CHAPTER II

LITERATURE REVIEW

Acquired Immunodeficiency Syndrome

AIDS is caused by HIV[1]. AIDS was first described as a new and distinct clinical entity in 1981 by Dr.Gottlielo's group[21-23]. The first cases were recognized because of unusual clustering of diseases such as Kaposi's sarcoma and pneumocystis pneumonia in young homosexual men. In 1982, AIDS cases were also reported in other populations such as IVDUs and hemophiliacs[24-26]. It is now known that HIV may be divided into 2 types. The first type of this virus, to be identified namely HIV-1, was isolated by Montagnier's group at the Institute Pasteur, Paris, in 1983 from a patient with lymphadenopathy syndrome, and was thus named lymphadenopathy-associated virus (LAV)[27]. It was more fully characterized by the same group and by Gallo *et al.*, in a report published in 1984, and named by Gallo as T-lymphotropic virus type III (HTLV-III)[28] and by Levy *et al.*, in San Francisco, AIDS-associated retrovirus (ARV) [29]. A second virus, HIV-2, was isolated from West African patients with AIDS or AIDS related complex (ARC) by Montagnier's group in 1986[30].

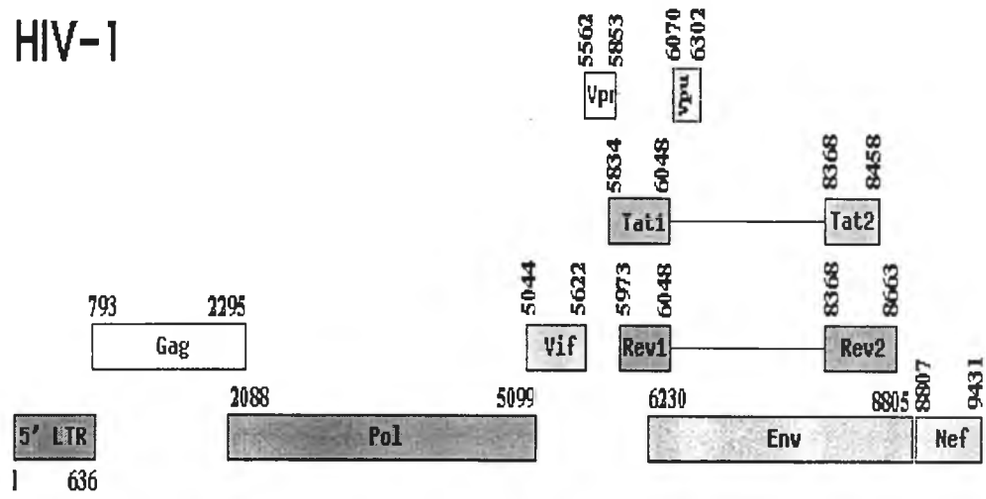
HIV-1 Biology[31-33]

HIV-1 is classified in family *Retroviridae*, subfamily *Lentivirinae* and genus *Lentivirus*. It has a complex genomic organization. Like all retroviruses, HIV-1 has a diploid single-strand plus-sense RNA genome, about 9.2 kilobases (kb) in length, within an icosahedral sphere with a diameter of approximately 100 nm. The outer coat of the virus, known as viral envelope, is composed of two layers of fatty molecules called the lipid bilayer membrane, taken from the bi-lipid membrane when a newly formed virus particle buds from a human cell. The lipid bilayer contains several cellular membrane proteins, including major histocompatibility antigens (MHC), actin and ubiquitin. In addition, the envelope contains viral envelope protein, a tetrameric protein complex, in which each subunit consists of two non-covalently-linked membrane proteins, glycoprotein 120 (gp120, the outer envelope protein) and glycoprotein 41 (gp41, the transmembrane protein). Gp41 anchors the glycoprotein complex to the surface of the virion.

Within the envelope of a mature HIV-1 particle is a cone-shaped capsid, located in the center of the virus, which consists of 2000 copies of another viral protein, p24.

The capsid surrounds two single strands of HIV RNA, each of which has a copy of the virus's nine genes (**Figure 1**).

Fig. 1 Genomic organization of HIV-1. Three structural genes: *gag, pol, env*; 6 accessory genes: *tat, rev, nef, vif, vpu* and *vpr*.



Structural genes : *env* , *gag* , *pol*
Accessory genes : essential regulatory genes (*tat* , *rev*)
: nonessential genes (*nef* , *vif* , *vpu* , *vpr*)

1. Structural genes

Gag (group antigen gene)

The *gag* gene encodes a precusor protein of 53 kilodaltons (p53) that is cleaved into four small products; matrix protein (p17), major capsid protein (p24) and nucleocapsid protein (p7/p9). These proteins constitute the core protein structure of the virus and also subserve nucleic acid and lipid membrane binding functions[34]. The gag proteins of HIV-1, as for other retroviruses, are synthesized as a polyprotein precursor that is subsequently cleaved during the maturation process. This facilitates the assembly of various components of a complex core structure in a three-dimensional configuration when they subsequently cleaved by specific protease acquire specialized functions.

Pol (polymerase gene)

The *pol* gene products are translated from the same genomic RNA message as the gag proteins but in a different overlapping reading frame[35]. *Pol* encodes three proteins; protease (PR), reverse transcriptase (RT) and integrase (IN), that are cleaved from a large precursor polypeptide[36]. HIV-1 protease plays a critical role in

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virus biology, acting specifically to cleave *gag* and *pol* precusor polypeptides into functionally active proteins. The RT of HIV-1 has been shown to be a magnesium 2+ (Mg^{2+}) requiring RNA-dependent DNA polymerase that is responsible for replicating the RNA viral genome[37]. The integrase protein, by analogy with other retroviruses, is important in proviral integration[38].

Env (envelope gene)

The *env* gene encodes a glycosylated polypeptide precusor (gp160) that is processed to form the exterior glycoprotein (gp120) and the transmembrane glycoprotein (gp41)[39]. Both of these constitute the viral envelope that possesses domains responsible for CD4 binding, fusion and epitopes primarily involved in viral-host immune interaction[40].

2. Accessory genes are divided into 2 groups

2.1 Essential regulatory genes[41]

Tat (transactivator of transcription)

Tat protein is transactivator protein to upregulate transcription from the viral promotor in the long terminal repeat (LTR) by binding at the trans-activating response element (TAR).

Rev (regulator protein of expression of viral proteins)

Rev protein promotes export of unspliced and singly spliced viral mRNAs from nucleus by binding at the RRE (rev-responsive element). *Rev* functions as a switch between the early synthesis of highly spliced mRNAs (encoding *tat, rev* and *nef*) and the later synthesis of unspliced (encoding the *gag* and *pol*) and singly-spliced (encoding *env, vpu, vif* and *vpr*) mRNAs.

Nef (negative effector)

Nef protein is required for efficient replication *in vivo* and down-regulates the surface expression of CD4 molecules[42].

2.2 Auxiliary genes

Vpu (viral protein U)

The vpu protein is located in the viral membrane and is essential for virion release and CD4 molecule down-regulation[43].

Vpr (viral protein R)

The protein product functions together with the matrix protein (MA) to lead the preintegration complex (proviral DNA) into nucleus and arrest cellular growth in G2 phase.

Vif (viral infectivity factor)

The protein product is required for virion infectivity, especially in peripheral blood mononuclear cells (PBMC).

The two ends of each strand of HIV-RNA contain a sequence called the LTR[44]. Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cells[42]. The core of HIV also includes protein p7, the HIV nucleocapsid protein; and three enzymes that carry out later steps in the virus's life cycle: RT, integrase and protease. Another HIV protein called p17, or the HIV matrix protein, lies between the viral core and the viral envelope.

Genetic Diversity of HIV-1

Since the discovery of HIV-1, there has been considerable interest in the genetic, antigenic and biological variation of this virus, particularly with regard to the relevance of this variability to HIV vaccine development[45]. Several factors are known to contribute to the generation of new variants and influence the speed with which these viruses evolve. One is the error-prone nature of the viral reverse transcriptase, which lacks proofreading functions and introduces substitutions at a rate of approximately 3 x 10^{-5} nucleotide changes per site per replication cycle[46]. A second factor is the high rate of virus production (up to 10^{10} virions per day) and the large numbers of replication cycles (approximately 300 per day) that sustain HIV-1 infection *in vivo*[47,48]. A third factor is *in vivo* selection pressure, which, for example, is responsible for the rapid emergence of drug resistant viral escape mutants[48]. Together, these mechanisms generate viral variants at extraordinary rates and represent a major force driving HIV-1 evolution in infected populations worldwide[47]. However, recent evidence suggests that recombination among highly divergent HIV-1 strains occurs quite frequently, indicating that this process may also contribute importantly to HIV-1 diversification.

Recombination is a fundamental property of retroviruses resulting from their dimeric RNA genome and RT that can switch between templates during proviral DNA synthesis[49,50]. In fact, it has been suggested that recombination is a necessary component of the viral replication strategy, since breaks in either of the two RNA

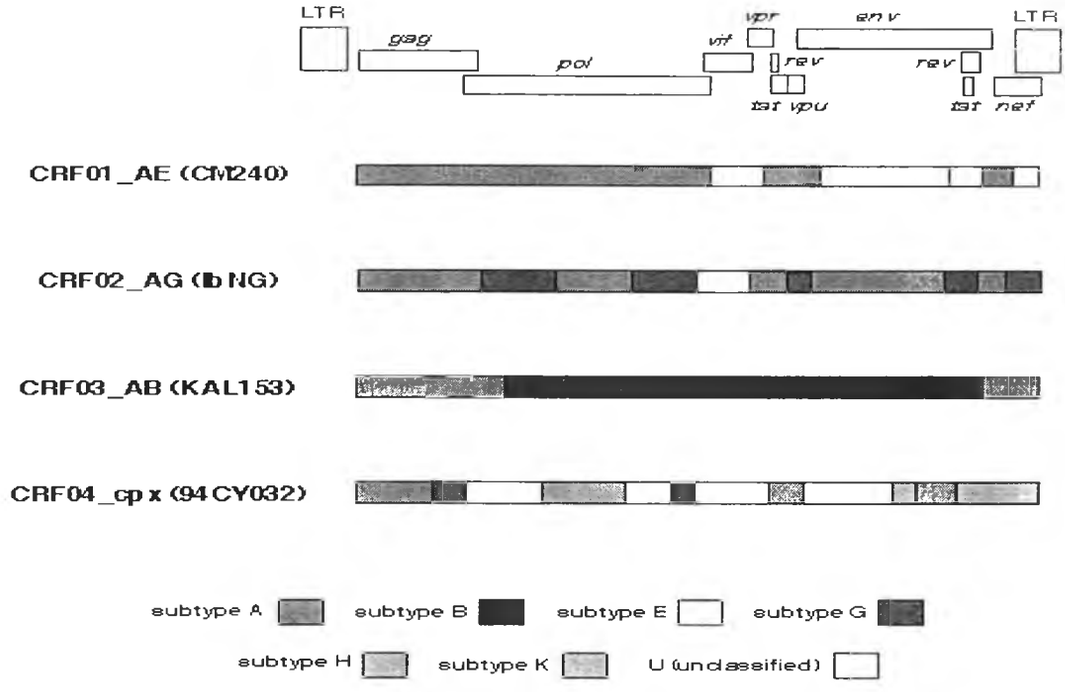
molecules are common[49]. In this process, the RT switches between heterogenous RNA templates as DNA synthesis proceeds. As the number of crossovers increases, so does the complexity of the resulting hybrid genome. Until recently, recombination in HIV-1 was thought to involve only the closely related members of the quasispecies that evolve within infected individuals over time[51]. However, analysis of sequences submitted to the HIV-1 database indicates that recombination also occurs between highly divergent strains of HIV-1, thus raising the possibility that HIV-1 variants can acquire biologically important traits through exchange of genetic material.

To date, based on phylogenetic analyses of the sequences derived from the HIV-1 *env* and *gag* genes, HIV-1 isolates can be classified into three broad groups, designated M, O and N[52], which are defined as distinct clusters on phylogenetic trees. Group M (Major) comprises the great majority of HIV-1 isolates and can be further assigned to 1 of 9 subtypes or genotypes or clades, designated A through K, excluding I [52]. The subtype I designation has been dropped from the nomenclature because the isolates from Cyprus and Greece which were earlier classified as recombinants of A, G and putative new subtype, I[53] have upon reanalysis with previously unavailable complete genome sequences, been revealed to be mosaics with regions associated with subtypes A, G, K, H and unclassified regions (**Figure 2**). Thus subtype I will be removed from the genetic classification system of HIV strains and the I regions will be relabelled as unclassified. The letter "I" will not be reused in the subtype nomenclature to avoid confusion with the existing literature. Group O (Outlier) was discovered more recently and thus far includes only a small number of viruses from Cameroon and Gabon[54]. Both groups are characterized by considerable sequence diversity. For example, env amino acid sequence variation within group M ranges from 3 to 23% among members of the same subtype and from 25 to 35% among members of different subtypes. Group O viruses exhibit a similar level of diversity but have not been classified into subtypes because of the small number of representatives characterized[55]. The two groups, M and O, differ by up to 47% in their env protein sequences. Recently, a new subgroup named N (non-M and non-O) has been identified from Cameroon[56]. Current studies suggest that the distribution of HIV-1 genetic subtypes can vary significantly from country to country and within population groups, can be associated with a particular mode of transmission, and can change over time.

The branching order of phylogenetic trees based on *gag* and *env* sequences in 1993 were very similar for major isolates except for MAL, an African isolate from Zaire, has *env* sequence in subtype D and *gag* sequence in subtype A[57], Thai isolates (CM240) has *env* sequence subtype E and *gag* sequence subtype A. The parental E virus has never been identified[58].

Because of full-length genomic sequencing is not hard to be performed, HIV-1 subtypes in group M currently consist of 9 subtypes (A, B, C, D, F, G, H, J, K) and 4 circulating recombinant forms (CRFs) which are AE (CM240) from southeast Asia, AG (IbNG) from west and central Africa, and AB (KAL153) from Russia and AGI (CY032) Cyprus and Greece. CRFs are recombinant viruses with following criteria: (1) at least two isolates should be sequenced in their entirely, (2) they should resemble each other but no other existing CRF in their subtype structure and (3) they should have been found in at least two epidemiological unliked individuals[59].

Fig. 2 Mosaic genome structures of the four currently recognized circulating recombinant forms: CRF01_AE, CRF02_AG, CRF03_AB, and CRF04_cpx (http://grinch.zoo.ox.ac.uk/HIV/Figure_4.html, 1995)



Most vaccine formulations currently in clinical trials contain immunogens derived exclusively from subtype B viruses, the predominant genotype in the United States and Europe[60]. By contrast, little emphasis has been placed on the development of candidate vaccines against non-subtype B viruses, although these cause the vast majority of HIV-1 infections in developing countries, where they continue to spread extremely rapidly e.g., subtype A in central Africa[61], subtype C in India[62,63] and subtype A/E in Thailand[45,64].

HIV-1 Subtypes in Thailand

A 1985 serosurvey of 600 individuals from high- and low-risk groups including prostitutes, IVDU, patients at sexually transmitted disease (STD) clinics, thalassemia patients and blood donors found only three positive tests[65]. The earliest recorded of HIV infection were predominantly in homosexual and bisexual men which later been overcome by rapid increase in infection in IVDU, second wave of infection. A sudden rise in an epidemic proportion among IVDUs in Bangkok was noted during 1988 with seroprevalence increasing from 16 to 44% in eight months of this year[66].

Later in 1989, a broad-based serosurvey showed significant HIV-1 prevalence in IVDU, prostitutes, prisoners, STD clinic attendees and blood donors, heterosexual transmission, third wave of infection. The prevalence of HIV-1 infection was range from 1% to 10% in thirteen provinces that were surveyed. While in Chiangmai, the second largest city located in the northern part of Thailand, during 1989, these two national surveys indicated 37 and 44% rates of seropositivity[67]. During 1980s, there were an influx of foreign drug smugglers, including some from Africa, who transported illegal opiates from the Golden Triangle to the United States and Europe, which might introduce subtype E to IVDUs and CSWs. By 1990, almost 15,000 cases of HIV-1 infection had been found in Thailand[68]. The fourth wave of HIV-1 infection in partners of men who visited CSWs occurred with the increasing rates in women attending antenatal clinics and the fifth wave of epidemic in pediatric cases has increased.

Two HIV-1 genotypes designated "A" and "B" were first detected in Thailand by C2-V3 *env* nucleotide sequences of HIV-1 isolated from various risk groups collected in 1991[69]. Thai genotype A was found predominantly among heterosexuals and Thai genotype B seen primarily in IVDUs. Thai genotype B was more closely related to North American isolates (85% sequence identity) than African isolates (75% sequence identity) but posses a GPGQ motif similar to African isolates. Thai genotype A differed from all previously identified subtypes and classified as subtype E, which later found in the Central African Republic[70,71]. The average intraperson variation for Thai genotype "A" (subtype E) and "B" (subtype B) was 2.0% and 2.7%, respectively. The average interperson variation for Thai genotype "A" (subtype E) and "B" (subtype B) was 3.8% and 3.7%, respectively, which was several fold lower than those observed in Africa and United States. The intersubtype variation of Thai genotype "A" and "B" was 18.1%, which showed that 2 HIV-1 subtypes entered the country independently from each other.

McCutchan *et al.*, also characterized HIV-1 isolates from Bangkok in 1992 by PCR typing and *env* nucleotide sequencing[72]. Two highly distinct HIV-1 variants named, according to city that virus isolated, Bangkok variants and Northern Thailand variants. Bangkok variants were isolates found 4/5 from Bangkok associated with IVDUs, which resembled subtype B in North American and Europe strains with V3 motif GPGR. All Northern Thailand variants with V3 motif GPGQ associated with sexual transmission and highly divergent from subtype B and later were named subtype A/E.

The HIV Life Cycle

Transmission

Transmission of HIV usually requires the transfer of body fluids. The most important of these are blood, semen and vaginal secretions that contain the virus, or may permit the transfer of cells, especially macrophages containing virus[73]. In general, HIV is transmitted between humans in three ways[74].

1. Sexual transmission : sexual contact with an infected person
   - male to male
   - male to female
   - female to male
2. Blood-born transmission : exposure to infected blood or blood component from an HIV-infected donor
   - Blood transfusion
   - Shared injection equipment
3. Vertical transmission
   - perinatally from an infected mother to her child
   - postpartum from nursing mother (presumably through breast feeding)

Sexual transmission accounts for the majority (75%) of cases of HIV infection worldwide. The number of unprotected sexual contracts, the stage of disease (which may dictate the viral load) and the existence of genital ulceration may all influence the risk of transmission.

Virus Entry

Cell-Surface Binding of HIV

Virus entry into cells requires the fusion of virus and cell membranes, a process that depends on binding of virus env proteins to specific host-cell surface receptors. The CD4 receptor for human and simian immunodeficiency viruses (SIV) has been well

characterized. CD4 contains four extracellular immunoglobulin (Ig)-like domains and is expressed at the surface of a subset of T-lymphocytes and some macrophages. Several independent lines of evidence demonstrated that CD4 serves as a binding receptor for HIV-1, including the observations that antibodies specific for CD4 specifically blocked viral infection, CD4 coprecipitates with gp120 after virus binding to cells, and HIV-1 is neutralized after incubation with soluble CD4 molecules, which bind with high affinity to gp120[75]. Formal proof that human CD4 is a receptor for HIV-1 was demonstrated by its ability to bind HIV-1 particles.

HIV Fusion with Cell Membrane

The post-binding events required for HIV-1 and cell membrane fusion are not well understood. HIV-1, like most other retroviruses, infects cells in a pH-independent manner that is consistent with direct fusion between viral and cell surface membranes[76-78]. This process is thought to be driven initially by conformational changes in env following CD4 binding that expose fusion peptide domains located at the amino terminus of gp41[79].

Experiments have led to the identification of human cell surface proteins in addition to CD4 that are crucial for HIV-1 entry. The seven-transmembrane receptor fusin is required for entry of T-cell tropic, but not macrophage-tropic, viruses[80]. A similar molecule, CCR5 (a receptor for the CC-chemokines regulated upon activation normal T expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α) and MIP-1β), is required for the entry of macrophage-tropic and non-syncytium inducing (SI) primary viral isolates, but not T-cell tropic viruses[81,82].

Reverse Transcription

The reverse transcription pathway generates a linear DNA copy of the viral RNA genome[83]. This step takes place within a viral nucleoprotein complex and requires the coordinated activities of RT (an RNA- and DNA-dependent DNA polymerase) and RNaseH, which degrades the RNA component of RNA-DNA hybrid molecules. HIV-1 RT is a heterodimer made up of two subunits, p66 and p51. The x-ray crystal structure of this important viral enzyme, complexed with nevirapine, a non-nucleoside RT inhibitor, has been determined[84]. Reverse transcriptase and RNaseH perform a series of "molecular gymnastics" that include several RNA template switches between each of the two copackaged viral RNA genomes to produce a linear viral DNA molecule flanked by long terminal repeat DNA sequences[83].

One feature of HIV-1 that makes it such a difficult target for therapeutic intervention is the high degree of nucleotide sequence variation between different viral strains[85]. The mutations incorporated by retroviral RTs include amino acid substitutions, frameshifts, and deletions of nucleotide sequence[86,87]. In addition, it appears that reverse transcription can lead to hypermutated proviruses that contain multiple G to A nucleotide substitutions[86-88]. Reverse transcription generates diverse viruses that can have altered cell-type tropisms and drug resistance and that perhaps can escape neutralization by the host immune system.

The only cellular factors known to be required for viral DNA synthesis are deoxyribonucleotides. At least in some cell types, however, this process might be subject to cell-cycle regulation; whereas dividing T-cells efficiently support the complete synthesis of HIV-1 DNA, T-cells resting *in vitro* contain mostly incomplete forms of reverse-transcribed viral DNA[89,90]. In contrast, full-length extrachromosomal DNA appears to be the predominant form of viral DNA found in resting T-lymphocytes purified from HIV-1-infected individuals[91]. It therefore remains to be seen if specific factors present in dividing T-cells are required for complete viral DNA synthesis.

Because the viral nucleoprotein complexes are rapidly transported to the host cell nucleus, the majority of viral DNA synthesis occurs within the nuclear compartment. Nuclear localization signals (NLS), responsible for uptake into the nucleus, have been defined on the vpr and matrix protein components of the intracellular complex[92-94]. The MA protein is associated with the viral nucleoprotein complex by binding directly with IN, an interaction regulated by phosphorylation of the C-terminal tyrosine residue of MA[95]. The NLS of the MA protein is recognized by alpha karyopherin, a protein that presumably delivers the viral complex to the nuclear pore[96].

Integration of Viral DNA into Cellular Genomic DNA

The nuclear viral complexes serve as the machines that integrate viral DNA into host-cell chromosomal DNA to form a provirus. This step is critically dependent on the activity of the viral IN protein and is essential for viral gene expression[97,98]. Retroviral DNA can be integrated into the host genome. HIV-1 can productively infect nondividing cells such as macrophages and monocytes[99-101], and viral genes are expressed following HIV-1 infection of target cells arrested in the G2 phase of the cell cycle[85]. Three different forms of viral DNA are observed in infected cells: linear DNA flanked by two LTRs, and two circular forms that contain either one or two LTRs. Linear viral DNA is found in the cytoplasm and nucleus of infected cells and is the direct substrate for integration[102-104]. Circular forms of viral DNA are found only in the

host-cell nucleus, their formation does not require IN, and they are most probably not significant for viral replication[105].

The viral vif and nef proteins may be important for early events of replication following viral entry but before integration. *Vif*-defective viruses have aberrant core structures and are arrested at a stage following viral DNA synthesis[106]. *Nef*-defective viruses appear to exhibit an earlier defect in reverse transcription[107].

Viral Protein Expression

Although the *gag* and *pol* regions of most retroviruses are in different translational reading frames (*pol* is in the -1 reading frame with respect to *gag*), both *gag* and *gag-pol* precursor proteins are synthesized inside the infected cell. *Gag-pol* fusion proteins result from a frameshift event in which ribosomes shift from the *gag* to the *pol* reading frame at a defined frequency. This event occurs at a specific shift site in the viral transcript (the consensus heptanucleotide shift site is X XXY YYZ, where X, Y, and Z can be any nucleotide). It also depends on a secondary RNA structure downstream from the shift site, either a stem loop or pseudoknot, thought to facilitate ribosomal pausing prior to frameshifting[108]. Ribosomal frameshifting is likely to be an important regulatory step, because alterations in the relative amounts of *gag* and *gag-pol* fusion proteins abrogate viral infectivity[109].

Virus Assembly

Viral genomic RNA is selectively taken up from the pool of cytoplasmic RNAs because the nucleocapsid (NC) gag protein recognizes specific cis-acting RNA packaging signals[110]. Because the sequences required for HIV RNA packaging are directly downstream of the major 5' splice donor of the virus, only unspliced genome-length viral transcripts can be packaged into viral cores. The packaging signal of HIV-1 forms part of the stable RNA secondary structure that is highly conserved between different viral isolates[111]. Two cysteine/histidine motifs in NC, with the consensus sequence Cys-X2-Cys-X4-His-X4-Cys (in which X can be any amino acid), are critical for viral RNA packaging[112].

Expression of Viral Envelope Proteins

Retroviral env proteins are synthesized in the endoplasmic reticulum (ER) of infected cells and are transported to the cell surface by the host-cell secretory pathway. Within the endoplasmic reticulum, monomers of gp160, the precursor HIV env protein, associate with BiP, a chaperone molecular, before folding and oligomerization[113]. HIV-1 env proteins are thought to assemble into either homotrimers or homotetramers.

Oligomeric gp160 complexes are transported from the ER to the Golgi apparatus, where gp160 is cleaved by a cellular proteinase to produce the surface gp120 and transmembrane gp41 subunits before transport to the cell surface[101]. The mature virions that are released from the virus producing cells are then competent to begin the replication cycle again in other target cells.

M-tropic and T-tropic HIV

Tropism refers to which cells an organism such as HIV prefers to infect. The type of HIV that infects monocytes and macrophages is called M-tropic virus (macrophage tropic) and uses CC chemokine receptor 5 (CCR5) as the major coreceptor. M-tropic HIV replicates in peripheral blood lymphocytes, but does not usually form syncytia in *in vitro* culture. For this reason, M-tropic HIV strains are often referred to as Non-syncytia-inducing (NSI) strains, although the terms are not interchangeable. The chemokines MIP-1α, MIP-1β and RANTES could block viral infectivity *in vitro*. These chemokines only block viral infectivity mediated by M-tropic virus.

Strains of HIV that infect T-cells and T-cell lines are refered to as T-cell line tropic (T-tropic). T-tropic HIV isolates are frequently SI. The major coreceptor for T-tropic virus is CXC chemokine receptor 4 (CXCR4). NSI strains of the virus are considered to be less virulent than SI strain. The presence of SI virus is associated with rapid progression and an unfavorable prognosis, and they tend to dominate in the later stages of HIV disease. The chemokine receptor CXCR4 is specific for the chemokine SDF-1 (Stromal cell-derived factor 1)[27,29,30,114,115].

The Immunopathogenesis of HIV Infection

The rate of progressin of HIV disease may be substatially different among HIV-infected individuals. Following infection of the host with any virus, the delicate balance between virus replication and immune response to the virus determines both the outcome of the infection, i.e. the persistence versus elimination of the virus, and different rates of disease progression[116-118].

Clinical Course of HIV Infection

The clinical course of HIV infection generally includes three phases or stages: (1) primary infection, (2) clinical latency, and (3) AIDS-defining illness. Such a course of infection is characterized of the so-called typical progressors who represent the

majority of HIV-infected individuals. The median time from initial infection to progression to AIDS in typical progressors is eight to ten years.

1. Primary Infection

Approximately three to six weeks after initial infection, 50-70% of HIV–infected individuals develop an acute mononucleosis-like syndrome. This period is associated with high levels of viremia, and within one week to three months there is an immune response to HIV. This immunity is apparently inadequate to suppress viral replication completely, since HIV expression persists in lymph nodes even when plasma viremia is difficult to detect. Detectable viremia declines markedly and stabilizes after the acute syndrome subsides. Although a substantial percentage of patients with HIV infection do not have a clinically recognizable acute syndrome after primary infection, the events described above probably occur even in the absence of symptoms.

2. Clinical Latency

Most patients have a period of "clinical latency" that lasts for years after primary infection. During this period virtually all patients have a gradual deterioration of the immune system, manifested particularly by the progressive depletion of CD4+ T cells. Although this depletion may occur even without large increases in plasma concentrations of virus, viral replication in lymphoid organs, together with the spectrum of immunologic events that are directly or indirectly triggered by the virus, may contribute to it. Thus, HIV disease is clearly progressive during the so-called latent period.

3. AIDS-defining Illness

An AIDS-defining illness or clinically apparent disease is the inevitable outcome of the progressive deterioration of the immune system that occurs in most patients with HIV infection. Exceptions to the direct correlation between deteriorating immune function and clinically apparent disease are progressive generalized lymphadenopathy; Kaposi's sarcoma, which can occur before the onset of severe immunosuppression; and neurologic disease that may reflect direct or indirect effects of the virus or its products on neurons. The profound immunosuppression that occurs during this phase of HIV infection is the end stage of the immunopathogenic events that began at the time of primary infection, and continued for years through the clinically latent with microbiologically active stages of infection.

HIV Disease Progression

There are 4 major patterns of HIV disease in regard to the role of disease progression.

1. Typical Progressors

The majority (70-80%) of HIV-infected individuals belong to the group of typical progressors. Following primary infection, as mentioned above, typical progressors experience a long period (up to six to eight years) of clinical latency. Despite the lack of symptoms, HIV disease is active as is indicated by the persistent replication of virus and by the progressive loss of CD4+ T cells. Individuals with CD4+ T cell counts > 500 cells/cu.mm generally remain free of symptoms, whereas the appearance of constitutional symptoms is generally more frequent in individuals with CD4+ T cell counts below 500 cells/cu.mm. Exceptions to this paradigm are subjects with CD4+ T cell counts higher than 500 cells/cu.mm who develop progressive generalized lymphoadenopathy, Kaposi's sarcoma, or neurologic diseases. Progression to clinically apparent disease or AIDS-defining illness generally occurs within eight to ten years in typical progressors. When CD4+ T cell counts are below 200 cells/cu.mm, the clinical picture may be characterized by severe and persistent constitutional signs and symptoms; at this level of CD4+ T cells, there is an increased susceptibility to opportunistic infections or neoplasms.

2. Rapid Progressors

A significant percentage (10-12%) of HIV-infected individuals experience an unusually rapid progression to AIDS within two to three years of primary infection. Rapid progressors may experience a prolonged acute viral syndrome and the period of true clinical latency may be absent or very brief. Downregulation of the initial burst of viremia may not be very efficient in rapid progressors; even after the initial decrease, the levels of viremia may rise rapidly. Inefficient control of the initial burst of viremia and rapid rise in viremia within the first or second year after primary infection reflect a poor control of HIV infection by the immune system. In this regard, a delay in the appearance of the primary immune response or a rapid disappearance of certain immune functions during the early stages of the chronic phase of infection may be detected in rapid progressors.

3. Long-term Nonprogressors (LTNP)

A small percentage (less than 5% on the basis of different cohorts) of HIV-infected individuals do not experience progression of disease for an extended period of time. Long-term nonprogressors by some definitions have CD4+ T-cell counts that are within the normal range and are stable over time; in addition, they generally have low levels of virologic parameters and preservation of lymphoid tissue architecture and immune function. From a clinical standpoint, long-term nonprogressors are asymptomatic; it seems that in these individuals, HIV infection has been arrested with regard to disease progression. It is unknown whether long-term nonprogressors have

experienced a primary infection similar to that of other groups of HIV-infected individuals, i.e. associated with an acute viral syndrome and burst of viremia.

4. Long-term Survivors

In a small percentage of subjects who experience progression of HIV disease within a period of time similar to typical progressors, both clinical and laboratory parameters, although abnormal, remain stable for an extended period of time. The mechanisms, either virologic or immunologic, that are responsible for preventing further progression of HIV disease are unclear at present; the possibility that changes in virus genotype and/or phenotype, as well as the possibility that preservation of certain HIV-specific immune responses are involved, is being investigated.

The Immune System[119,120]

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, and viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill virus-infected cells. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins.

The Organs of the Immune System

Bone Marrow

All the cells of the immune system are initially derived from the bone marrow. They form through a process called hematopoiesis. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells of the immune system or into precursors of cells that migrate out of the bone marrow to continue their maturation elsewhere. The bone marrow produces B cells, natural killer cells, granulocytes and immature thymocytes, in addition to red blood cells and platelets and cells of the monocyte/macrophage lineage.

Thymus

The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus. Through a remarkable maturation process sometimes referred to as thymic education, T cells that are beneficial to the immune system are spared, while those T cells that might

evoke a detrimental autoimmune response are eliminated. The mature T cells are then released into the bloodstream.

Spleen

The spleen is an immunologic filter of the blood. It is made up of B cells. T cells, macrophages, dendritic cells, natural killer cells and red blood cells. In addition to capturing foreign materials (antigens) from the blood that passes through the spleen, migratory macrophages and dendritic cells bring antigens to the spleen via the bloodstream. An immune response is initiated when the macrophage or dendritic cells present the antigen to the appropriate B or T cells. This organ can be thought of as an immunological conference center. In the spleen, B cells become activated and produce large amounts of antibody. Also, old red blood cells are destroyed in the spleen.

Antigens: The Signalers

Antigens are the fingerprints of immunity. They are identifying molecules that reside on the surface of cells and, like fingerprints, are unique to the cells that bear them. All cells of the body have antigens that signal "self-self-self"-- a message that they are part of the body and therefore not to be attacked.

Microorganisms, viruses, or any agent that invades the body also have identifying antigens on their surfaces, which signal "foreign-foreign-foreign" to the immune system, readying it for immediate attack.

If the immune system overreacts to an outside antigen, the result is an allergy. When the immune system reacts inappropriately to self antigens, the result is an autoimmune disorder. If the immune system fails to react properly to an outside agent—such as a virus or bacterium--the result is an infection. Finally, if the immune systems fail to identify and destroy the body's cells after they become malignant, the result is cancer-cell development and, possibly, the growth of tumors.

Antibodies: The Keys

Antibodies are the body's complement to antigens. Each antigen may be thought of as a unique lock, and the protein molecules called antibodies (or immunoglobulins) as custom-made keys for every variation of lock. Antibodies are marvels of immunity that can fit into the "keyhole" of any one of millions of different antigens. Each one has a unique molecular configuration, and antibodes can be produced that latch perfectly onto every conceivable antigen.

Antibodies are carried throughout the body by white blood cells called lymphocytes, the prime movers of the immune system. Lymphocytes are divided into two main classes--T-lymphocytes and B-lymphocytes (T-cells and B-cells, for short). It is the B-cells that manufacture, display, and secrete antibodies.

Lymph Nodes

The lymph nodes function as an immunologic filter for the body fluid known as lymph. Lymph nodes are found throughout the body. Composed mostly of T cells, B cells, dendritic cells and macrophages, the nodes drain fluid from most of the tissues. Antigens are filtered out of the lymph in the lymph node before the lymph is returned to the circulation. In a similar fashion to the spleen, the macrophages and dendritic cells that capture antigens present these foreign materials to T and B cells, consequently initiating an immune response.

The Cells of the Immune System

Thymus-derived Lymphocyte (T Cell)

T cells are responsible for cell-mediated immunity, the branch consisting of subgroups of interacting cells. T-cells are so named because they "develop" in the thymus, a walnut-sized gland located under the breastbone. Although all immune cells are "born" in the bone marrow, different types follow different developmental pathways. T cells migrate to the thymus. There, with the aid of various thymic hormones, immature T cells grow, learn to recognize and attack antigens, and develop a range of specialized activities. The thymus is thus the master gland of cell-mediated immunity. Mature T cells are harbored in the spleen and lymph nodes. As with B-cells, the T-cell line also generates memory cells that prime the body for repeat attacks by known antigens.

T lymphocytes are usually divided into two major subsets that are functionally and phenotypically different. The T helper subset, also called the CD4+ T cell, is a coordinator of immune regulation. The main function of the T helper cell is to augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection.

Another important type of T cell is called the T killer/suppressor subset or CD8+ T cell. These cells are important in directly killing certain tumor cells, viral-infected cells and sometimes parasites. The CD8+ T cells are also important in down-regulation of immune responses. Both types of T cells can be found throughout the body. They often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites

where activation occurs, but they are also found in other tissues of the body, most conspicuously the liver, lung, blood, and intestinal and reproductive tracts.

Natural Killer Cells

Natural killer cells, often referred to as NK cells, are similar to the killer T cell subset (CD8+ T cells). They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and virus-infected cells, most notably herpes and cytomegalovirus-infected cells. NK cells, unlike the CD8+ (killer) T cells, kill their targets without a prior "conference" in the lymphoid organs. However, NK cells that have been activated by secretions from CD4+ T cells will kill their tumor or virus-infected targets more effectively.

NK cells have the capacity to recognize viruses and cancer cells without having encountered them before, without having antigens presented by other cells, and without a specific cognate receptor. Through mechanisms not fully understood, NK cells recognize virus-infected and cancer cells, killing them efficiently. In animal studies, NK cells have been shown responsible for stopping the spread of cancer cells throughout the body. Immunologists suspect that NKs serve the same function in humans, as well.

Bursa-derived Lymphocyte (B Cell)

The major function of B lymphocytes is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. Antibodies are specialized proteins that specifically recognize and bind to one particular protein. Antibody production and binding to a foreign substance or antigen often is critical as a means of signaling other cells to engulf, kill or remove that substance from the body.

B cells circulate throughout the body with antibody molecules on their surfaces. When they pick up the signal of a particular antigen, they multiply and transform into plasma cells, which then produce large quantities of precise antibodies that hook onto the foreign antigens. Antibodies function not only to neutralize foreign substances or microbes, they activate other cellular components of the immune system.

Certain B cells also remember their encounters with foreign agents. As a result, antibodies are produced swiftly when the same antigen is encountered.

Granulocytes or Polymorphonuclear (PMN) Leukocytes

Another group of white blood cells is collectively referred to as granulocytes or PMNs leukocytes. Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophils, based on their staining characteristics with

certain dyes. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using powerful enzymes.

Macrophages

Macrophages are important in the regulation of immune responses. They are often referred to as scavengers or antigen presenting cells (APC) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis and are also secretory.

Dendritic Cells

Another cell type, described only recently, is the dendritic cell. Dendritic cells, which also originate in the bone marrow, function as antigen presenting cells. In fact, the dendritic cells are more efficient APCs than macrophages. These cells are usually found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen. However, they are also found in the bloodstream and other tissues of the body. It is believed that they capture antigens or bring them to the lymphoid organs where an immune response is initiated. Unfortunately, one reason, so little is known about dendritic cells is that they are extremely hard to isolate, which is often a prerequisite for the study of the functional qualities of specific cell types. Of particular issue here is the recent finding that dendritic cells bind high amounts of HIV, and may be a reservoir of virus that is transmitted to CD4+ T cells during an activation event.

The Immune Response

An immune response to foreign antigen requires the presence of an APC, (usually either a macrophage or dendritic cell) in combination with a B cell or T cell. When an APC presents an antigen on its cell surface to a B cell, the B cell is signalled to proliferate and produce antibodies that specifically bind to that antigen. If the antibodies bind to antigens on bacteria or parasites it acts as a signal for PMNs or macrophages to engulf (phagocytose) and kill them. Another important function of antibodies is to initiate the "complement destruction cascade". When antibodies bind to cells or bacteria, serum proteins called complement bind to the immobilized antibodies and destroy the bacteria by creating holes in them. Antibodies can also signal natural killer cells and macrophages to kill virus- or bacteria-infected cells.

If the APC presents the antigen to T cells, the T cells become activated. Activated T cells proliferate and become secretory in the case of CD4+ T cells, or, if they are CD8+ T cells, they become activated to kill target cells that specifically express the antigen presented by the APC. The production of antibodies and the activity of CD8+ killer T cells are highly regulated by the CD4+ helper T cell subset. The CD4+ T cells provide growth factors or signals to these cells that signal them to proliferate and function more efficiently. This multitude of interleukins or cytokines that are produced and secreted by CD4+ T cells is often crucial to ensure the activation of natural killer cells, macrophages, CD8+ T cells, and PMNs.

Cytotoxic T Lymphocyte

CTLs are important in defence against virally-infected cells[1], in rejection of foreign tissue grafts and possibly also in immune responses to certain tumor types. CTLs are capable of killing targets expressing a specific antigen. They are found predominantly among the CD8+ population of T cells. The pathway for antigen presentation to CTLs uses the TCR which interacts with peptide antigen (9-12 amino acids) derived from internally synthesised proteins (the endogenous pathway of antigen presentation) and presented in the groove of an major histocompatibility complex (MHC) class I molecule[1,8,9]. Target peptides for CTLs are fragment of virus or tumor antigens.

CD8+ T cells, thus interact with antigen through the presentation of peptides in the groove of class I MHC molecules. The induction of a CTL response is dependent upon CD4+ T lymphocytes to provide help, in the form of activation stimuli. The life cycle of a CTL up to the point of killing is as follows. CD8+ T lymphocytes with the potential to become CTLs exit the thymus expressing their specific TCR but cannot lyse target cells at this stage. In the periphery, when a cytotoxic response is required, the pre-CTLs are activated by CD4+ T lymphocytes, which are involved in an immune reaction complementary to that of the CTL. For example, a CD4+ Th lymphocyte is primed to response to viral peptides after viral particles have been ingested and presented by macrophages at the site of infection. In turn, the activated Th cell activates virus-specific CTLs. The main cytokine signal is interleukin 2 (IL-2), but IFN-γ and IL-6 are also stimulators of CTLs. Activation of CTLs requires the typical two signal activation process of presented antigen and co-signal.

Following activation, the CTL must prepare the machinery required to lyse target cells. First, membrane bound granules appear in the cytoplasm. Second, there is

cytokine production and release. The killing by CD8+ T cells will be MHC class I restricted and the killing requires cell-cell contact. Only those cells to which a CTL becomes attached are killed. There is no 'bystander death' because the lytic molecules are released only at the site of TCR contact. Finally, CTLs are not themselves damaged during the process.

Recent studies of CTL have defined two major mechanisms of cytotoxicity. The first involves exocytosis of the contents of cytoplasmic granules from the CTL toward the target cell. The second involves engagement of a tumor necrosis factor receptor (TNFR)-like molecule on the target cell (e.g., Fas [CD95] or TNFR) by its CTL ligand (FasL or TNF, respectively). Both induce apoptosis in the target cell.

Granule-mediated Cell Death[121]

CTL granules are vectorially secreted into the intercellular space formed during conjugation of the CTL and target cell, and lysis is often associated with the formation of membrane lesions on the target cell. The granules of CTL contain a number of proteins, including a pore-forming protein termed perforin, and a family of serine proteases coined granzymes. Perforin causes osmotic damage due to its binding of phosphorylcholine headgroups, polymerization, and subsequent pore formation in the lipid bilayer of the target cell. Perforin is found essentially in CTL (including γδ T cells) and NK cell granules, although macrophage precursors also appear to express perforin and may be cytolytic under the appropriate conditions. CTL-mediated target cell death generally involves changes such as chromatin condensation, extensive membrane blebbing and ultimately nuclear DNA fragmentation (apoptosis). These events clearly occur sometime before appreciable perforin-mediated cell lysis and purified perforin alone is incapable of causing DNA fragmentation. Indeed, it is likely that a supplementary role is played by granzymes in target cell killing and this postulate is supported by much *in vitro* and *in vivo* experimental evidence. Granzymes are the major protein complements of CTL and NK cell granules and they synergize with perforin to trigger an internal disintegration pathway in the target cell. Most notably, the killer cell serine protease granzyme B shares Asp-ase specificity with an increasingly large family of cell death cysteine proteases (termed caspases), including the interleukin-1β-converting enzyme (ICE)-like family members. Granzyme B appears to trigger an endogenous cell death cascade by activating key target cell caspases. Collectively, these data indicate that granzyme B can greatly amplify the activation of key signaling components shared by other forms of cell death (including the Fas/TNFR pathways) and directly contribute to apoptotic nuclear morphology.

Lymphocyte Receptor-mediated Cell Death[121]

Previous observations of target cell death in the absence of calcium 2+ (Ca^{2+}), granule exocytosis or perforin suggested the existence of alternative pathways of CTL-mediated cytotoxicity. Rouvier *et al.*, demonstrated that Ca^{2+}-independent lysis was accounted for by CTL cross-linking with the target cell Fas receptor. Cloning and characterization of Fas indicated that it belonged to the TNFR superfamily of molecules. Mutational analysis of the cytoplasmic domains of these receptors has indicated a region in both Fas and TNFR1 that is conserved and necessary for transduction of the apoptotic signal. Signaling via Fas or TNFR leads to apoptotic death cell, with characteristic cytoplasmic and nuclear condensation and DNA fragmentation. This death process is rapid (within several hours), occurs in the absence of RNA or protein synthesis and can also be triggered in target cell by monoclonal antibodies to Fas. Cytoplasmic caspases, including those with adaptor function (i.e., association with Fas via adaptor Fas-associated death domains [FADD]) and Asp-ase activity (e.g., FADD-like ICE [FLICE] proteases), are involved in Fas and TNFR cell death pathways and many of the signaling molecules in these apoptotic pathways have been characterized. Triggering of these pathways requires cross-linking of Fas or TNFR, and similar to TNF, the soluble from of FasL has a trimeric structure in solution. In addition, the FasL is a CTL surface receptor of the TNF family. FasL expression appears constitutive in NK cells and can be rapidly induced in T cells by TCR engagement. Among the classical $CD4^+$ T helper subpopulations, Th1 cells can express FasL and lyse in a Fas-based manner more readily than Th2 cells. By contrast, $CD8^+$ T cells may predominantly display TNF-mediated cytotoxicity.

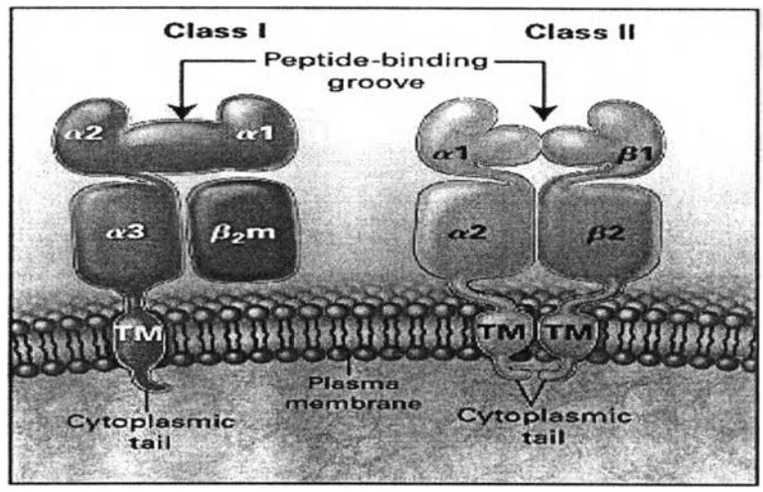
These two pathways of cytotoxicity are distinguishable not only by the molecular interaction at the contact zone between CTL and target, but also by the initiation of distinct downstream events in the target cell. Fas-mediated cytolysis is dependent on the proteolytic action of caspases, whereas lysis via granule exocytosis does not require caspases. The finding that two CTL pathways initiate distinct molecular events in target cells is probably relevant for the role of these CTL mechanisms in host responses to microbial pathogens.

Human Leukocyte Antigen (HLA) [122]

MHC is a region of highly polymorphic genes whose products are expressed on the surfaces of a variety of cells. The genes that controlled such immune responses were called immune response (*Ir*) genes. It was later shown that *Ir* genes controlled the activation of helper T lymphocytes, which were necessary for antibody responses to protein antigens. In the human called that HLA. The central role of MHC genes in

immune responses to protein antigens was explained in last 1970s, with the demonstration that antigen-specific T lymphocytes do not recognize antigens in free or soluble form but recognize portions of protein antigens (i.e., peptides) that are non-covalently bound to MHC gene products. In other words, MHC molecules provide a system for displaying antigenic peptides to T cells. This allows T cells to survey the body for the presence of peptides derived from foreign proteins. There are two different types of MHC gene products, called class I and class II MHC molecules (class I and class II HLA molecules) shown in **Figure 3**.

Fig. 3 HLA class I and class II structure
(Klein J. and Sata A., NEJM 2000; 343: 702-709)



Class I HLA Molecules

All class I molecules contain two separate polypeptide chains: an HLA-encoded α or heavy chain of about 44 kilodaltons (kD) and a non-MHC-encoded β chain was called β_2 microglobulin (β_2m) about of 12 kD in human. The α chain is formed by a core polypeptide of about 40 kD and contain one N-linked oligosaccharides. Each α chain is oriented so that about three quarters of the complete polypeptide. The β chain interacts non-covalently with the extracellular portion of the heavy chain and has no direct attachment to the cell.

Class II HLA Molecules

All class II MHC molecules are composed of two non-covalently associated polypeptide chains. In general, the two class II chains are similar to each other in overall structure. The α chain (32 to 34 kD) is slightly larger than the β chain (29 to 32 kD) as a result of more extensive glycosylation. In class II molecules, both polypeptide chains contain N-linked oligosaccharide groups, both polypeptide chains have extracellular amino termini and intracellular carboxy termini, and over two thirds of each chain is located in the extracellular space. The two chains of class II molecules are encoded by different MHC genes, and, with few exceptions, both class II chains are polymorphic.

In humans, the HLA is located on the short arm of chromosome 6. β_2m is encoded by a gene on chromosome 15. The HLA is very large (about 3500 kb) and is organized as follows: (1) class II genes (HLA-DP, HLA-DQ, HLA-DR) (2) complement genes (3) heat shock protein and cytokine (TNF, lymphotoxin (LT) and LT-β) genes, and (4) class I genes (HLA-B, HLA-C, HLA-A)

Expression of MHC Molecules

The expression of MHC molecules on different cell types detemines whether or not T lymphocytes can interact with foreign antigens present on the surface of these cells. CD8+ cytolytic T lymphocytes recognize foreign antigens such as viral polypeptides when they are bound to class I MHC molecules. The ability of CTLs to lyse a virally infected cell is a direct function of the quantity of class I molecules expressed. In contrast, CD4+ Th lymphocytes recognize antigens bound to class II MHC molecules.

The peptide-binding region of class I molecules is formed by the α1 and the α2 segments of the heavy chain. It consists of a cleft measuring approximately $25A^{o}x10A^{o}x11A^{o}$ with α helical sides and an eight-strand β-plated sheet floor. The cleft can accommodate a peptide of about 9 to 11 amino acid residues. Peptides bind to class I molecules largely through allele-specific motifs, which fit into complementary pockets formed by polymorphic residues of the class I molecule. The analogous cleft of class II molecules is formed by the α1 and β1 domains of the two chains. The cleft in class II molecules is open at the ends so that class II molecules can accommodate larger peptides of 10 to 30 or more amino acid residues. In both class I and class II molecules, the polymorphic amino acid residues located in the peptide-binding region determine the specificity of peptide binding and T cell antigen recognition

Currently, HLA allele frequencies vary greatly among different ethnic groups. For the common HLA class I molecules around the world, a 17% prevalence of HLA-A2 and 11% of HLA-A28 are found in North America, 30% HLA-A35 is found in South America, 17% HLA-A2 is found in Africa and 33% HLA-A11, 25% HLA-A2 and 14% HLA-A24 and HLA-A33 are found in Southeast Asia and Thailand[10].

HIV-1 infection is associated with a vigorous HLA class I restricted CTL response[123]. By analogy to a number of animal models of viral infection, these CTL are likely to play an important role as a host defense in infected persons and are likely to be an important component of an effective AIDS vaccine. A large number of laboratories have now contributed to the identification of peptides containing HIV-1 CTL epitopes. In terms of class I-restricted CTL epitopes, studies have shown that these peptides are typically 8-12 amino acids in length and have certain anchor residues to bind into specific pockets of the MHC class I molecule due to hydrogen bonding. These pockets, designated A, B, C, D, E and F, are located in the antigen binding site on the class I molecule formed by two α-helicles, with the floor of the groove formed by a β-sheet structure[124]. As the pockets are located at the edge of the peptide binding groove, the anchor positions in the peptides are often in positions 1, 2 and 9 of the processed peptide[125]. The characterization of the antigenic epitopes involed in CTL induction is important not only for a better understanding of disease pathogenesis, but also for possible vaccine development. Since the first description of HIV specific CTL, the efforts of many laboratories have led to the identification of a large number of epitopes involved in the HIV-1 specific CTL response in infected persons[126,127]. The Los Alamos HIV Molecular Immunology Database contains all reported sequences with CTL activity, regardless of their definition as optimal epitopes. A summary of those sequences is accessible in this database. It contains more than 200 peptides, which contain putative HLA class I restricted epitopes.

CTL and HIV-1[128,129]

CTLs are believed to mediate antiviral activity against HIV. This was first reported in 1986, when investigators noted that depletion of CD8 cells PBMCs of HIV-infected subjects resulted in a marked increase of viral replication in the remaining CD4 cells. In 1987, it was first reported that many HIV-infected persons have virus-specific CTLs, and that these cells are present at unprecedented levels compared to those in other chronic viral infections. Further work has characterized the specific HIV antigens that are targeted by CTLs and quantitated responses in those infected. The evidence is found that they control viral replication by at least two mechanisms: direct antigen-

specific cytolysis, which appears to be required for optimal suppression, and release of soluble antiviral factors.

Soluble Inhibitory Factors

CTL are activated to kill virus-infected cells, they produce soluble factors which have been shown to inhibit HIV replication. The first soluble antiviral factors identified from CTLs were the chemokines MIP-1-α, MIP-1-β, and RANTES, which were found to block M-tropic but not T-tropic strains of HIV. SDF-1α and SDF-1β, could block replication of T-tropic HIV strains *in vitro*. The release of these factor is triggered when the TCR recognizes an infected cell.

MIP-1-α, MIP-1-β, and RANTES are known to be produced by CD8 cells. But, SDF-1-α and SDF-1-β, although active against T-tropic strains of HIV *in vitro*, do not appear to be produced by CD8 cells.

HIV-Specific Cytolysis

CD8 CTLs lyse target cells expressing foreign antigen. Most cells express MHC class I, which presents peptides from self or foreign proteins. Formation of these complexes begins with newly translated proteins being cleaved into small peptides in proteasomes. The resulting peptides are transported by the transporter associated with processing (TAP) complex into the ER, where they encounter nascent MHC molecules. Peptides that fit a certain motif (epitopes) then bind to the MHC. Epitopes are usually approximately nine amino acids in length and express certain residues that serve as anchors, allowing each peptide to sit in a cleft in the MHC. Anchor residues vary according to the MHC molecule, each of which has its own motif (there are three MHC class I loci in humans, and thus up to six different alleles). After the peptide-MHC complex is formed, it is presented on the cell surface.

Cells expressing foreign peptide-MHC complexes can be recognized and lysed by CTLs through very specific interaction. Each CTL expresses a surface receptor, the TCR, that can bind specifically to a particular MHC-peptide complex. This binding is restricted by both peptide and MHC molecule. Whereas the outer amino acids of the peptide bind to MHC, the central amino acids protrude from the cleft of the MHC molecule and contact the TCR during engagement. Even single amino acid alterations in these residues potentially can completely abrogate binding. Engagement of the TCR triggers a signal to lyse the target cell via release of proteolytic enzymes, as well as to induce cell death (apoptosis).

So, CTL can kill the infected cell through a direct recognition mediated by the TCR on the CTL. CTL are able to recognize infected cells before progeny virus have been produced. This is a very important point, since this is a time that the virus is uncoated and particularly vulnerable. If the infected cell is lysed before progeny are produced, then the virus will be eliminated. These differ among individuals, depending on the particular class I molecules expressed on the surface of the cells in a given person. Each HLA class I allele is slightly different, and therefore the viral peptides that can bind in the peptide binding groove are different. This is the phenomenon of so-called "class I restriction". For example, the p17 peptide sequence SLYNTVATL has been shown to be HLA A2 restricted, meaning that it is presented at the cell surface as a complex with HLA A2 allele.

Through genetic rearrangements of the TCR genes, a tremendous diversity of CTL specificities is generated. CTL-recognizing self-peptides are presumably deleted in the thymus during development. In response to new foreign proteins, previously naive CTLs that recognize the new antigens are selectively activated through the TCR. These CTLs then proliferate and become activated killer cells.

In vitro, CTL activity is detected with chromium-release assays, in which target cells containing an intracellular chromium radioisotope are exposed to CTLs. Subsequent measurement of chromium release is an indicator of target cell lysis. Autologous B lymphoblastoid cell lines (BLCL) are the usual target cells for these assays, since they are easily obtained and maintained in culture. Entire HIV proteins may be expressed by infecting those BLCLs with recombinant vaccinia viruses (rVV), or synthetic epitopes can be added directly to the target cells.

HIV-specific CTL activity appears to be correlated with control of disease. In primary infection, generation of HIV-specific CTL responses is temporally correlated with a drop in viraemia, suggesting that CTLs inhibit viral replication *in vivo*. Furthermore, warning of CTL responses has been correlated with disease progression; recent data indicate an inverse correlation of virus-specific CTL activity with plasma viral load. Other studies suggest that some exposed yet uninfected persons, including health care workers with percutaneous exposure, sexual partners of infected persons, and prostitutes in epidemic areas, may have such responses as well.

Techniques for Measurement of Cytotoxic T lymphocyte[130]

CTLs are believed to play critical roles in the control of many viral infections, including of HIV. For this reason, there has for many years been much interest in developing assays to detect the presence of CTLs. At least 4 techniques use to detect CTL activities have been described, i.e., the classical chromium-51 release assay, ELISPOT assays, intracellular IFN-γ assays and HLA-peptide tetrameric complex assay [13].

Classical Chromium-51 Release Assay[131]

For many years, the standard assay for cytotoxic function has been the chromium release assay. First, described by Burnner and co-workers, the assay involves the radiolabelling of target cells with sodium chromate ($Na_2{}^{51}CrO_4$) The radiolabelled target cells are then incubated with the test effector cells population for a short period (4 hours). The amount of 51Cr released into the supernatant is then quantified, to provide a measure of target cells lysis.

ELISPOT Assays [132]

The ELISPOT assay is an adaptation of the enzyme-linked immunosorbent assay (ELISA), which measures the local concentration of cytokines (i.e., INF-γ) that are released from an activated CD8+ T cell. In the ELISPOT method, cells that have been stimulated with antigen *in vitro* were incubated in nitrocellulose-lined microtitre wells, which have been pre-coated with anti-cytokine antibody. After incubation (for several hours or days), the local production of cytokines around "producing cells" can be visualised by adding a second antibody that is labelled with enzyme alkaline phosphatase or horseradish peroxidase, and then adding a substrate that is enzymatically converted into an insoluble coloured product. Cytokine-producing cells can then be visualised as "spots".

Intracellular IFN-γ Assays[133]

IFN-γ production at the level of the single CD8+ T cell can be analysed using flow cytometry to detect intracellular cytokines. T cells are stimulated *in vitro* (typically with phorbolmyristate acetate (PMA) and ionomycin(I)), and for at least some of the stimulation period monensin or brefeldin A (BFA) are present to block the transport of cytokines through the golgi apparatus, and therefore prevent the secretion of cytokines. The T cells are then fixed, permeabilised (to allow cytokine-specific antibodies to enter the cell), and stained for the presence of intracellular cytokines using directly conjugated anti-cytokine antibodies.

HLA-peptide Tetrameric Complex (Tetramer) Assay[134]

Recent technological advances have now made possible one of the long-term goals of T-cell assays, namely to identify individual T cells on the basis of the specificity of binding to the MHC-peptide complex. The complex (via biotin) can then be attached to another polypeptide streptavidin. Because each streptavidin molecule has four biotin-binding sites, a tetrameric complex is produced, which contains four MHC class I molecules; this complex has a greater affinity for T cells. The presence of the fluorochrome allows T cells that have bound the tetramer to be detected by flow cytometry.

Table 1. Comparison of each technique for detect of CTL activity

Measurement of CTL	Cell type measurement (function)	Time (day)	price	HLA Typing Required	Assay type	Radiolabelled	sensitivity
1. Cr-51 assay	Target (lysis)	14	Low	No / Yes	Qualitative	Yes	$1/10^4$
2. ELISPOT	Effector (IFN-γ secretion)	2	High	No / Yes	Quantitative	No	$1/10\text{-}5x10^5$
3. Tetramer	Effector (Binding)	2	High	Yes	Quantitative	No	$1/10\text{-}5x10^5$
4. Intracellular IFN-γ assay	Effector (IFN-γ expression)	2	High	No / Yes	Quantitative	No	Not Available

Evidence of HIV-Specific CTL Responses

Viral infection induces CD8+ CTLs responses by presenting viral antigen on MHC class I molecules at the surface of infected cells. A vigorous CTL response is also associated with HIV infection. HIV specific CTL are thought to play an important role in the control of the virus during clinical latency and may influence the course of disease development.

Several studies have demonstrated that, In 1995, Lamhamedi *et al.*, examined the specificity of HIV-specific CTL responses in acute infection found that seven out of nine donors had detectable CTL activity in the first four weeks after seroconversion[135]. In 1994, Mackewicz *et al.*, showed that CD8+ cell-mediated suppression of HIV replication has been described early in HIV infection, before the development of neutralizing antibody response. In these studies CD8+ suppressive activity was most

marked before seroconversion, and showed an inverse correlation with plasma viral load in three out of seven subjectss[136]. In 1996, Harrer *et al.*, found that CTL effector have been detected in LTNP with low levels of plasma viraemia[137]. In 1997, Michael R Betts *et al.*, to examine cross-clade HIV-specific CTL activity in peripheral blood of eight Zambian individuals infected with non-B-clade HIV-1. They found that six of eight C-clade HIV-infected individuals elicited CTL activity specific for recombinant vaccinia virus-infected autologous targets expressing HIV *gag-pol-env* derived from B-clade HIV-1 (IIIB). These data demonstrate that HIV clade C-infected individuals can mount vigorous HIV clade B-reactive CTL responses[13]. In 1998, Stephen McAdam *et al.*, to evaluate cross-clade recognition of p55 antigen by CTL in persons infected with diverse clades of HIV-1. The result showed that Uganda patients were detected cross-reactive CTL responses that recognized gag proteins from clades A of HIV-1 other than those with which they were infected[14]. In the same year, Julia A. Lynch *et al.*, studied cross-clade CTL response to HIV-1 proteins among HLA disparate North Americans and Thais. These studies suggest that gag epitopes are common across a wide range of HLA alleles and sufficiently conserved among several HIV-1 clades to make gag an important component antigen in a broadly immunogenic vaccine[12]. Sarah L. Rowland-Jone *et al.*, to determine CTL responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. They found that CTL responses have been defined to novel epitopes presented by HLA class I molecules associated with resistance to infection in the cohort, HLA-A6802 and HLA-B18[19]. Florence Buseyne *et al.*, studied CTL cross-reactivity between HIV-1 subtypes within a group of infants infected with either HIV-1 B or non B clade. This data showed that cross-clade specific CTL responses were identified in this cohort at a rate of 88% for pol, 83% for nef, 67% for gag and 55% for env[17]. CTL responses in HEPS[18,19] and in uninfected children born to HIV-1-infected mothers[20] have also been reported.

Many researchers feel that a natural infection with a quasispecies of a dynamic virus such as HIV-1 may lead to good polyclonal cross-reactive cellular immune responses, whereas a vaccine based on a single sequence (in a recombinant vector or a DNA vaccine for example), may induce less cross-reactivity.

AIDS is severe T cell immunodeficiency caused by infection with HIV. This virus has a tropism for CD4+ T lymphocytes, causing depletion of the cells by direct lysis as well as several indirect mechanisms that lead to death, defective maturation, and abnormal function of uninfected T cells. The depletion of T cells results in greatly increased susceptibility to infection by a number of opportunistic microorganisms, including *Pneumocystis carinii*, mycobacteria and various fungi and viruses. In addition, patients have an increased incidence of tumors, particularly Kaposi's sarcoma

and Epstein-Barr virus-associated B cell lymphomas, and frequently develop an encephalopathy, the mechanism of which is not fully understood. Despite enormous effort, and effective cure or prophylactic vaccine for this disease is not available.

The developement of an effective HIV vaccine that is broadly applicable to populations exposed to multiple HIV-1 clades is problematic. First, the antigenic diversity among different isolates and clades and the varying genetic makeup among target populations might impede the developement of an effective immunogen[138,139]. Second, the correlates of protective immunity in HIV-1 infection remain undefined. Although many factors may contribute to protection[140], the current consensus suggests that an effective HIV vaccine will need to induce virus-specific CTLs.

Thus, to develope an effective CTL inducing HIV-1 vaccine, studies to investigating cross-clade CTL activities were studied, CTL epitope mapping in populations infected with different HIV-1 subtypes, non-B in particular, and with different HLA polymorphic are warranted.