3. Review of Literature
3.1 History of HIV/AIDS

The Acquired Immune Deficiency Syndrome (AIDS) first came to the notice of physicians and epidemiologists in 1981 in San Francisco, USA when a handful of homosexual men in cities presented with *Pneumocystis carinii* pneumonia and Kaposi’s sarcoma (KS). These diseases were previously extraordinarily rare in young adults and indicated that some kind of immune deficiency was occurring in gay men. The first full case description already showed a selective depletion of CD4+, T-helper lymphocytes in the peripheral blood (Gottlieb *et al.*, 1981). It was soon noted that a larger proportion of gay men suffered from generalized, extended lymphadenopathy and the disease was initially called the ‘gay-related immune deficiency syndrome’ (GRID). In 1981, it was more appropriately renamed AIDS, and the *San Francisco Chronicle* newspaper published a description of the ‘seven deadly symptoms’ associated with the disease: a fever persisting for more than 4 or 5 days; unexplained weight loss of 10 to 20 pounds in a few months; general aches and pains similar to an acute viral syndrome for more than 10 days; sore or swollen lymph glands for more than a week; appearance of blue or purplish spots on the skin (now recognized as Kaposi’s sarcoma); herpes sores that worsen and persist for more than 5 weeks; and loss of sensory or motor ability or defects in mental or neurological function.

By early 1982, investigators at the Center for Disease Control (CDC) in Atlanta, USA, detected similar cases among injecting drug users, and recipients of blood transfusions and blood products, especially of pooled clotting factors administered for haemophilia. These observations indicated that AIDS was not simply a consequence of gay lifestyle, but was caused by an infectious agent spreading by sexual and parenteral transmission. For infectious disease clinicians, the challenge was to identify the causative microbial agent. Various candidate microbes were postulated as the cause of AIDS, including human T lymphotropic virus (HTLV), African swine fever virus, and a possible fungal infection secreting an immunosuppressive factor like cyclosporin.

3.1.1 Discovery of HIV

Within 2 years after AIDS was defined, a virus was recovered from a person with the lymphadenopathy syndrome which many considered a pre-condition of AIDS. The virus, isolated by Francoise (Barre-Sinoussi *et al.*, 1983) had the unusual
characteristic of infecting peripheral blood mononuclear cells (PBMCs) and causing cytopathic effects within 6 or 7 days. Examined by electron microscopy, it had the morphology of a budding retrovirus, later recognized as a lentivirus (Gonda et al., 1985). This virus was unlike the HTLV, a previously described human retrovirus that can cause leukemia (Poiesz et al., 1980). This lymphadenopathy-associated virus (LAV) did not establish a transformed state in CD4+ cells, but caused cell death after high-level replication (Barre-Sinoussi et al., 1983). After this description of LAV (Barre-Sinoussi et al., 1983), two other groups reported the isolation of retroviruses from AIDS patients. The first described a virus which they called HTLV III, because they believed it to be part of the HTLV family of oncogenic retroviruses (Gallo et al., 1984a), and the second, working with subjects from San Francisco, noted the presence of retroviruses with characteristics of cytopathic agents such as LAV (Levy et al., 1984). Thus, it was concluded that they could not be transforming viruses like HTLV and were called AIDS-associated retroviruses (ARV) (Levy et al., 1984). With time, the retroviruses isolated by all three research groups were found to have similar features, although being somewhat distinct—a characteristic now further appreciated through other research findings (Levy et al., 1998). These viruses were renamed the human immunodeficiency virus (HIV) (Coffin et al., 1986).

3.1.2 Origin of HIV-1

The HIV strains arose from cross-species transmission of non-human primate lentiviruses (Hahn et al., 2000). Evidence for cross-species transmission became apparent in 1985, when studies of West African sex workers revealed the presence of antibodies that were more highly reactive with proteins of simian immunodeficiency virus (SIV) on Western blots than with those of HIV-1 (Barin et al., 1985). This observation led to the discovery and isolation of HIV-2 (Brun Vezinet et al., 1987). Genetically HIV-2 is closely related to the SIV strains than to HIV-1. SIVsm, isolated from sooty mangabeys (Cerocebusatys), is very closely related to HIV-1 isolated from West African humans, and it appears that the sooty mangabey is the source of HIV-1 infection in humans. In contrast, the non-human primate sources for HIV-1 appears to be the chimpanzee, Pan troglodytes (Gao et al., 1999). All HIV-1 strains known to infect humans are closely related to SIVcpz strains.
3.2 Classification

HIV-1 is an enveloped RNA virus belonging to the lentivirus genus in the family Retroviridae (Chiu et al., 1985). HIV-1 reverse transcribes its genome to form copy DNA (cDNA), which integrates into the genomic DNA of the host as a provirus. In vitro cytopathicity, lack of oncogenecity, the establishment of chronic infections, and relatively slow rates of development of disease characterize lentiviruses.

3.2.1 Genotypes, Serotypes and Antigenicity

There are three distinct genetic groups of HIV-1, designated M, O and N. Group M viruses dominate the pandemic. This group is divided into genetic subtypes or clades (A through D, F through H, J, and K) based on nucleotide sequences relatedness. Certain viral isolates appear to be recombinants containing sequences from more than one subtype. These are designated CRF (circulating recombinant form, Robertson et al., 1995a). For example, the former subtype E, prevalent in Thailand, is now believed to be a recombinant between subtypes A and E, and its proposed designation is CRF01-AE. Mosaic viruses that contain parts resembling four or more subtypes are given the suffix cpx for complex; for example, the former subtype 1 has been given the designation CRF04-cpx to indicate that it is complex circulating recombinant form. There are no clearly defined HIV-1 serotypes.

The humoral immune response relevant to immunity concerns the viral envelope glycoprotein (Env), which is exposed on the surfaces of infected cells and virions. The HIV-1 Env is composed of a surface domain (gp120) and a transmembrane domain (gp41), which are non-covalently associated and form trimeric spikes. The peptide sequence of Env contains conserved (C) and variable (V) regions. Env has several features that render it a suboptimal antigen and immunogen (Kwong et al., 1998; Wyatt et al., 1998). The surface of gp120 is heavily glycosylated, and this glycosylation reduces the antigenicity of Env, presumably by allowing the molecule to appear to the immune system as “self”. Regions of Env that are conserved because of their involvement in the binding interactions with cellular molecules required for infectivity is poorly accessible to antibodies. For example, the binding site on gp120 for CD4 is recessed and surrounded by variable
glycosylated regions. Similarly, the binding site for coreceptors is masked by the variable loops V2 and V3 until CD4 is engaged (Rizzuto et al., 1998).

3.2.2 Phenotypes
HIV-1 can also be classified according to phenotype, which does not relate directly to their major genotypic classification. Thus within each HIV-1 subtype there are virus isolates that are syncytium inducing (SI) or are non-syncytium inducing (NSI) for CD4 cells in vitro. Most primary, transmitting HIV-1 strains have an NSI phenotype, while SI substrains tend to appear in uninfected individuals later as they progress to AIDS. As described later, this phenotypic classification is related to cellular tropism for macrophages or T cell lines and to which kind of chemokine receptor the virus uses to gain entry into cells. Most NSI strains utilize the CCR5 coreceptor and are known as R5 viruses, whereas most SI strains utilize the CXCR4 coreceptor and are known as X4 viruses (Berger et al., 1998).

3.3 Epidemiology
3.3.1 Distribution and Geography
The AIDS pandemic can be viewed as a composite of multiple epidemics, each occurring in specific geographic regions and populations. Group M viruses are responsible for the vast majority of HIV-1 infections. Viruses belonging to the distinct subtypes or clades within group M have been isolated in geographically distinct regions of the world. Subtype C is currently predominant in the global epidemic. In the United States, Europe and Australia, subtype B predominates, illustrating a founder effect in which one or several viral variants were introduced and then disseminated through the population (Myers et al., 1992; Foley et al., 2000). Subtype A predominates in West Africa, subtypes A and D predominate in East Africa, and subtype C predominates in Southern Africa. Variants of HIV-1 appear to quickly expand to become the major subtype when introduced into a specific population or geographic area. For example, subtype B viruses predominated among intravenous drug users in Bangkok, while CRF-01AE (formerly designated subtype E) recombinant viruses spread throughout the country by heterosexual contact. No differences in biology or pathogenesis of these subtypes have yet been reported.
The distribution of HIV-1 subtypes is complex and probably reflects a stochastic dissemination. Multiple subtypes cocirculate within areas of Central Africa, Southeast Asia, and South America. In regions where different subtypes are prevalent, recombination between subtypes is apparent (Robertson et al., 1995b). The incidence of co-infection and subsequent recombination may be high in areas such as Africa and Southeast Asia, where multiple subtypes of HIV-1 circulate simultaneously. For example, in Cameroon, mixed infection was observed in approximately 10% of cases and included co-infection with two subtypes within group M, co-infection with M and O viruses, and even co-infection with HIV-1 and HIV-2 (Takehisa et al., 1998). Recombination events have been described between all of the HIV-1 subtypes belonging to group M. Recombination has also been described between group M and O viruses.

3.3.2 Incidence and prevalence of infection

According to World Health Organization estimates over 2.1 million [1.9-2.4 million] people were estimated to have died (Fig.3.3.2a) and 33.2 million [30.6-36.1 million], were living with HIV infection or AIDS in 2007 (Fig.3.3.2b) (UNAIDS, 2007). The incidence of persons become infected with HIV on a worldwide scale is estimated to be over 68,000 per day while over 57,000 die with AIDS per day. Approximately 95% of all HIV infections have occurred in young and middle aged adults in the developing world.

Sub-Saharan Africa remains the most affected region in the global AIDS epidemic. More than two thirds (68%) of all HIV-positive people live in this region while more than three quarters (76%) of all AIDS deaths occurred in 2007. It is estimated that 1.7 million [1.4 million-2.4 million] people were newly infected with HIV in 2007, bringing total number of people living with the virus to 22.5 million [20.9 million-24.3 million]. Unlike other regions, the majority of people living with HIV in sub-Saharan Africa (61%) are women.

In Asia, national HIV prevalence is highest in South-East Asia, with wide variation in epidemic trends between different countries. While the epidemics in Cambodia, Myanmar and Thailand all show declines in HIV prevalence, those in Indonesia (especially in the Papua province) and Vietnam are growing. Although the proportion of people living with HIV in India is lower than previously estimated, its epidemic continues to affect large numbers of people. Overall in Asia, an estimated 4.9 million [3.7 million-6.7 million] people were living with
Chapter 3: Review of Literature

Figure 3.3.2a: Estimated adult and child deaths from AIDS during 2007
(From www.unaids.org)

Figure 3.3.2b: Adults and children estimated to be living with HIV in 2007
(From www.unaids.org)
HIV in 2007, including the 440,000 [210,000–1.0 million] people who became newly infected in the past year. Approximately 300,000 [250,000–470,000] died from AIDS-related illnesses in 2007 (UNAIDS, 2007).

3.3.3 HIV/AIDS in India

Prevalence trends in India vary greatly between states and regions. Even in the four southern states (Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu) where the large majority of people living with HIV are residing, HIV prevalence varies and the epidemic tends to be concentrated in certain districts (NACO, 2005a). Reported adult HIV prevalence in six states included in the recent national population-based survey (NFHS-3, 2007) varied from 0.07% in Uttar Pradesh to 0.34% in Tamil Nadu, 0.62% in Maharashtra, 0.69% in Karnataka, 0.97% in Andhra Pradesh, and 1.13% in Manipur. Prevalence in all other states together was 0.13%. An earlier analysis of sentinel surveillance data also showed that HIV prevalence in southern states was about five times higher than in northern states in 2000–2004 (Kumar et al., 2006) (Fig. 3.3.3). However, pockets of high HIV prevalence (mainly among population groups at high risk of exposure to HIV) have also been identified in states where overall prevalence is generally low, warning against complacency.

Data from the expanded 2006 sentinel surveillance show stable or declining prevalence among pregnant women in Tamil Nadu, Maharashtra, Karnataka, and Andhra Pradesh, but high HIV prevalence among sex workers, and rising HIV prevalence among injecting drug users and men who have sex with men in a few states. Unprotected sex between sex workers and their clients, and their respective other sex partners is a key risk factor for HIV transmission (Kumar et al., 2005). Prevention programmes focusing on sex workers show some success and HIV prevalence is on the decline among sex workers in areas that have been the focus of targeted prevention efforts, especially in Tamil Nadu and other southern states. However, prevention efforts are often complicated by the varied nature of commercial sex (Char et al., 2003).
Figure 3.3.3: Estimated adult HIV prevalence (15-49 years), by state, India 2006 (From www.unaids.org)
3.4 Transmission

Transmission of HIV occurs through direct contact with infected body fluids, including blood, blood products, semen (Ho et al., 1984), vaginal and cervical secretions (Vogt et al., 1986), amniotic fluid (Mundy et al., 1987), and breast milk (Thiry et al., 1985). Despite detection of HIV-1 nucleic acids in saliva and tears, there have been no documented cases of transmission via these fluids. Transmission most commonly occurs during sexual contact with the exchange of semen, genital secretions, or blood from an infected individual to the uninfected partner. Unprotected receptive anal intercourse, with associated mucosal trauma and exposure to infected blood, carries the highest risk of sexual transmission. However, in the majority of instances, transmission from male to female and from female to male takes place during vaginal intercourse, although cases have occurred after fellatio. Infection may be facilitated by the presence of underlying sexually transmitted disease including chancroid, herpes genitalis, and syphilis, which disrupt the integrity of the skin or mucosal lining (Creenbalt et al., 1988).

Infection may also occur through direct inoculation of infected blood, transfusion of infected blood products, transplantation of infected tissue, or reuse of contaminated needles. The risk of HIV-1 transmission following occupational percutaneous exposure to infected blood is approximately 0.3% (Gerberding et al., 1987). The likelihood of transmission may be influenced by many factors including the type of needle (hollow versus solid bore), the depth of penetration, the volume of the inoculum, and the amount of infectious virus in the inoculum.

HIV-1 is also transmitted from an infected mother to her child during pregnancy, delivery, or breast-feeding. The risk of maternal-fetal transmission is 13 to 40% (European Collaborative Study, 1991), but this can be significantly reduced by prophylactic treatment of the mother and the newborn with antiretrovirals (Connor et al., 1994) or by suppression of virus replication by treatment of mothers. Unfortunately, the beneficial effects of perinatal prophylaxis with antiretroviral therapy can be completely lost by subsequent infection of the infant during breastfeeding.
Chapter 3: Review of Literature

3.5 Composition of Virus

3.5.1 The morphologic structure of HIV-1

HIV-1 viral particles have a diameter of 100 nm and are surrounded by a lipoprotein membrane. Each viral particle contains 72 glycoprotein complexes, which are integrated into lipid membrane, and are each composed of trimers of an external glycoprotein gp120 and a transmembrane spanning protein gp41 (Fig. 3.5.1).

![Figure 3.5.1: Structure of an HIV virion particle](From www.hivmedicine.com)

The bonding between gp120 and gp41 is only loose and therefore gp120 may be shed spontaneously within the local environment. Glycoprotein gp120 may also be detected in the serum (Oh et al., 1992) as well as within the lymphatic tissue of HIV-infected patients (Sunila et al., 1997). During the process of budding, the virus may also incorporate different host proteins from the membrane of the host cell into its lipoprotein layer, such as HLA class I and II proteins, or adhesion proteins such as ICAM-1 that may facilitate adhesion to other target cells. The matrix protein p17 is anchored to the inside of the viral lipoprotein membrane. The p24 core antigen contains two copies of HIV-1 RNA. The HIV-1 RNA is part of a protein-nucleic acid complex, which is composed of the nucleoprotein p7 and
the reverse transcriptase p66 (RT). The viral particle contains all the enzymatic equipment that is necessary for replication: a reverse transcriptase (RT), an integrase p32 and a protease p11 (Gelderblom et al., 1993).

3.5.2 The organization of the viral genome

The genomic organization of the HIV-1 proviruses is shown in Fig. 3.5.2 and consists of mainly three genes: gag (group-antigen), pol (polymerase) and env (envelope) (Wong-Staal et al., 1991). The "classical" structural scheme of a retroviral genome is 5' LTR-gag-pol-env-LTR 3'. The LTR ("long terminal repeat") regions represent the two end parts of the viral genome, that are connected to the cellular DNA of the host cell after integration and do not encode for any viral proteins.

Figure 3.5.2: Genomic organization of HIV-1 (From www.hivmedicine.com)

The gag and env genes code for the nucleocapsid and the glycoproteins of the viral membrane; the pol gene codes for the reverse transcriptase and other enzymes. In addition, HIV-1 contains six genes (vif, vpu, vpr, tat, rev and nef) in its 9kB RNA that contributes to its genetic complexity. nef, vif, vpr and vpu were classified as accessory genes in the past, as they are not absolutely required for replication in vitro. However, the regulation and function of these accessory genes and their proteins have been studied and characterized in more detail in the past few years. The accessory genes, nef, tat and rev, are all produced early in the viral replication cycle.

Tat and rev are regulatory proteins that accumulate within the nucleus and bind to defined regions of the viral RNA: TAR (transactivation-response elements) found in the LTR; and RRE (rev response elements) found in the env gene, respectively.
and is essential for viral replication in almost all in vitro culture systems. *Tat* and *rev* stimulate the transcription of proviral HIV-1 DNA into RNA, promote RNA elongation, enhance the transportation of HIV RNA from the nucleus to the cytoplasm and are essential for translation. *Rev* is also a nuclear export factor that is important for switching from the early expression of regulatory proteins to the structural proteins that are synthesized later.

*Nef* may induce down-regulation of CD4 (*Aiken et al.,* 1994) and HLA class I molecules (*Collins et al.,* 1998) from the surface of HIV-1-infected cells, which may represent an important escape mechanism for the virus to evade an attack mediated by cytotoxic CD8+ T-cells. *Nef* may also interfere with T-cell activation by binding to various proteins that are involved in intracellular signal transduction pathways (*Peter et al.,* 1998).

*Vpr* seems to be essential for viral replication in non-dividing cells such as macrophages. It may stimulate the HIV-LTR in addition to a variety of cellular and viral promoters. *vpr* was shown to be important for the transport of the viral pre-integration complex to the nucleus (*Miller et al.,* 1997) and may arrest cells in the G2 phase of the cell cycle.

*Vpu* is important for the virus "budding" process, because mutations in *vpu* are associated with persistence of the viral particles at the host cell surface. *Vpu* is also involved when CD4-gp160 complexes are degraded within the endoplasmic reticulum and therefore allows recycling of gp160 for the formation of new virions (*Bour et al.,* 1995; *Cullen et al.,* 1998).

*vif* plays important role in supporting viral replication (*Mariani et al.,* 2003). *Vif*-deficient HIV-1 isolates do not replicate in CD4+ T-cells, some T cell lines ("non-permissive cells") or in macrophages. *Vif*-deficient isolates are able to enter a target cell and initiate reverse transcription, but synthesis of proviral DNA remains incomplete.

### 3.6 The HIV replication cycle

HIV-1 is an enveloped virus that enters the organism via mucous membranes or intravenously. Env consists of gp120 (SU, surface subunit) and gp41 (TM, transmembrane sub-unit). Dendritic cells play an important role in capturing and transporting the virus to lymphoid organs. They express dendritic cell-specific
transporting the virus to lymphoid organs. They express dendritic cell-specific intracellular adhesion molecule 3(ICAM-3)-grabbing integrin (DC-SIGN), which binds gp120 and stores viral particles in an infectious form (Geijtenbeek et al., 2000b). Gp120 then binds CD4 on the surface of macrophages and T lymphocytes (Landau et al., 1988), which is one of the receptors for HIV-1 (Maddon et al., 1986) (Fig. 3.6).

![Diagram of the replicative cycle of HIV-1](image)

**Figure 3.6: The replicative cycle of HIV-1 (From Freed et al., 2006)**

Upon a conformational change in gp120 (Wu et al., 1996; Trkola et al., 1996), Env interacts with the CC chemokine receptor 5 (CCR5: R5, macrophage tropic or non-syncytium inducing (NSI) strains) (Deng et al., 1996), or CXC chemokine receptor 4 (CXCR4: X4, T cell tropic or syncytium inducing (SI) strains) (Feng et al., 1996). These interactions prompt a conformational change in gp41, which mediates the fusion between the virus and the host cell (Chan et al., 1998). Once
genomic RNA into the double stranded cDNA. During this stage, HIV-1 has to counteract two types of host factors that block retroviral infection: TRIM5 and APOBEC family members. The completion of reverse transcription gives rise to the HIV-1 pre-integration complex (PIC), which is composed of double-stranded viral cDNA, integrase (IN), matrix (MA), Vpr, RT, and the high-mobility group DNA-binding protein, HMGI (Y) (Miller et al., 1997). The bulky PIC slides down microtubules and traverses the nucleus with the help of multiple nuclear localization signals (NLSs) in IN, MA, RT, and Vpr proteins (Fouchier et al., 1999). With the help of IN, the linear double stranded DNA then integrates predominantly into transcriptionally active regions of the genome (Schroder et al., 2002).

5' and 3' -ends of the provirus contain identical long terminal repeats (LTRs). Despite conventional promoter and enhancer sequences, the HIV-1 LTR only promotes the synthesis of short transcripts in its basal state. For the efficient elongation of transcription, its enhancer must be occupied or Tat must be synthesized.

Tat binds the transactivation response (TAR) RNA stem loop with the help of the positive transcription elongation factor b (P-TEFb). P-TEFb, which contains cyclin T1 and cyclin dependent kinase 9 (Cdk9), phosphorylates the C-terminal domain (CTD) of RNA polymerase II and negative effectors, thus allowing transcription to elongate on the viral genome (Garber et al., 1999). One full-length genomic mRNA (9 kb) gives rise to 46 different spliced products of two types, singly spliced (4.4 kb) and multiply spliced (2 kb) transcripts (Purcell et al., 1993), whereas the latter exit the nucleus freely, the former require Rev and the Rev response element (RRE) RNA structure in the middle of the env gene for their transport into the cytoplasm (Pollard et al., 1998). Rev also binds CRM1 and this export requires Ran GTP. Upon the hydrolysis of GTP to GDP in the cytoplasm, HIV-1 transcripts are released and translated (Cullen et al., 2003). Alternative splicing and transport compete in this process, and if splicing is too efficient, insufficient genomic RNA is present in the cytoplasm for virion assembly.

After viral proteins are translated in the cytoplasm, they are assembled into new virions in lipid rafts on cellular membranes (Nguyen et al., 2000). In T and fibroblastic cells, the assembly and release occur at the cell surface. In
macrophages and dendritic cells, HIV-1 assembles on the endosomal membranes and buds into multivesicular bodies (MVBs) (Raposo et al., 2002). Eventually, these organelles fuse with the plasma membrane and viral particles are released. Of interest, p6 (L, late domain) of Gag binds components of ESCRT machinery and assembles MVBs at these sites of virus budding (Garrus et al., 2001; Martin-Serrano et al., 2001; Strack et al., 2003; von Schwedler et al., 2003). The further maturation of virions occurs after the formation of active protease dimers, which cleave Gag and Pol polyprotein precursors into their functional subunits. The virus assumes its mature shape with a clearly defined inner cigar-shaped core and outer dodecahedral envelope.

3.7 Pathogenesis of HIV infection

Viral infections are often transmitted through the mucosa. HIV-1 enters through intact or damaged mucosal epithelium and infects the underlying Langerhans cells and macrophages in the submucosa of the rectum or vagina. However, studies indicate that CD4+ cells are also preferentially infected initially (Stahl-Hennig et al., 1999; Veazey et al., 1997). The infected cells disseminate to regional lymph nodes where viral replication occurs (Spira et al., 1996).

3.7.1 Primary HIV infection

A general viremia follows, accompanied by lymphadenopathy, fever and flu-like symptoms in 50 to 70 percent of the cases. The infection is widely disseminated during the primary infection, as seen by the dramatic loss of CD4+ T cells, leading to an initial immunodeficiency. The seeding of virus during primary infection, especially in the lymphoid organs, may influence the subsequent course of HIV-1 infection. Moreover, as CTL responses develop, viremia falls dramatically (Koup et al., 1994). Strong HIV-1 specific antibody responses develop during the first weeks after initial infection (seroconversion) but antibody present immediately after infection is not neutralizing (Moore et al., 1994) (Fig. 3.7). However, this immunity is apparently inadequate to suppress viral replication completely, since HIV-1 expression persists in lymph nodes even with undetectable viremia.
3.7.2 Latency

The term clinical latency is misleading. After primary infection, a mounting of HIV specific humoral and cell mediated immune response is initiated successfully and virus is not readily detectable in plasma. However, this period is characterized by a vigorous battle between the virus and the immune responses (Fig.3.7). An enormous amount of virus and CD4 cells are killed each day and outbreaks of transient viremia are commonly observed during this time. Although CD4 levels are maintained for a variable period of time, continuous depletion of CD4 cells is seen in most patients.

![Figure 3.7: Immune responses and viremia during HIV infection](From www.msu.edu/course/isb/202/ebertmay/images/Hiv-timecourse.png)

3.7.3 Progression of HIV infection

Advances have been made in elucidating the genetic, immunologic, and virologic factors in HIV-infected individuals who either progress rapidly or do not progress to AIDS. In addition, natural immune responses to HIV may be protective in rare individuals, as evidenced by the detection of HIV-specific immune responses in HIV-negative individuals who have been exposed to the virus many times (Mazzoli et al., 1997; Heeney et al., 1999; Williams et al., 1999). The clinical
progression in HIV-1 infection follows a number of diverse courses. Rapid progressors constitute 10% of HIV infected individuals and develop AIDS within 2 to 3 years of HIV infection. Approximately 5 to 10% of HIV-infected subjects, nonprogressors, are clinically asymptomatic after 7 to 10 years. The remaining subjects with HIV-infection, typical progressors, develop AIDS within a median time of approximately 10 years from initial infection. Approximately 10 to 20% of HIV infected individuals will be AIDS-free 20 years after infection (Haynes et al., 1996).

3.7.4 Development of clinical disease

The progressive deterioration of the immune system that occurs in most patients with HIV infection inevitably leads to an AIDS-defining illness. Opportunistic infections or neoplasms, as well as severe and persistent constitutional signs and symptoms, are included for the diagnosis of AIDS (CDC, 1985; CDC 1992). HIV related oral manifestations are prevalent during late progression to AIDS (Lucht et al., 1991). In addition, infection of the CNS may lead to distinct HIV-1 associated diseases, including the HIV associated dementia complex, vacuolar myelopathy, and sensory neuropathy (Price et al., 1996a; Price et al., 1996b).

Several disease classifications have been introduced since the first description of AIDS in 1981 (Gottlieb et al., 1981). The currently used Centers for Disease Control and prevention (CDC) 1993 classification (Table 3.7.4) is based on a combination of clinical and CD4 T-cell count categories that define nine mutually exclusive stages.
### Table 3.7.4: CDC 1993 classification of HIV infection

<table>
<thead>
<tr>
<th>CD4 cell category</th>
<th>Clinical category</th>
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<tbody>
<tr>
<td></td>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 (≥500/µl; ≥29% of lymphocyte count)</td>
<td>A1</td>
</tr>
<tr>
<td>2 (200-499/µl; 14-28% of lymphocyte count)</td>
<td>A2</td>
</tr>
<tr>
<td>3 (&lt;200/µl; &lt;14% of lymphocyte count)</td>
<td>A3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Asymptomatic, acute (primary) HIV or PGL.

<sup>b</sup>Symptomatic; not category A or C conditions (see Table 3.7.4a)

<sup>c</sup>AIDS indicator conditions (see Table 3.7.4b)

(Adapted from Principles and Practice of Clinical Virology, 4th Edition)

### Table 3.7.4a: Signs and conditions defining HIV clinical category B

Symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical category C and that meet at least one of the following criteria: (i) attributed to HIV infection or indicative of a defect in cell-mediated immunity or (ii) considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples include the following:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe) or cervical carcinoma in situ
- Constitutional symptoms, such as fever or diarrhea for > 1 month
- Hairy leukoplakia, oral
- Herpes zoster (singles) involving at least two distinct episodes
- Idiopathic thrombocytopenia purpura
- Listeriosis
- Pelvic inflammatory disease
- Peripheral neuropathy

(Adapted from Principles and Practice of Clinical Virology, 4th Edition)
| Table 3.7.4b: Signs and conditions defining HIV clinical category C  
(AIDS indicator diseases) |
<table>
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<tbody>
<tr>
<td>Candidiasis of bronchi, trachea, or lungs</td>
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<tr>
<td>Candidiasis, esophageal</td>
</tr>
<tr>
<td>Cervical cancer, invasive</td>
</tr>
<tr>
<td>Coccidiodomycosis, disseminated or extrapulmonary</td>
</tr>
<tr>
<td>Cryptococcosis, extrapulmonary</td>
</tr>
<tr>
<td>Cryptosporidiosis, chronic intestinal (&gt; month’s duration)</td>
</tr>
<tr>
<td>Cytomegalovirus disease (other than liver, spleen, or nodes)</td>
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<tr>
<td>Cytomegalovirus retinitis (with loss of vision)</td>
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<tr>
<td>Encephalopathy, HIV-related</td>
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<tr>
<td>Herpes simplex: chronic ulcers</td>
</tr>
<tr>
<td>Histoplasmosis, disseminated or extrapulmonary</td>
</tr>
<tr>
<td>Isosporiasis, chronic intestinal (&gt;1 months duration)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>Lymphoma, Burkitt’s</td>
</tr>
<tr>
<td>Lymphoma, immunoblastic</td>
</tr>
<tr>
<td>Lymphoma, primary, of brain</td>
</tr>
<tr>
<td>Mycobacterium avium complex, disseminated or extrapulmonary</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)</td>
</tr>
<tr>
<td>Mycobacterium, other species or unidentified species, disseminated or extrapulmonary</td>
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<td>P. carinii pneumonia</td>
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<tr>
<td>Progressive multifocal leukoencephalopathy</td>
</tr>
<tr>
<td>Salmonella septicemia, recurrent</td>
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<tr>
<td>Toxoplasmosis of brain</td>
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<tr>
<td>Wasting syndrome due to HIV</td>
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</tbody>
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(Adapted from Principles and Practice of Clinical Virology, 4th Edition)
3.8 Cell tropism and HIV receptors
HIV mainly infects CD4+ cells by binding to CD4 as a receptor (Dalgleish et al., 1984). However, many of these cells are not lymphocytes. For example, monocyte, macrophages, dendritic cells (langerhans cells) and some brain cells, such as the microglia, express the CD4 receptors, and many are infectible with HIV. It is likely that infection of these cell types plays a major role in the pathogenesis of disease. HIV can infect some macrophages and dendritic cells without killing them, and hence they may form an important reservoir for persistent infection. Some isolates preferentially infect monocytes compared to T cell lines, and vice versa. The molecular changes that determine this remarkable difference in tropism are small. Changes in the long terminal repeat may affect the envelope, including the V3 loop, as well as nuclear factors.

The CD4 cell surface antigen is necessary but not sufficient for HIV infection (Dalgleish et al., 1984; Maddon et al., 1986). While CD4 is required for high affinity binding of gp120, coreceptors are required for subsequent steps leading to fusion between the viral envelope and cell membrane. The coreceptors were identified in 1996 to be chemokine receptors and help to explain the differential tropism for lymphocytes and macrophages. Primary, NSI (non-syncytium inducing) strains of HIV mainly utilize CCR5, which is the receptor for the chemokine MIP-1 alpha, MIP-1beta and RANTES, whereas SI (syncytium inducing) strains utilize CXCR4, the receptor for stromal derived factor 1 (SDF-1) (Fig.3.8).

![Figure 3.8: Cellular tropism of HIV virus](image)

The discovery that chemokine receptors act as coreceptors to CD4 (Feng et al., 1996; Weiss et al., 1996) helped to explain why certain beta -Chemokines can
inhibit HIV-1 infection invitro (Cocchi et al., 1995). Modified chemokines and small molecular receptor blockers can potently inhibit HIV-1 entry and may have promise in therapy (Schols et al., 1997; Simmons et al., 1997; Donzella et al., 1998).

The importance of the CCR5 coreceptor for NSI viruses in HIV transmission and disease progression is borne out in people with mutations in the gene for this receptor. In Caucasians populations a deletion in CCR5, rendering it non-functional, occurs frequently; people homozygous for this ‘delta 32’ mutation are resistant to NSI HIV-1 infection, even when exposed, for example by regular sexual contact with an HIV carrier (O’Brien et al., 1997).

3.9 Risk factors for HIV/AIDS

A risk factor is anything that increases the chance of getting a disease such as HIV/AIDS. There are several risk factors that have been found to increase the chances of developing HIV/AIDS. The established risk factors for HIV/AIDS are given below

3.9.1 Biological risk factors:

Many biological risk factors (both one’s own and one’s partners) make it easier for HIV to enter the body, including:
✓ Presence of other STIs/STDs
✓ Structure of the vagina and of the anus
✓ Viral load
✓ Tissue/membrane vulnerability
✓ Genetic character of the virus
✓ Genetic character of the host

3.9.2 Psychological factors

Individual psychological factors shape HIV risk behaviors. These include:
✓ Personality
✓ Beliefs about HIV/AIDS
✓ Risk perception
✓ Communication styles with sexual partners
✓ Mental health disorders
✓ Depression and psychological distress
3.9.3 Demographic and population-based factors

HIV risk behaviors are shaped in the context of demographic factors and population-based factors. These include:

✓ Race/ethnicity
✓ Age
✓ Sexual orientation
✓ Gender or transgender
✓ Migration
✓ The number of HIV positive people in the population
✓ The frequency of risky behaviors in the population

3.9.4 Social and cultural factors

HIV risk behaviors are shaped by social and cultural factors:

✓ Inequality
✓ Discrimination
✓ Stigma
✓ Cultural rituals
✓ Economics
✓ Individuals and social poverty
✓ Community transitions
✓ The availability and accessibility of medical and social services

3.10 Host genetic factors and HIV-1 infection

The primary risk factors for HIV-1 infection are unprotected sexual intercourse, sharing of syringes, and being an infant born to an infected mother. In most cases, behavioral modification remains a foremost priority with regard to prevention of infection. The importance of biological and genetic differences between individuals in explaining differential susceptibility to HIV-1 infection is largely unknown.

The course of HIV-1 infection varies widely even among individuals with similar risk exposure levels (Mazzoli et al., 1997; Goh et al., 1999; Plummer et al., 1999). There is considerable heterogeneity among individuals in infection susceptibility, in the time required to deplete the CD4 T-lymphocytes population and to develop AIDS-defining diseases (Phair et al., 1992; Detels et al., 1994; Pisani et al., 2000). For example some sex workers and homosexual men have remained uninfected.
Despite repeatedly engaging in unprotected sexual intercourse with HIV-1-infected partners or constantly engaging in high-risk behavior (Detels et al., 1994; Shearer et al., 1996; Plummer et al., 1999). A small fraction of HIV-1-infected individuals remain clinically and immunologically healthy for 10 years or more after seroconversion. Conversely, the disease of another significant fraction of patients is characterized by extremely rapid progression within a period as short as 1 year. The possible role of host genetics in determining susceptibility to HIV-1 exposure is also suggested by the differential immunological responses that individuals have during the course of infection. Although a myriad of social and economic factors strongly influence the HIV-1 pandemic, virologic and host genetic factors probably account for a portion of the observed epidemiological heterogeneity in infection susceptibility and in progression rate (Cheng-Mayer et al., 1991; Shioda et al., 1992; Richman et al., 1994; Kanki et al., 1999; O’Brien et al., 2000; Renjifo et al., 2001; Gonzalez et al., 2001; Roof et al., 2002). Immunologic and genetic studies of high-risk exposed uninfected, such as discordant couples who have unprotected sex and commercial sex workers have helped to elucidate protective mechanism for HIV-1 infection.

Numerous genes have been implicated in the outcome of HIV infection, leading to the characteristic variability of disease progression following HIV infection. The genes that have been identified are involved in several stages of HIV replication, including viral entry, immune regulation following infection and adaptive immunity to HIV.

3.10.1 AIDS Restriction Genes (ARGs)

The role of host cellular factors and their genetic variation in modulating HIV infection and disease progression is well known. A vast literature of AIDS pathogenesis has suggested several candidate genes including HIV-1 coreceptors, their ligands, cytokines and their receptors, transcription factors, immune response genes, other factors that participate in HIV-1-mediated immune destruction (Table 3.10). Among these several genes, genetic association analysis of several large AIDS cohorts implicates that some genes called as AIDS Restriction Genes (ARGs; human genes with polymorphic variants that influence the outcome of HIV-1 exposure or infection) have considerable translational impact on individual and population sensitivity to AIDS (O’Brien et al., 2004).
### Table 3.10: Human genes that limit AIDS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Mode</th>
<th>Effect</th>
<th>Mechanism of Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV entry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR5</td>
<td>Δ32</td>
<td>Recessive</td>
<td>Prevent infection</td>
<td>Knockout CCR5 expression</td>
<td>Dean et al., 1996</td>
</tr>
<tr>
<td></td>
<td>A32</td>
<td>Dominant</td>
<td>Prevent lymphoma</td>
<td>Decrease available CCR5</td>
<td>Dean et al., 1999</td>
</tr>
<tr>
<td></td>
<td>A32</td>
<td>Dominant</td>
<td>Delay AIDS</td>
<td>Decrease available CCR5</td>
<td>Dean et al., 1996</td>
</tr>
<tr>
<td>CCR5</td>
<td>P1</td>
<td>Recessive</td>
<td>Accelerates AIDS</td>
<td>Increase CCR5 expression</td>
<td>Martin et al., 1998</td>
</tr>
<tr>
<td>CCR2</td>
<td>l64</td>
<td>Dominant</td>
<td>Delay AIDS</td>
<td>Interact with and reduce CXCR4</td>
<td>Smith et al., 1997</td>
</tr>
<tr>
<td>CCL5</td>
<td>In1.1C</td>
<td>Dominant</td>
<td>Accelerates AIDS</td>
<td>Decrease RANTES expression</td>
<td>An et al., 2002</td>
</tr>
<tr>
<td>CXCL12</td>
<td>3’A</td>
<td>Recessive</td>
<td>Delay AIDS</td>
<td>Impede CCR5-CXCR4 transition</td>
<td>Winkler et al., 1998</td>
</tr>
<tr>
<td>CXCR6</td>
<td>E3K</td>
<td>Dominant</td>
<td>Accelerates PCP</td>
<td>Alter T-cell activations</td>
<td>Duggal et al., 2003</td>
</tr>
<tr>
<td>CCL2-CCL7-CCL11</td>
<td>H7</td>
<td>Dominant</td>
<td>Enhance infection</td>
<td>Stimulate immune response</td>
<td>Modi et al., 2003</td>
</tr>
<tr>
<td><strong>Cytokine anti-HIV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL10</td>
<td>5’A</td>
<td>Dominant</td>
<td>Limit infection</td>
<td>Decrease IL-10 expression</td>
<td>Shin et al., 2000</td>
</tr>
<tr>
<td></td>
<td>5’A</td>
<td>Dominant</td>
<td>Accelerates AIDS</td>
<td>Decrease IL-10 expression</td>
<td>Shin et al., 2000</td>
</tr>
<tr>
<td>IFNG</td>
<td>-179T</td>
<td>Dominant</td>
<td>Accelerates AIDS</td>
<td></td>
<td>An et al., 2003</td>
</tr>
<tr>
<td><strong>Acquired immunity, cell mediated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA</td>
<td>A,B,C</td>
<td>Homozygous</td>
<td>Accelerates AIDS</td>
<td>Decrease breadth of HLA class 1 epitope recognition</td>
<td>Carrington et al., 1999</td>
</tr>
<tr>
<td></td>
<td>B*27</td>
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<td>Delay HIV-1 escape</td>
<td>Carrington et al., 2003</td>
</tr>
<tr>
<td></td>
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<td>Delay AIDS</td>
<td>Delay HIV-1 escape</td>
<td>Carrington et al., 2003</td>
</tr>
<tr>
<td></td>
<td>B*35-Px</td>
<td>Codominant</td>
<td>Accelerates AIDS</td>
<td>Deflect CD8-T cell clearance of HIV-1</td>
<td>Gao et al., 2001</td>
</tr>
<tr>
<td><strong>Acquired immunity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR3DS1</td>
<td>3DS1</td>
<td>Epistatic with HLA-Bw4</td>
<td>Delay AIDS</td>
<td>Clear HIV+ HLA cells</td>
<td>Martin et al., 2002</td>
</tr>
</tbody>
</table>

(Adapted from O’Brien et al., 2004)
3.10.2 C-C chemokine receptor 5 (CCR5)

The entry of HIV-1 into its target cells is mediated by the viral envelope glycoproteins such as gp120, which binds the cellular receptor CD4. However, HIV-1 also needs coreceptor for entry into target cells. In 1996, chemokine receptor CCR5 was reported to be HIV-1 coreceptor used by R5 strains. CC chemokine CCL5 (or RANTES [regulated on activation normal T cell expressed and secreted]), CCL3 (or MIP-1α [macrophage inflammatory peptide 1α]), and CCL4 (MIP-1β) are natural ligands of CCR5 and competitively inhibit the replication of R5 viruses.

The CCR5 gene is located in the 3p21.3 region of the human genome, together with other inflammatory chemokine receptor genes. The whole coding sequence of CCR5 is located within a single exon, but introns interrupt the 3' untranslated region of the gene (Fig. 3.10.2). Genetic studies have reported various mutations in the promoter and coding region of CCR5 gene which were associated with varied clinical outcomes in HIV-1 infection.

![Figure 3.10.2: Structure of the CCR5 locus, single nucleotide polymorphisms and main haplotypes (From Arenzana et al., 2006)](image)

3.10.2.1 CCR5delta32

The identification of CCR5 as a major co-receptor for macrophage-tropic HIV-1 strains stimulated the search for mutations of the gene that would impair the receptor function and represent a molecular basis for the resistance to HIV infection characterizing some individuals. Sequencing of the CCR5 coding region
in randomly selected or exposed uninfected individuals allowed to identify a first mutant allele of the CCR5 gene bearing a 32 bp deletion in a region corresponding to the second extracellular loop of the receptor (Fig. 3.10.2) (Samson et al., 1996; Liu et al., 1996). This mutation has likely been caused by replicative slippage due to the presence of a direct repeat flanking the deleted region (Samson et al., 1996). This mutant encodes a truncated receptor with only four transmembrane segments. The CCR5Δ32 mutant is not functional as a chemokine receptor, it is not expressed at the cell surface, neither in natural leukocyte populations, nor on transfected cell lines. The improperly folded truncated receptor is retained in the endoplasmic reticulum, where it can be detected by monoclonal antibodies (Rana et al., 1997). Homozygotes for the Δ32 mutation are highly protected against HIV infection (Samson et al., 1996; Liu et al., 1996; Dean et al., 1996) and peripheral blood mononuclear cells (PBMCs) from homozygous individuals are uninfectable with M-tropic HIV-1 strains, while entry and infection by T-tropic strains is unaffected (Samson et al., 1996; Rana et al., 1997). The strong protection of homozygotes was confirmed in subsequent studies, including in situations of massive parenteral contact with the virus (Kupfer et al., 1999), but a few seropositive individuals were reported as homozygous for Δ32, demonstrating that protection is incomplete (Balotta et al., 1997; Theodorou et al., 1997; O’Brien et al., 1997; Biti et al., 1997; Michael et al., 1998; Heiken et al., 1999; Kuipers et al., 1999; Sheppard et al., 2002). In some of these cases, the HIV strain was characterized as using exclusively CXCR4 as co-receptor (O’Brien et al., 1997; Michael et al., 1998; Sheppard et al., 2002). These rare cases of initial transmission and propagation of X4 HIV viruses further underline the preponderant role played by CCR5 or R5 viruses in the initiation of HIV infection and the paradox of the inefficient transmission or evolution of X4 viruses despite the constitutive expression of CXCR4 in CD4-expressing cells and the abundance of these HIV target cells (Margolis et al., 2006).

Heterozygotes were found to display slower progression to clinical stages of AIDS (Dean et al., 1996). This association was found in most cohorts (Huang et al., 1996; Stewart et al., 1997; Zimmerman et al., 1997; Eugen-Olsen et al., 1997; Michael et al., 1997; Balfe et al., 1998; Hendel et al., 1998; Mummididi et al., 1998; Magierowska et al., 1999b; Mas et al., 1999; Meyer et al., 1999; Ioannidis...
et al., 2001; Pasi et al., 2000; Marmor et al., 2001), but not all (Eskild et al., 1998; Wilkinson et al., 1998; Schinkel et al., 1999). Some studies suggested that heterozygotes could be partially protected against HIV infection (Eugen-Olsen et al., 1997; Hoffman et al., 1997; Philpott et al., 1999; Pasi et al., 2000; Marmor et al., 2001), but this was not confirmed in numerous other studies, and this effect must be considered as mild. Since heterozygotes for the Δ32 allele represent up to 30% in some populations, it may have a significant impact on the average progression to AIDS in these populations.

CCR5 levels at the surface of leukocytes was found to be reduced in Δ32 heterozygotes as compared to homozygotes for the wild-type allele, affecting ex vivo infection of lymphocytes by M-tropic HIV-1 strains (Wu et al., 1997). This observation suggests that regulation of CCR5 expression does not compensate for the nonfunctional allele. It was also proposed that CCR5 expression in heterozygotes was decreased by more than 50% on average, suggesting that the variant receptor could act as a dominant negative mutant. CCR5 is indeed well characterized as forming homodimers, as well as heterodimers with CCR2 (Springael et al., 2005). Dimerization of the mutant receptor with the wild-type receptor was therefore proposed as a potential mechanism preventing the normal traffic of the wild-type receptor to the cell surface, and its retention in the endoplasmic reticulum (Benkirane et al., 1997). More recently, a dominant negative effect of Δ32 acting on CCR5 and CXCR4 was further suggested, resulting in ineffective infection by X4 viruses as well of primary cells expressing both co-receptors (Agrawal et al., 2004). Extensive analysis of CCR5 surface expression in Δ32 heterozygotes has however revealed that expression is on average half of what is found in wtCCR5 homozygotes (Mariani et al., 1999; Venkatesan et al., 2001). The partial resistance to the virus can therefore be attributed to a gene dosage effect rather than a dominant negative property of the Δ32 mutant. The slower replication of M-tropic HIV-1 strains in cells expressing functional CCR5 from a single allele was confirmed in SCID mice grafted with human leukocytes derived from Δ32 heterozygotes (Picchio et al., 1997).

### 3.10.2.2 CCR5 promoter

A number of promoter variants have been described, all consisting in single base substitutions in the downstream promoter region, the first exon of the major
transcript, or the following intron (Fig.3.10.2) (Mummidi et al., 1998; McDermott et al., 1998; Martin et al., 1998; Gonzalez et al., 1999). Similarly, a number of haplotypes were determined, and these were associated with differential transcriptional regulation, as various polymorphisms affected the binding sites of transcription factors such as NF-κB (Mummidi et al., 2000). The frequencies of the CCR5 haplotypes in various populations were estimated, and their association with AIDS progression analyzed. By including the Δ32 and CCR2-64I variants, Martin (Martin et al., 1998) identified six major haplotypes in Caucasians, one of them (CCR5P1) being associated with AIDS progression. Individuals carrying CCR5 and CCR2 wild type alleles and homozygous for CCR5P1 were found to progress more rapidly to AIDS. Although striking differences on CCR5 expression was found within individuals carrying each genotype, comparative functional testing of the polymorphism-encoding promoter regions, or direct measure of CCR5 expression on white blood cells from analysis in healthy volunteers carrying P1/P1, P1/P4 or P4/P4 genotypes, did not allow to demonstrate significant modification of CCR5 expression levels (Martin et al., 1998). Similarly, an association with disease progression was also reported for an A/G polymorphism (59029 base pair, Genbank U95626), located in the first CCR5 intron. In the absence of Δ32 or 64I mutations, 59029-A/A individuals progressed more rapidly to AIDS and/or death (McDermott et al., 1998; Clegg et al., 2000; Knudsen et al., 2001). A reporter-gene expression system showed reduced transcriptional activity for the promoter region encoding the 59029G substitution (McDermott et al., 1998), and this allele was associated with lower CCR5 expression on PBMC (Salkowitz et al., 2003). As the 59029A/G polymorphism (McDermott et al., 1998), is one of the main determinants of the P1 allele (303A/G in Martin et al., 1998)), the conclusions of these studies are essentially concordant.

Other studies (Mummidi et al., 1998; Gonzalez et al., 1999; Tang et al., 2002) have identified additional haplotypes that dissect further the P1/59029A haplotype: HHG*1, HHG*2 (carrying the Δ32 allele), HHF*1, HHF*2 (linked to the CCR2-64I allele) and HHE. Caucasians are characterized by high frequencies of a small number of haplotypes (HHC/P4, HHE and to a lower extent HHG*2 and HHF*2), while populations originating from Africa display a more
widespread distribution pattern of haplotypes. The spectrum of CCR5 haplotypes associated with disease acceleration or retardation was found to differ between African Americans and Caucasians. As an example, the CCR2-64I allele was associated with delayed progression to AIDS in African-Americans, but not in Caucasians. Also, haplotype HHE (included in P1) was associated to rapid progression in Caucasians, but not in African-Americans, while haplotype HHC (P4) was deleterious for African-Americans but not for Caucasians. The phenotypic effect of Δ32 was also shown to depend, in large part, on the identity of the partner allele. In agreement with this, it was recently demonstrated that the combination of the CCR5 Δ32/−2459A and CCR5 wt/−2459G haplotypes results in relatively weak CCR5 expression, and significant protection against HIV transmission (Hladik et al., 2005).

3.10.3 Chemokines Polymorphism
Chemokines are chemoattractant cytokines, that are secreted by cells and serve to regulate chemotaxis (the movement of cells) and adhesion. Once secreted, chemokines attach to other cells via chemokine receptors present on the target cell surface. Eighteen chemokine receptor have been identified, each of which can accept more than one chemokine (Hoffman et al., 2005). The beta-chemokines MIP-1α(CCL3), MIP-1β(CCL4), and RANTES (CCL5) are the natural ligands of CCR5. Two additional variants named CCL3L1 and CCL4L1, encoded by genes arising from the duplication of CCL3 and CCL4, respectively, have also been described (Menten et al., 2002). The role of β-chemokines in HIV infection was first proposed in a seminal article in which the anti-HIV-1 effect of these molecules was reported just a few months before the discovery of CCR5 as co-receptor for HIV-1 (Cocchi et al., 1995). Soon thereafter, several reports found inverse correlations between the levels of β-chemokines in plasma and the rate of disease progression (Ullum et al., 1998; Saha et al., 1998).

3.10.3.1 MIP-1αP (Macrophage Inhibitory Protein-1αP; CCL3L1)
It is widely accepted that genome duplication is a corner-stone mechanism in the adaptability and evolution of organisms, in particular of primates (Bailey et al., 2002; Ciccarelli et al., 2005; Li et al., 2005). Segmental genome duplications lead to regions that exist in at least 2 copies in the haploid human genome. The
duplication events concern roughly 5% of the human genome, and the duplicated regions range between one and several hundred kilobases displaying high identity rates. Some of these regions encompass genes, many of which are either partially deleted or otherwise degenerated into pseudogenes, as the duplication may not be beneficial, or even disadvantageous for the organism. However, under the driving force of adaptative evolution, the function of duplicated genes are sometimes preserved and, eventually, the two copies may acquire distinct physiological roles. Gene duplication usually results in overdosage of the corresponding product, which may, in some circumstances, play an etiologic role in a human disease. However, in other cases, in particular for genes involved in host defense, duplications are thought to play a protective mechanism in the maintenance of immunity.

The duplication of the CCL3L1 gene represents an overwhelming example in support of this assumption. The CCL3 gene arose, together with the related genes CCL4 and CCL5, by duplication of an ancestral gene (Fig.3.10.3.1a). CCL3 and CCL4 were in turn duplicated further, and non-allelic copies (CCL3L1 and CCL4L1, respectively) are found in variable numbers in the human genome (Menten et al., 2002;Townson et al., 2002). The two non-allelic CCL3 isoforms encode highly related proteins (CCL3 and CCL3L1, previously called MIP-1α and MIP-1αP) displaying more than 90% identity (Irving et al., 1990). The CCL3L1 product, is the most potent CCR5 agonist, and the most efficient inhibitor of R5 HIV entry.

Figure 3.10.3.1a: Organization of CCL3L1 gene (From Shao et al., 2007)
The CCL3L1 gene located on chromosome 17q11.2, encodes a 93-amino acid preprotein MIP-1alpha. It contains 3 exons and spans about 1.9 kb (Menten et al., 2002). The mature protein contains 70 amino acids and has a calculated molecular mass of about 7.8 kD. CCL3L1 shares 96% amino acid identity with CCL3. Although the CCL3 gene exists as a single copy per haploid genome, the copy number of the CCL3L1 gene varies among individuals.

Recently, a study performed in more than 1000 individuals belonging to 57 human populations showed inter-individual and inter-population differences in the number of CCL3L1 copies (Gonzalez et al., 2005). The number of copies ranged from 2 to 10, with higher figures in African populations than in non-Africans. The study also investigated the relationship between the number of CCL3L1 copies and the phenotype of susceptibility to HIV infection and AIDS evolution in a large cohort of HIV+ and HIV- individuals with different ethnic origins. The hypothesis was that gene overdosage of CCL3L1 may account for a reduced susceptibility to contract HIV and develop AIDS. The findings revealed that lower number of copies correlated with reduced susceptibility to HIV/AIDS. However, by itself, the number of copies did not determine HIV susceptibility. Rather, it is dependent on how many copies an individual carries as compared to other individuals within the same population. This susceptibility is even greater in individuals who also possess disease-accelerating CCR5 genotypes. This study proved that the number of copies influences the level of chemokine and CCR5-cell surface expression in CD4+ T lymphocytes, and that low CCL3L1 gene dosage was associated with a higher viral set point and greater subsequent T cell loss.

It has been previously shown that high CCR5 ligand and/or low CCR5 receptor expression correlates with HIV/AIDS protection. Indeed, a number of exposed uninfected individuals were reported to exhibit high endogenous levels of CCR5-binding chemokines (Zagury et al., 1998). Thus the protective phenotype against HIV/AIDS associated to higher CCL3L1 copy number is likely accounted by the binding and occupancy of CCR5 by the CCL3L1 product and its subsequent CCR5 endocytosis, which reduces its availability to R5 HIV viruses (Fig.3.10.3.1b). Gonzalez provided direct evidence for the correlation between gene dosage and host defense against pathogens. It also extends the notion that in general and in particular for CCR5-dependent HIV/AIDS susceptibility, ethnicity
is a critical parameter to understand the complex relationship between genotype and phenotype (Gonzalez et al., 2001).

Figure 3.10.3.1b: CCL3L1-mediated down-modulation of CCR5 (From Mackay et al., 2005)

3.10.3.2 RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted; CCL5)

RANTES encoded by CCL5 gene is a CC chemokine, located on chromosome 17q11.2-q12, that chemoattracts leukocyte. It plays a critical role in T-lymphocytes activation and proliferation. It is produced principally by CD8+ T-lymphocytes, platelets and epithelial cells (Cocchi et al., 1995; Moriuchi et al., 1997). It is one of the natural ligands for the chemokine receptor CCR5 and potently suppresses in vitro replication of the R5 strains of HIV-1 (Cocchi et al., 1995; Arenzana-Seisdedos et al., 1996). RANTES acts by blocking binding of the HIV envelope gp120 to CCR5 and by reducing surface levels of CCR5 (Mack et al., 1998). CD4 + T cells from highly exposed-uninfected individuals produce increased amounts compared with random blood donor controls (Paxton et al., 1996a; Paxton et al., 1996b; Furci et al., 1997).

Two single nucleotide polymorphisms (SNP), -403 G/A and -28C/G, in the promoter region of RANTES were initially identified by Liu et al in Japan (Fig.3.10.3.2) (Liu et al., 1999a). The -403A-28G haplotype was shown to be associated with delayed disease progression in HIV-1 infected Japanese, but
Chapter 3: Review of Literature

exerts no influence on the incidence of HIV-1 infection (Liu et al., 1999a). In European-American population, the compound genotype -403G/A -28C/C was reported to be resistant to AIDS progression in one study (McDermott et al., 2000), but not in another (Gonzalez et al., 2001). These RANTES polymorphisms have no effect on HIV-1 infection and disease progression in African-Americans (Gonzalez et al., 2001). Recently, 3 SNPs (-403A in the promoter, In 1.1C in the first intron, and 3'222C in the untranslated region) are found to be associated with increased frequency of HIV-1 infection. Moreover, In 1.1 C allele or haplotypes display a strong association with rapid progression to AIDS among HIV-1 infected African-Americans and European-Americans (An et al., 2002). These and other RANTES SNPs may also influence the varied epidemiology of HIV-1 infection throughout the world (Gonzalez et al., 2001; An et al., 2002).

There is relatively a little information describing variation in the RANTES gene and the association with HIV-1 infection in Chinese population (Liu et al., 2003, Zhao et al., 2004). Liu et al. identified 6 genotypes of RANTES promoter -403 and -28 in the Han Chinese group. RANTES genotypes AC/AG, AC/GC, AG/GC, GC/GC were associated with reduced susceptibility to HIV-1 infection (Liu et al., 2003). However, there was no significant difference in the allele frequencies between living with HIV-1 and HIV negative individuals. There were significant differences of RANTES In1.1C between HIV-1 infected and healthy individuals in males, suggesting that the In 1.1 C-bearing genotypes could increase susceptibility to HIV-1 infection. No such significance was found in females.

![Figure 3.10.3.2: Four SNPs (-403G/A, -28C/G, In 1.1T/C and 3'222T/C) in RANTES gene (From An et al., 2002)](image)

A study conducted on 1082 Chinese blood donors from northern and southern China and 249 HIV patients from southern China indicated that Chinese AIDS
frequency of the -403G allele and haplotype I, -403 G/-28C (p<0.05), and a lower frequency of the -403 A/A genotype (p<0.01). Symptomatic patients had a higher frequency of -28G allele and a lower frequency of the -28 C/C genotype (p<0.01). These results suggest that -403G may be associated with increased susceptibility to HIV infection, while -28G may be associated with advanced disease progression (Zhao et al., 2004).

3.10.4 HIV-1 Transreceptors

Until recently, the cellular entry, molecules for HIV-1 were known to be the T cell surface marker CD4, one of the immunoglobulin superfamily, and the chemokine receptor CXCR4 or CCR5. HIV-1 entry into the target cells can be influenced by several other HIV-1 receptors. Recently, two HIV-1 receptor: DC-SIGN and DC-SIGNR were identified, which plays key role in dissemination of HIV-1. They are novel class of HIV-1 receptor because they do not allow viral infection but binds HIV-1 and enhances its infection of T cells in trans. besides their role in adhesion processes, DC-SIGN and DC-SIGNR also functions as an antigen receptor that captures and internalizes antigens for presentations. Strikingly, HIV-1 circumvents processing after binding DC-SIGN/R and remains infectious for several days after capture.

3.10.4.1 DC-SIGN (DC-specific ICAM3-grabbing nonintegrin; CD209)

Dendritic cell (DC) interaction with HIV is relevant in the pathogenesis of AIDS, favoring both the initial establishment and spread of the infection and the development of antiviral immunity (Pope et al., 1994; Pope et al., 1995). Immature DC (iDC), capable of capturing antigens are present in the skin and mucosa and therefore could be among the first cells encountering HIV (Hussain et al., 1995). Mucosal and blood DC represent the first HIV-1 targets following sexual transmission (Geijtenbeek et al., 2000c) and transmission via blood (Engering et al., 2002a) respectively. Both myeloid DC (myDC) and plasmacytoid DC (pDC) express the required receptors for HIV-1 entry, i.e., CD4, CXCR4, and CCR5, suggesting that they could be infected, although with a lower efficiency than CD4+ T cells or macrophages (Sallusto et al., 1998). In addition, the mechanisms whereby DC promotes HIV-1 replication may vary in different sites. In peripheral lymph nodes during acute infection, virus may be transmitted via DC to T cells in the T cell areas, whereas in chronic infection virus may be transmitted
to T cells in the T cell areas, whereas in chronic infection virus may be transmitted primarily via DC interacting with T cells in germinal centers. At mucosal surfaces, activated DC can support virus replication and transmit virus to adjacent T cells (Steinman et al., 1999).

In addition to CD4, CCR5, and CXCR4, iDC express DC-SIGN (CD209), a mannose-binding, C-type lectin that binds gp120 and might play an important role in virus capture and transmission (Geijtenbeek et al., 2000a). DC-SIGN is expressed in DC derived from blood monocytes or found in lymphoid tissues and beneath genital surfaces (Jameson et al., 2002). The HIV-1 virus can exploit DC migration by binding DC-SIGN on the surface of immature DC via gp120, thus hitch-hiking to its target, the CD4+ T cell (Fig.3.10.4.1a).

![Figure 3.10.4.1a: DC-SIGN mediated HIV-1 transmission](image)

Binding of gp120 by DC-SIGN does not lead to viral fusion; however, the virus is internalized through DC-SIGN into a low-pH compartment in which incoming virus escapes lysosomal degradation and remains infectious for several days before transmission to T cells (Engering et al., 2002b). It has been recently demonstrated that DC-SIGN-mediated HIV internalization is indispensable for both trans-enhancement of T cell infection and retention of viral infectivity. Moreover, long-term transfer of HIV to T cells requires viral fusion that occurs exclusively through DC infection and transmission of viral progeny. Both DC-
SIGN-mediated cis infection of DC and trans-enhancement of T cell infection may occur in vivo, either in mucosal surfaces or in lymph nodes (Burleigh et al., 2006). DCSIGN, encoded by CD209 gene, located on chromosome number 19p13.3, is a type II transmembrane protein, consisting of 404 amino acids with 3 distinct domains: an N-terminal cytoplasmic region, a neck region containing one incomplete seven complete tandem repeats of the 23 amino acid sequence, and a C-terminal domain with homology to C-type lectins (Fig.3.10.4.1b).

Polymorphism analysis of the numbers of repeats present in this neck region suggested that tandem repeats in the neck region are variable and predominantly consisted of 7 repeats among the Caucasians (Bashirova et al., 2001). However, novel variations in the DC-SIGN repeat region were identified which were rare. Cohort studies of HIV-1 seronegative, HIV-1 seropositive and repeatedly exposed seronegative individuals suggest that heterozygosity in the DC-SIGN repeat region may have protective effect on transmission of HIV-1 (Liu et al., 2004) whereas no associations could be established in recent studies on Thais individuals (Wichukchinda et al., 2007).

3.10.4.2 DC-SIGNR (DC-specific ICAM3-grabbing nonintegrin related; CD209L)

The DC-SIGN related lectin DC-SIGNR (also termed L-SIGN), is also a type II C-type lectin receptor and functions as an HIV-1 transreceptor similarly to DC-SIGN. DC-SIGNR shares 77% amino acid identity with DC-SIGN. The main

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**Figure 3.10.4.1.b: Structure of DC-SIGN/R (From Pohlman et al., 2001)**
difference between DC-SIGNR and DC-SIGN is their distribution on different cell types. DC-SIGN is expressed on DC, whereas DC-SIGNR is expressed primarily on sinusoidal and endothelial cells (Soilleux et al., 2000; Bashirova et al., 2001; Pohlman et al., 2001a). DC-SIGNR interacts with the same spectrum of pathogens as DC-SIGN and might particularly promote spread of viruses that target liver and lymph nodes (Baribaud et al., 2002). DC-SIGNR enhances HIV infection of liver sinusoidal endothelial cells (LSEC), which are permissive (Stefan et al., 1992) and might constantly release progeny virions into the blood stream.

The domain organization of DC-SIGNR is similar to previously described domain of DC-SIGN (Fig.3.10.4.1b). The extracellular portion of DC-SIGNR consists of an extended neck region formed by seven complete and one incomplete repeat of a 23 amino acid sequence and a C-terminal carbohydrate recognition domain (CRD) that interacts with glycans on viral envelope glycoprotein (Soilleux et al., 2000). The N-terminal domain is located in the cytoplasm and is followed by a transmembrane domain, which anchors the protein in the cytoplasmic membrane.

In contrast to DC-SIGN, the neck region of DC-SIGNR is polymorphic and can harbor between 3-9 repeats unit (Bashirova et al., 2001; Mummidi et al., 2001). The DC-SIGNR allele with 7 repeat unit is most common among Caucasians (54%) and is thus considered wild type (wt), followed by the alleles 5 and 6 repeat units which were detected in 29% and 12% respectively of the individuals analyzed in one study (Bashirova et al., 2001). In some recent studies, DC-SIGNR homozygous 7/7 repeat in the neck region was associated with an increased risk of HIV-1 infection, whereas heterozygous 7/5 repeat was correlated with resistance to HIV-1 infection (Liu et al., 2006). Earlier studies could not found any association between DC-SIGNR repeat region polymorphism and HIV-1 infection (Lichterfeld et al., 2003).

3.10.5 APOBEC3G (apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like-3G)

Host cells are endowed with another mechanism to halt HIV-1 infection before viral cDNA integration occurs into the host chromosome. The human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like-3G (APOBEC3G), formerly known as CEM15, is an endogenous inhibitor of HIV-1 replication (Harris et al., 2003; Mangeat et al., 2003; Zhang et al., 2003). In the
uracil in the negative-sense single-stranded DNA, resulting in G to A hypermutations in the complementary, positive sense DNA strand (Fig. 3.10.5). This hypermutation leaves the viral cDNA vulnerable to degradation by nucleases. Those cDNAs that manage to integrate into the host chromosomes carry multiple mutations that may result in aberrant viral products (Lecossier et al., 2003; Mariani et al., 2003; Yu et al., 2004b). Recent reports appear to suggest that APOBEC3G also exerts antiviral activities through mechanisms independent of its cytidine deaminase activity (Turelli et al., 2004; Newman et al., 2005). APOBEC3G can be found in cells in two different forms of low and high molecular-mass (LMM and HMM, respectively). APOBEC3G in LMM form is enzymatically active and restricts HIV-1 infection. The HMM form is a catalytically inactive ribonucleoprotein complex that appears to protect against mobilization of endogenous cellular retroelements such as Alu and hY (Chiu et al., 2006; Schumacher et al., 2005; Esnault et al., 2005). This APOBEC3G-induced mechanism of restriction of Alu and hY mobilization is mediated by sequestration of the RNA retroelements into HMM complexes, which are kept away from the L1-dependent retrotransposition machinery (Chiu et al., 2006). APOBEC3G and related deaminases may also protect against other retroviruses and hepatitis B virus, which also relies on a reverse transcription step to complete its life cycle (Mangeat et al., 2003; Turelli et al., 2004).

![Figure 3.10.5: APOBEC3G mediated deamination](image)

The Vif protein binds APOBEC3G in virus producer cells and targets it for degradation in the proteasome, thus preventing APOBEC3G incorporation into
absence of the viral protein Vif, APOBEC3G is incorporated into HIV-1 particles in the producer cell, and during reverse transcription deaminates cytosine bases to uracil in the negative-sense single-stranded DNA, resulting in G to A hypermutations in the complementary, positive sense DNA strand (Fig. 3.10.5). This hypermutation leaves the viral cDNA vulnerable to degradation by nucleases. Those cDNAs that manage to integrate into the host chromosomes carry multiple mutations that may result in aberrant viral products (Lecossier et al., 2003; Mariani et al., 2003; Yu et al., 2004b). Recent reports appear to suggest that APOBEC3G also exerts antiviral activities through mechanisms independent of its cytidine deaminase activity (Turelli et al., 2004; Newman et al., 2005).

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Figure 3.10.5: APOBEC3G mediated deamination (From www.gladstone.ucsf.edu)
virions (Marin et al., 2003; Yu et al., 2004a; Mehle et al., 2004a). To induce APOBEC3G degradation Vif binds the cellular proteins Cul5, elonginB, elonginC and Rbx1, to form a cullin5-based E3 ubiquitin ligase complex that leads to polyubiquitination and ultimately to proteasomal degradation of APOBEC3G and APOBEC3F, which also displays anti-HIV activity (Yu et al., 2004c; Mehle et al., 2004b).

The importance of the APOBEC3G-mediated innate response against HIV-1 is underscored by the existence of both APOBEC3G and Vif genetic variants influencing disease progression. Studies with long-term non-progressor (LTNP) have revealed an association between low viral load and a serine residue at position 132 of Vif. When tested in vitro, HIV-1 carrying Ser132 displayed a five-fold decrease in replication in PBMC, as compared to virus containing the wild type allele (Arg) at the same position (Hassaine et al., 2000). Another study revealed the presence of a two-amino-acid insertion in the Vif protein of a mother's virus and her child's, both LTNP. This amino acid insertion results in an aberrant Vif protein that severely impairs replication when expressed in a recombinant HIV-1 (Alexander et al., 2002). Furthermore, Vif-defective proteins have been isolated from strains of other LTNP individuals (An et al., 2004). The reason why some of these LTNP Vif variants do not revert to encode a functional product is not understood. It is possible that Vif plays other roles in viral replication that constrain its ability to mutate. Alternatively, the genetic background of these LTNP's may have weakened the virus' ability to replicate and mutate in these subjects.

With regards to APOBEC3G, it is plausible that genetic variants with altered inhibitory activity or partially resistant to Vif might alter the course of HIV-1 disease. A variant of APOBEC3G common in African-Americans has been identified, which contains a non-synonymous substitution. This allele carries Arg instead of His at position 186 (186R) and is strongly associated with faster decline of CD4 T cells and accelerated progression to AIDS (An et al., 2004). An extensive analysis of a French cohort failed to identify significant associations between 29 APOBEC3G polymorphisms and disease progression, although discrepancies with previous studies may be explained by the different variables used to analyze disease progression (Do et al., 2005). Another study identified an APOBEC3G variant (C40693T) in a cohort of EU Caucasians subjects. This allele...
was found to be strongly associated with increased risk of infection (Valcke et al., 2006). A recent report revealed an inverse correlation between APOBEC3G mRNA levels and viral load in infected individuals, with the highest APOBEC3G levels observed in LTNP patients (Jin et al., 2005). However, no associations with specific variants in the APOBEC3G gene have been identified in these individuals.