Chapter 1

Introduction
In 1981, Acquired Immunodeficiency Syndrome (AIDS) was first described, as a distinct clinical disease among young men having sex with men (MSM), who were diagnosed either with *Pneumocystis carinii* pneumonia and/or a rare type of skin cancer, Kaposi’s sarcoma, seen only in immuno-compromised persons. They showed unique pattern of underlying immuno-suppression without any identifiable cause (Gottlieb et al., 1981). Similar cases were subsequently reported in haemophilia patients who had received blood products (Davis et al., 1983) and intravenous drug users (IDUs) who shared needles and syringes (Boasso et al., 2007). 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 recommended giving the AIDS virus a separate name, the Human Immunodeficiency Virus (HIV) (Coffin et al., 1986).

Although AIDS was first identified in 1981, the earliest evidence of HIV infection was from an archived serum sample stored in 1959 in Kinshasa (Democratic Republic of Congo) which showed the presence of HIV specific antibodies (Park et al., 2005). A few more cases of AIDS were identified between the year 1972 and 1976 in USA and Haiti retrospectively (Korber et al., 2000). 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 SIV<sub>sm</sub>, a lentivirus from the sooty mangabey (*Cercocebus atys*) of West Africa (Hahn et al., 2000).

Genetic diversity is a hallmark of HIV. The extreme heterogeneity of HIV is a result of high mutation rate (0.2-2 mutations per genome per cycle) due to lack of proofreading ability of the reverse transcriptase, high replication rate and propensity for recombination. Based on the phylogenetic analysis of numerous isolates obtained from diverse geographical regions, HIV is divided into types, groups, subtypes, genotypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs) (Fig. 1.1 and Fig. 1.2).

**Figure 1.1:** HIV subtypes

![HIV subtypes diagram](image)

The virus strains belonging to the same subtype differ up to 20% in their envelope protein whereas differences between strains of different subtype may be up to 35% (Gaschen et al., 2002). 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 (Facchetti et al., 1988), different subtypes (Lal et al., 2005), within subtype (Rousseau et al., 2007) as well as among recombinant strains of HIV-1 (Park et al., 2005) has been reported. 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). These viruses with mosaic genome are called as Unique Recombinant Forms (URFs) and when a certain URF is obtained from three or more epidemiologically unlinked individuals, they are referred as Circulating Recombinant Forms (CRF). Circulating recombinant forms represent mosaic viruses that are transmitted from one person to other and the transmission continues in the population. Till date 48 major CRFs have been documented within HIV-1 M group (http://www.hiv.lanl.gov/content/hiv-db/CRFs/CRFs.html). In the East and Southeast Asia, CRF01_AE is highly predominant. The CRF01_AE, CRF07_BC, CRF08_BC, CRF33_01B and CRF34_01B were confirmed to be originated in Asia while new unique recombinant forms (URFs) are continually being identified and their complexity continues to increase (Thomson and Najera, 2005). These URFs may later become new CRFs after circulating in particular geographical areas.

UNAIDS global estimates indicate that the total number of people living with HIV in 2008 was 33.4 million (31.1-35.8 million) and 2 million (1.7-2.4 million) people died of AIDS. Of the 2.7 million (2.4-3 million) people who were newly infected with HIV in 2008, (i.e. over 7400 new infections in a day) more that 96% are from low and middle income countries.
In India, the first case of HIV infection was detected in 1986 from Tamil Nadu. Since then, the spread of HIV epidemic in India is a major public health concern. Although the proportion of people living with HIV in India is lower than previously estimated, the epidemic continues to affect large numbers of people. More accurate estimates of HIV indicate that approximately 2.4 million (2 million–3.1 million) people in India were living with HIV in 2008 (UNAIDS 2009 report). Genotypically more than 90% of the strains tested from India belonged to subtype C although some B/C and A/C recombinants have also been identified (Cecilia et al., 2000; Lole et al., 1999). Sequence analysis for most subtype C isolates has revealed that these viruses display certain unique genetic characteristics that, by altering biological activity, may have contributed to the success for the predominance of this subtype. Examples include the presence of three or four NF-κB enhancer copies in the long terminal repeat region, Tat and Rev proteins that are prematurely truncated, and a 15-bp insertion of the 5' end of the vpu reading frame. Subtype C also shows a preference for CCR5
coreceptor usage, and the variable V3 loop is relatively conserved in subtype C compared to other subtypes (Shankarappa et al., 2001).

HIV is transmitted by sexual contact, contaminated blood and from mother to child during pregnancy, birth or breast-feeding. 85% of HIV-1 subtype C transmission is via sexual transmission i.e. mucosal routes. In the mucosa, the virus initially interacts with dendritic cells (DCs), which carry the virus to draining lymph nodes. This enables the infection of T cells, especially the T cells that are activated by interaction with DCs. The transport of the virus to lymph nodes results in subsequent systemic viral dissemination to lymphoid tissue, a high viral load in blood and seeding of the virus to central nervous system. CD4+ T cells are the primary target cells for HIV and infection with HIV produces a prolonged, gradually progressive disease which leads to multiple infections with opportunistic microorganisms, many types of cancer, and eventually death. The initial asymptomatic period may last 10 or more years depending on the virulence of the viral strain and the immune status of the patient. With time, the functional capacity of the immune system is impaired and clinical signs and symptoms of AIDS become apparent. Neither a vaccine nor a totally efficient treatment is available, so the development of AIDS is believed to be invariably fatal (Fauci, 1988).

The critical loss of CD4+ T cells during progression to AIDS is the immunologic hallmark of HIV-1 pathogenesis, resulting in susceptibility to opportunistic infections. There is a rapid and dramatic loss of CD4+ T cells in lymphoid tissues (LT) during acute infection with both HIV-1 (Barber, 2001; Mildvan et al., 1982) and SIV (Natarajan et al., 2002; Vivier et al., 2008). During the initial phase of the infection, there is also a heavy loss of CD4+ T cells in gut-associated lymphoid tissue (Dandekar, 2007; Zeitz et al., 1998). Although partial repopulation with T helper cell is observed after the acute phase, a continuous gradual loss of CD4+ T cell occurs throughout chronic disease, which is accelerated during AIDS (Bonaparte and Barker, 2003). Several mechanisms have been proposed to explain this
depletion during chronic HIV-1 disease, ranging from direct cytopathic effects of HIV-1 infection on CD4+ T cells (Barber, 2001) to HIV-induced immune activation of T helper cell death (Bonaparte and Barker, 2004). However, the frequency of infected circulating CD4+ T cells is too low to account for the loss of CD4+ T cells during the chronic phase (Tasca et al., 2003). Furthermore, because HIV-1 activates both CD4+ and CD8+ T cells (Fauci et al., 2005), T cell activation solely does not account for the selective depletion of CD4+ T cells.

Pathogenesis of HIV infection is considered multi-factorial; because no unique immune alteration has been identified that can fully explain the plethora of adaptive and innate immune dysregulations described so far. Apart from CD4+ T cells, HIV can interact with other cells like natural killer cells (NK cells), plasmacytoid and conventional/myeloid dendritic cells (pDCs and mDCs). The virus seems to have adapted to the unique biological characteristics of these cells. Activation of natural killer (NK) cells and production of type I interferon (IFN-α/β) by plasmacytoid dendritic cells (pDC) are the main effector arms of innate antiviral responses (Barber, 2001). HIV infected cells appear to be resistant to NK cell-mediated killing despite markedly reduced expression of MHC class I (Major histocompatibility class I) (Bonaparte and Barker, 2003; Bonaparte and Barker, 2004; Tasca et al., 2003). The reasons for this dysfunction are not fully understood, but probably depend on a combination of increased expression of iNKR (inhibitory NK cell receptors) and concomitant low expression of receptors with activating function (Fauci et al., 2005). Both cytolytic activity and cytokine production of NK cells are affected possibly as a consequence of HIV-mediated signalling through CCR5 and CXCR4 and of general immune activation of the host, rather than infection of a small subset of CD4-expressing NK cells (Fauci et al., 2005; Kottilil et al., 2003; Kottilil et al., 2004; Valentin et al., 2002).

Clinical studies have demonstrated reduced pDC frequencies from the blood of HIV infected patients along with reduced IFN-α secretion (Fauci
et al., 2005; Granelli-Piperno et al., 2004; Kamga et al., 2005; Kottilil et al., 2003; Kottilil et al., 2004; Valentin et al., 2002) however, recent hypothesis suggests that the immune dysfunctions during HIV infection are due to chronic immune activation and that pDCs play a very important role in it. The interaction of CD4 on pDCs with gp120 on HIV may lead to continuous pDC activation for a prolonged period of time which may result in long-term suppression of T cell responses, causing a state of immune deficiency, similar to that observed during chronic HIV-disease (Fallarino et al., 2007; Mellor and Munn, 2003; Mellor and Munn, 2004; Tailor et al., 2006). In addition to pDCs, other DC subtypes also contribute the disease pathogenesis. DCs are among the first cellular targets of HIV-1 during sexual transmission. DCs promote viral replication by two mechanistically distinct processes; they are susceptible to direct infection and they capture virions and then pass them on to CD4+ T cells. The latter mechanism has generated particular interest because after capturing virions, DCs send signals to T cells that promote their ability to replicate the virus.

Despite 25 years of dedicated research towards understanding the biology of HIV pathogenesis, our understanding of how the virus interacts with the immune system and its consequences are still limited. It is becoming apparent that viral pathogenicity is a consequence of both the direct effects of viral replication on infected cells and its indirect effects on uninfected cells. Complete comprehension of the complex virus-host interactions will help in identifying ideal targets for immune-based therapies and protective strategies. The interaction between dendritic cells and HIV is of prime importance because these cells are mediators of innate as well as adaptive immune response. In this thesis we have focused on two aspects of DC-HIV interaction; one, mechanisms responsible for the depletion of circulating DC subtypes and effect of HIV infection of expression of genes important for DC function, especially co-stimulatory molecules.