HIV-1 Genes and gene products	Role and Function
1.	*gag* (group specific antigens)	p55 myristoylated protein precursor, which is processed to p17 (Matrix), p24 (Capsid), p7 (Nucleocapsid), and p6 proteins, by the viral protease. Gag associates with the plasma membrane, where virus assembly takes place.
2.	*pol*	The genomic region encoding the viral enzymes protease, reverse transcriptase, and integrase. These enzymes are produced as a Gag-Pol precursor polyprotein, which is processed by the viral protease.
3.	*env*	Env is synthesized as precursor (gp160), which is processed to give surface glycoprotein gp120 and the transmembrane glycoprotein gp41. The mature gp120-gp41 proteins are bound by non-covalent interactions and are associated as a trimer on the cell surface. Gp120 contains the binding site for the CD4 receptor and co-receptors for HIV-1. Essential for virus entry and attachment to host cell.
4.	*tat*	Transactivator of HIV gene expression. It is a regulatory gene, localized in the nucleus and acts by binding to the TAR RNA element and activating transcription initiation and elongation from the LTR promoter, preventing the 5' LTR AATAAA polyadenylation signal from causing premature termination of transcription and polyadenylation.
5.	*rev*	The second necessary regulatory factor for HIV expression. A 19-Kd phosphoprotein, localized primarily in the nucleolus/nucleus, Rev acts by binding to RRE and promoting the nuclear export, stabilization, and utilization of the viral mRNAs containing RRE.
6.	*vif* (Viral infectivity factor)	A 23-Kd cytoplasmic protein that promotes the infectivity but not the production of viral particles. Vif prevents the action of the cellular APOBEC-3G protein, which deaminates DNA: RNA heteroduplexes in the cytoplasm.
7.	VPR (viral protein R)	Vpr is a 96-amino acid (14-Kd) protein localized in nucleus and is incorporated into the virion. Proposed functions for Vpr include the targeting the nuclear import of preintegration complexes, cell growth arrest, transactivation of cellular genes, and induction of cellular differentiation.
8.	*vpu* (viral protein U)	Vpu is unique to HIV-1, SIVcpz (the closest SIV relative of HIV-1), SIV-GSN, SIV-MUS, SIV-MON and SIV-DEN. Vpu is a 16-kd (81-amino acid) type I integral membrane protein with at least two different biological functions: degradation of CD4 in the ER, and enhancement of virion release from the plasma membrane of HIV-1-infected cells.
9.	*nef*	A multifunctional 27-Kd myristoylated protein present predominantly in the cytoplasm and associated with the plasma membrane via the myristoyl residue linked to the conserved second amino acid (Gly). Nef down regulates CD4, the primary viral receptor, and MHC class I molecules, and these functions map to different parts of the protein. Nef interacts with components of host cell signal transduction and clathrin-dependent protein sorting pathways. It increases viral infectivity.

HIV-1 Life cycle:

The HIV-1 life cycle, can be split into an early and a late phase of replication. The early steps begin with the attachment of the virion at the cell surface, and finish with the integration of the proviral DNA into the host genome. The succeeding late part of the viral replication extends until virion release.

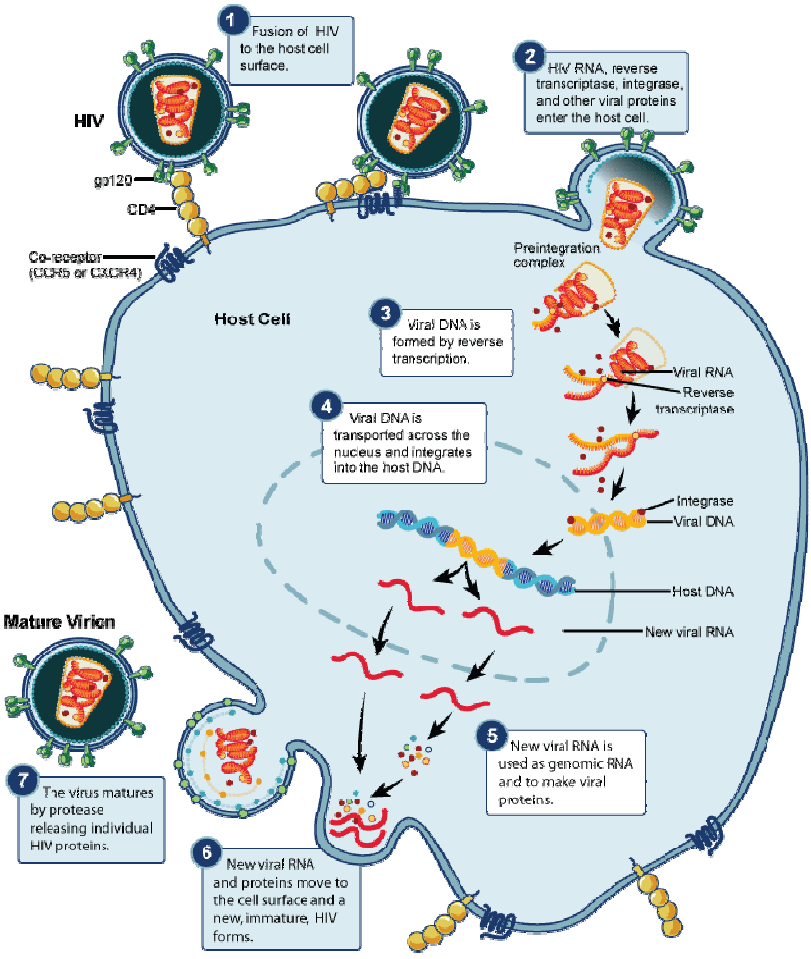

Figure 1.5 Overview of HIV-1 Replication Cycle (*www.niaid.nih.gov*)

Once the infection is initiated, the HIV-1 particle travels, mainly in the blood, lymph or lymphatic tissues, until it attaches to the cell surface of its target cells. These target cells are resting or activated $CD4^+$ T lymphocytes and macrophages, which are non-dividing immune cells that also express CD4. HIV-1 life cycle includes following steps (Figure.1.5):

Virus attachment to the host cell and entry:

Attachment of HIV is mediated by interaction between the extracellular domain (gp120) of Env gp and the CD4 antigen present on the surface of susceptible cells followed by interaction with coreceptors (members of the seven membrane-spanning CC or CXC families of chemokine receptors). The two major coreceptors for HIV infection are CXCR4 and CCR5. Once the gp120 of virus envelope binds to CD4 receptor and coreceptor of target cell, it induces a conformational change in gp41 that leads to fusion of viral and cell membranes. The membrane fusion event enables the viral core to gain entry into the host cytoplasm.

HIV-1 uses CCR5 as preferential co-receptor for entry into the target cell at the time of transmission but in some cases HIV-1 isolated from patients during late stage of infection uses CXCR4 as coreceptor. Therefore based on phenotype, HIV-1 isolates are classified as CCR5 tropic (R5 phenotype) and CXCR4 tropic (X4 phenotype). HIV-1 isolates that use both i.e. CCR5 & CXCR4 coreceptors (R5/X4 phenotype) as well as coreceptors other than CCR5 and CXCR4 have been also reported.

Viral uncoating and viral DNA synthesis by reverse transcription:

Uncoating of viral capsid occurs within the cytoplasm of infected cell. It is said that un coating is promoted in response to multiple successive changes in the cellular environment, sequential contact with different cellular factors, and through the molecular rearrangements that accompany reverse transcription, thus triggering progressive or stepwise conformational changes and disassembly. After uncoating, there is formation of virus reverse transcription complexes (RTCs) and pre-integration complexes (PICs). RTCs are simply defined as HIV-1 complexes that undergo reverse transcription, during which they convert their single-stranded positive RNA viral genome into double-stranded DNA. The RTC genomes are thus either RNA or RNA-DNA intermediates of reverse transcription. In contrast, PICs no longer contain any RNA but only the double-stranded DNA. PICs are per definition integration-competent HIV-1 complexes and can integrate efficiently into a target DNA *in vitro*.

Reverse transcription involves firstly the formation of the minus strand strong-stop DNA, a strand transfer event, and the synthesis of the minus strand DNA with concomitant degradation of the RNA template. In the HIV-1 genome, two polypurine tracts (PPT), the central PPT (Cppt) and 3' PPT, resist degradation by Rnase H and serve

as primers for synthesis of plus-strand DNA. Reverse transcription proceeds with synthesis of plus-strand DNA, involves a second strand transfer event, and terminates at a central termination sequence (CTS) in the centre of the genome. The initiation of plus-strand synthesis at the Cppt, as well as the 3' PPT, leads to a discrete plus-strand displacement of 100 nucleotides in the centre of the genome. The final product of HIV-1 reverse transcription is therefore a linear double-stranded (ds) DNA with a central DNA Flap. Upon DNA Flap formation and completion of reverse transcription, the viral complex becomes a PIC, competent for import into the nucleus and integration within the host cell chromatin **(Arhel *et al.,* 2010)**.

Integration of viral DNA into host cell chromosome:

The PIC moves towards nucleus using microfilaments and microtubules and enters into the nucleus using nuclear import pathway. After translocation into the nucleus, the integrase cleaves the 3' termini of the viral double-stranded DNA to generate two nucleotide 5' overhangs at each end. Subsequently the integrase triggers trans-esterification reaction in which the 3' hydroxyl group attacks phosphodiester bonds of chromosomal DNA and joins viral DNA to host DNA. The viral DNA is randomly integrated at many chromosomal locations. The integrated form of the virus is called provirus. The non-integrated linear DNA is circularized, which reduces signal for apoptosis by reducing number of linear DNA molecules.

Transcription of viral RNA:

The provirus remains latent or is actively transcribed depending on chromatin structure around the integration site and metabolic status of the host cell. The virus may remain latent due to integration into areas of repressed heterochromatin or due to absence of factors such as nuclear factor Kb (NF-Kb) and Nuclear Factor of Activated T cells (NFAT), which act as transcriptional enhancers. In an activated cell, NF-Kb and NFAT bind to enhancer sequence of LTR and promote viral transcription. Host cell RNA Polymerase II binds to transcription initiation site and begins transcription but fails to elongate efficiently in absence of viral Tat protein. Tat protein binds to TAR (first 45 nucleotides of viral m-RNA, which is target sequence for viral transactivation) and prevent premature termination of transcription.

Processing of viral RNA transcripts, nuclear export and expression of viral proteins:

Viral RNA transcripts are completely spliced incompletely spliced or remains unspliced. The viral Rev protein plays an important role in controlling the splicing mechanism and transport of viral RNA transcripts to cytoplasm. In the cytoplasm these RNA molecules are translated into viral proteins. Multiply spliced m-RNA molecules encode Nef, Tat and Rev whereas incompletely spliced RNA transcripts encode Env, Vif, Vpr and Vpu proteins. The unspliced RNA transcripts encode Gag (p55) and Gag-Pol (p160) precursor proteins. The Gag-Pol precursor is produced by ribosomal frame shifting near the 3' end of *gag*.

Assembly, budding and maturation of HIV virus particles:

The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by protease and processed into the two HIV envelope glycoproteins gp41 and gp120. These are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. The enzyme protease plays a vital role at this stage of the HIV life cycle by chopping up long strands of protein into smaller pieces, which are used to construct mature viral cores. Assembly of HIV-1 is directed by the Gag protein and it contains all of the determinants necessary for assembly as Gag alone is capable of forming non-infectious viral like particles. In addition, Gag recruits other HIV-1 proteins, especially Env and viral RNA into nascent virions. Gag is synthesized as a polyprotein precursor, $Pr55^{gag}$, and is cleaved into its component subunits by HIV-1 protease. Approximately 2000 Gag proteins, 200 Gag-Pol proteins, two unspliced viral RNA and other proteins (Vif, Vpr and Nef) assemble below the cell membrane **(Wilk *et al.*, 2001)**. These assembled viral components form immature virion which buds out of the host cell using cellular ESCRT (Endosomal Sorting Complex required for Transport) that mediates outward vesiculation **(Marsh and Thali, 2003)**. In this process, host cell plasma membrane embedded with gp120 and gp41 proteins form HIV-1 envelope and encloses the nucleocapsid. After budding the viral protease enzyme cleaves Gag and Gag-Pol precursor proteins. The Gag precursor protein is cleaved into p24, p17 and other subunits whereas Gag-Pol precursor protein is cleaved into reverse transcriptase, protease and integrase. This results in the formation of mature, infectious virion **(Wilk *et al.*, 2001)**.

Pathogenesis of HIV-1:

The key characteristic of HIV-1 infection is gradual loss of $CD4^+$ T lymphocytes resulting in severe immunosuppression and ultimately the development of AIDS and increased susceptibility to opportunistic infections. Profound loss of CD4+ T cells was reported in early description of AIDS **(Lane and Fauci, 1985)**. $CD4^+$ T cell count is one of the strongest predictors of clinical disease **(Phillips and Lundgren, 2006)**. The natural history of HIV-1 infection is characterized by an acute and primary phase that lasts for few weeks, followed by clinically latent phase that lasts for several years and ultimately by immune deficiency syndrome **(Rowland-Jones, 2003)** (Figure 1.6).

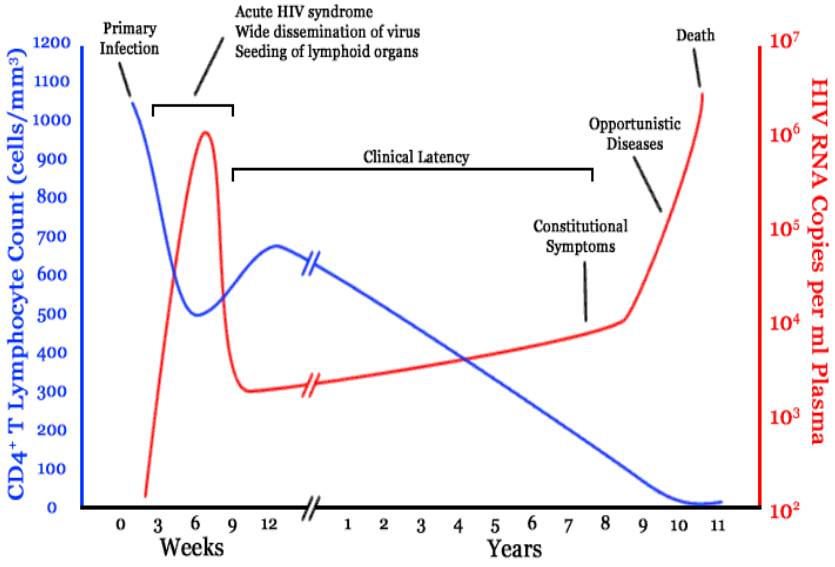

Figure 1.6: Schematic representation of natural history of HIV-1 infection (*Fields Virology, 2005*)

Acute phase:

Within 2-3 weeks after acquisition of HIV-1 infection, virus becomes well established in the lymphoid tissue. Plasma viral RNA level increases exponentially with a doubling time of 10 to 20 hrs **(Fiebig *et al.*, 2003)**. Plasma viral RNA level is highest at the start (primary stage) and end of infection (AIDS), whereas low and relatively steady level is maintained in between for a variable number of years (Figure 1.6). This steady state of viraemia (viral set point) is achieved within 6 to 12 months of infection and may depend on various host (immune response and genetic factors such as HLA) and viral factors as

yet not fully understood. There is massive infection and loss of $CD4^+$ T cells predominantly in lymphoid tissues of gastro-intestinal track (**Brenchley *et al.*, 2004; Mattapallil *et al.*, 2005; Mattapallil *et al.*, 1998; Veazey *et al.*, 1998**).

Clinically latent phase:

Acute primary HIV-1 infection is followed by a long period of clinical latency (usually 7 to 10 years). Although patients in this stage of infection do not show clinical disease and plasma viral RNA level is often low, virus multiplication continues in the lymphoid tissue. Ho *et al.* showed that there is daily production and clearance of 0.05×10^9 to 2×10^9 HIV-1 virions and daily turnover of $CD4^+$ T lymphocytes ranges between 0.2×10^9 to 5.4×10^9 cells (**Ho *et al.*, 1995**). This turnover in the $CD4^+$ cells leads to steady decline in peripheral $CD4^+$ T cell count leading to immune suppression. The rate of $CD4^+$ T cell decline may vary leading to slow, average or rapid disease progression. There are three general patterns of disease progression that occur following infection: 1) typical progressor; 2) rapid progressor; and 3) long-term non-progressor. About 10% of the HIV-infected individuals progress to AIDS within 2 to 3 years after infection and are referred as *rapid progressors*. About 5-10% individuals do not show clinical disease even after 10 years of infection (**Cao *et al.*, 1995; Lefrere *et al.*, 1997**). These individuals show less than 5% decline in $CD4^+$ T cell count annually with plasma viral RNA level undetectable or very low without anti-retroviral therapy. These individuals are called *Long-Term Non-Progressors (LTNPs)*. Majority of infected persons may lead to AIDS stage within 7 to 10 years.

Clinical AIDS is established when an infected individuals circulating $CD4^+$ T cell level drops below 200 cells/µl or if they exhibit one of the AIDS defining clinical conditions as defined by the Centre for Disease Control and Prevention (CDC). The patients acquire various opportunistic infections and malignancies such as, Candidiasis of respiratory tract & lungs, Coccidioidomycosis (disseminated or extra pulmonary), Cryptococcosis (extra pulmonary), Cytomegalovirus disease (other than liver, spleen, or nodes), Herpes simplex infection (with chronic ulcer and greater than 1 month's duration), Histoplasmosis (disseminated or extra pulmonary), Isosporiasis (chronic intestinal with greater than 1 month's duration), Kaposi's sarcoma, Burkitt's Lymphoma, Lymphoma of brain, Mycobacterium avium complex, Mycobacterium tuberculosis (pulmonary & extra pulmonary) and wasting syndrome. Patients in this stage show highest risk of death.