# CHAPTER II

# LITERATURE REVIEW

## History

Acquired immunodeficiency syndrome (AIDS) was first recognized as a new and distinct clinical entity in 1981.[1,2,3] The first few cases were recognized because of unusual clusters of rare diseases such as Kaposi's sarcoma and pneumocystis carinii pneumonia in young homosexual men. AIDS cases were soon reported in other populations as well, including intravenous (IV) drug[46] users and hemophiliacs.[47,48,49] The observation that T-lymphocytes bearing the CD4 marker were depleted in these cases reinforced the conclusion that the disease arose from immune system failure. In 1982, Centers for Disease Control (CDC) of the United States of America named this kind of immune deficiency as Acquired Immune Deficiency Syndrome or AIDS.[46]

## Etiology

The first indication that AIDS could be caused by a retrovirus came in 1983, when Barre-Sinoussi et al. at the Pasteur Institute[50] recovered a reverse transcriptase containing virus from the lymph node of a man with persistent lymphadenopathy syndrome (LAS). In addition, the characteristics described for the retrovirus recovered by the Pasteur Institute group[50] included some which had been reported for the human T-cell leukemia virus (HTLV).[51] Thus, many investigators decided initially that the lymph node isolate was a member of human retrovirus group. In support of this conclusion was the

concomitant publication by Gallo et. al. reporting an isolation of HTLV from individuals with AIDS.[52]

Further studies in 1983 by Montagnier and co-workers clarified certain questions in relation to the LAS agent. The results indicated that this human retrovirus, although similar to HTLV in infecting $CD4^+$ lymphocytes, had quite distinct properties. Their virus was later called lymphadenopathy associated virus (LAV).[53] In early 1984, Gallo and associates reported the characterization of another human retrovirus distinct from the previously known HTLVs that they named HTLV-III. [54,55,56,57] It was isolated from peripheral blood mononuclear cells (PBMC) of adult and pediatric AIDS patients.

Levy et al. [58] also reported at that time the identification of retroviruses which they named the AIDS-associated retroviruses (ARV). This retrovirus showed some cross-reactivity with the French LAV (BRU) strain. Within a short period, the three prototype viruses (LAV, HILV-III and ARV) were recognized as members of the same group of retroviruses and their properties identified them as lentivirinae. In 1986, the International Committee on Taxonomy of virus recommended a separate name for the AIDS virus, the human immunodeficiency virus (HIV).[59] Soon after the discovery of HIV-1. HIV-2, a different subtype, was identified in western Africa.[60] Both HIV subtypes can lead to AIDS, although the pathogenic course with HIV-2 is mostly longer.

**HIV-1 Biology**

HIV-1 is the prototypical member of the Lentivirinae subfamily. Retroviruses are RNA viruses that replicate via DNA intermediates using the

viral enzyme reverse transcriptase (RT). After a long incubation period, lentiviruses cause clinical syndromes, which are characterized by a protracted symptomatic phase.

The mature virion of HIV-1 is an icosahedral sphere of diameter approximately 100 nm. The outer envelope is formed from the host cell proteins and spikes of the viral envelope glycoprotein (gp120 and gp41). Under the lipid bilayer, are the internal structural capsid and core proteins, p17, p24, p9, p7. These proteins enclose two copies of the single-stranded RNA genome, and multiple reverse transcriptase (RT) molecules, in the centre of the virus particle.

The HIV-1 genome contains at least nine different genes, of which the major three are common to all retroviruses. These are "gag" (coding for structural proteins), "pol" (coding for the viral enzymes, protease, RT and integrase) and "env" (coding for the envelope glycoproteins). The gag and pol regions of the genome are translated into large polyprotein precursors, which are subsequently cleaved into mature structural proteins and enzymes by a specific virally-encoded protease which is a product of the pol gene. The two env proteins, gp120 and gp41, are cleaved from a larger precursor (gp160) by a cellular enzyme.

Of the other HIV-1 gene products, tat, rev, vpr and nef intervene to regulate the virus life cycle. The accessory gene products, vif vpu and nef, are believed to function in virus infectivity, and in virus particle maturation. The viral genes are flanked at each by long terminal repeat sequences (LTRs). The LTRs contain binding sites for cellular proteins that are able to activate transcription, and are also under the control of viral signals. The complex regulation of HIV allows the virus to establish latency, then respond rapidly to

various signals and synthesize high levels of viral proteins and new virions.[61,62,63,64,65]

## HIV Life Cycle

### Transmission

HIV-1 has been found to be present in many body fluids and tissues. It can only be transmitted, however, by blood and blood products,[66,67] during sexual intercourse,[68,69,70] and from mother to child, either[71,72,73] directly or rarely through breast feeding.[74]

### Target cells

HIV only infects certain types of cells. Generally, these are cells that possess the CD4 antigen on their external surface. Most of these cells belong to the immune system, namely CD4 T-lymphocytes, or T-helper cells.[76] It is these CD4 T-lymphocytes that have been shown to be the predominant cell harbouring HIV-1 in the peripheral blood of infected individuals.

Several other cell types, particular those that belong to the mononuclear phagocyte lineage which bear the CD4 antigen, have been shown to be infected with HIV *in vivo*. These include white blood cells such as monocytes, which mature to form macrophages, Langerhans cells of the skin, follicular dendritic cells in the lymph nodes, alveolar macroghages in the lung, retinal cells and cells of the uterine cervix. In addition, HIV may infect microgial cells of the central nervous system which do not bear CD4 surface proteins.[77,78,79]

Studies in vitro reveal that the virus may cause death of infected CD4 T-lymphocytes. However, blood monocytes are relatively refractory to its

cytopathic effects. This suggests that persistence of HIV in circulating monocytes could lead to widespread dissemination in the body.

**Attachment and Entry.**

In order to enter a host cell, the viral particle must bind with the CD4 molecule on the host cell membrane. The CD4 molecule acts as a receptor for a glycoprotein on the viral envelope termed gp120.[80,81] Once this binding is completed, the virus particle is thought to enter the cellular cytoplasm by 'virus-to-cell fusion', where the viral membrane joins with the host cell membrane, injecting the core of the virus into the cell. This process involves a second viral envelope glycoprotein termed gp41.[82]

When the core of the virus is within the cellular cytoplasm, the viral RNA is translated into DNA. The core of the viral particle contains two strands of RNA which are used as templates for the formation of DNA: a process which is achieved by the action of the viral enzyme reverse transcriptase. The resulting DNA, referred to as a 'provirus' or 'proviral DNA', passes into the cell nucleus and is randomly inserted into the host cell DNA by the action of another viral enzyme called integrase (or endonuclease). The integration of the viral genetic material usually occurs within 2-10 hours after infection.[82]

**Replication**

Replication begins with the production of RNA copies of the provirus. This is accomplished by the action of nucleotide sequences in the 'long terminal repeats (stretches of virus encoded DNA at each end of the provirus), which direct host cell enzymes to copy only the proviral part of the cellular DNA. Some of the RNA will be used as the genetic material of new virus particles and some will be used to make structural proteins of the new virus

particles or regulatory proteins, which work to control viral replication. The new virions are produced from multiple copies of the viral proteins. These proteins are first formed as large precursors-- long chain protein molecules-- which are then specifically cleaved to become the viral enzymes and structural proteins of the new virions. The assembly of the new virus particle begins with two of the precursor proteins collecting at the edge of the cell where they join together and attach themselves to the host cell membrane. They begin to form a spherical structure which bulges outwards from the cell membrane and draw two strands of viral RNA into it.

An enzyme called protease, which is located on one of the precursor protein molecules, carries out the final steps of protein cleavage as follows : first, it cuts itself free from the protein molecule , then, the protease works to cleave all the other viral components from the protein chain. The remaining protein segments form a protein coat (inner 'capsid') that surrounds the RNA and other viral enzymes. A third structural protein, the envelope protein, is made and transported to the cell surface independently. It contains the envelope glycoproteins, which together with elements from the host cell membrane totally enclose the new virus particle. The completed virion then leaves the cell in process known as "budding".[83]

**Genetic control of HIV**

HIV-1 contains three main genes characteristic of all retroviruses. These genes are called : 'gag', which encodes core proteins of the virus; 'pol', which encodes viral enzymes; and 'env'which encodes proteins of the envelope surrounding the virus particle.[61]

Unlike other retroviruses ; however, HIV also contains at least six other genes.[84] Three of these genes are involved in the control of replication of the virus. Working individually and together, these genes can code for sudden rapid replication, steady replication, controlled replication, or quiescence. One of these regulatory genes, called 'tat' is produced early in the replicative cycle. It boosts the production of other viral regulatory proteins and speeds up the replicative process. Another regulatory gene called 'rev' plays an essential role in the process of differential protein production. It enhances the production of either structural proteins, or regulatory proteins. In this way, it can control the shift from silent infection to active viral growth. The 'nef' gene is produced during the initial phase of proviral gene expression[85] and has been described as having a negative effect on viral replication through the inhibition of the viral gene 'transcriptase'.[86,87,88]

**Latency, Activation and Trigger Factors.**

Following primary infection with HIV, most individuals do not develop symptoms for many years. Exactly what is happening during this asymptomatic period and what causes sudden clinical deterioration is not yet clearly understood. Although small amounts of virus are present during the asymptomatic phase, the virus does not appear to be rapidly replicating. Deterioration is, however, characterized by intensive viral replication. A variety of different factors may trigger this replication. Individuals with HIV are often co-infected with other viruses. Active replication of one or more of these possible co-infecting virus may play a role in precipitating HIV replication and enhancing disease progression. Gene products from herpes simplex virus,[89] cytomegalovirus,[90] hepatitis B virus,[91] human herpes virus type 6[92] and HTLV-1,[93] have been described to stimulate HIV-1 expression of viral genes in cell cultures. Additionally, any antigen that would usually

elicit an immune response from the T4-lymphocytes may also lead to active replication following stimulation of the T4-lymphocyte. Once a T4-lymphocyte has been stimulated by the presentation of an antigen, it usually responds by proliferation, which involves transcription of its own genetic material to form new T4-lymphocytes. This process begins when specific cellular proteins bind at the 'initiation sites' on the genome. The HIV provirus, which is integrated into the genome of infected T4-lymphocytes, contains similar initiation sites on its long terminal repeats for which the cellular proteins may mistake these for their own initiation sites. One particular cellular protein that is thought to increase transcription, appear to increase its binding to the long terminal repeats following stimulation of the infected T4-lymphocyte. The activation of this protein may, therefore, lead to increased replication of HIV and thus triggering progression of the disease.

**Immunopathogenesis of HIV**

The fundamental abnormality in HIV-infected individuals is a progressive decline in the number of CD4+ T lymphocyte.[94] CD4+ T lymphocytes are crucial for appropriate immunological response against a widerange of pathogens as well as malignant tumors. The interaction of CD4+ T cells with B cells, natural killer cells, cytotoxic T cells, and monocyte/macrophages is effected primariry by the release of cytokines and other soluble factors, and, to a lesser extent, by direct cell-to-cell contact. In addition, CD4+ T cells secrete factors that affect the growth and differentiation of other lymphoid cells and hematopoietic cells. It also affect the function of nonlymphoid cells as well. As a result, it is clear that either qualitative or quantitative abnormalities in the CD4+ T cell population can have profound effects on human immune system function.[95]

The pivotal event in the immunopathogenesis of HIV infection is the binding of the HIV virion to the CD4+ T cell. HIV binds to the cell via the specific, high-affinity interaction of the external envelope glycoprotein, gp120, with the CD4 molecule that is also present on the surface of monocyte/macrophages.[96,97,98,99] HIV may cause a qualitative decrease in CD4+ Tcell function, via the binding of gp120 to the CD4 molecule with disruption of the CD4-class II MHC association,[100] in addition to its effect on the absolute numbers of CD4+ T cells. Inappropriate cell signaling via interaction of the HIV envelope protein (gp120) and CD4 molecule could also render the cell anergic.

A central theme in HIV immunopathogenesis is the ability of the virus to undermine the very pathways that are designed to defend the host against HIV and other invading pathogens. For example, the typical function of the lymph node is to kill antigens (e.g., foreign microbes) from the peripheral blood and trap it within the processes of the follicular dendritic cell (FDC) network.[101,102] Trapping of the antigen in the germinal center of the lymph node allows the germinal center B cells and CD4+ T cells in the paracortical areas to undergo antigenic stimulation as part of the initiation of an appropriate immune response. However, it is becoming increasingly apparent that the trapping of HIV in the lymph node germinal center may also play an important role in HIV immunopathogenesis.[103,104]

Another example of the subversion of the immune system by HIV is the manipulation of the molecular mechanisms of immune cell activation. The molecular basis for the activation of T cells involves the induction of cellular proteins that bind to the host cell genome and stimulate transcription of specific genes that are essential for T cell responses. One of these cellular proteins is

nuclear factor (NF-KB), which binds to NF-KB binding sites in the promoter region of the interleukin 2 gene as well as a variety of other cellular genes.[105] However, because the HIV genome also contains NF-KB sites, the induction of these cellular proteins results in the enhancement of HIV expression in addition to T cell activation. In addition, uninfected, activated CD4+ T cells are more susceptible to productive infection after exposure to HIV.[106,107]

**Depletion of CD4+ T cells during clinical latency.**

Many of the mechanisms of CD4+ T cell depletion that have been proposed to account for the dramatic decline in CD4+ T cells after acute HIV infection may also be responsible for the slow, insidious fall in CD4+ T cells that occurs throughout the period of clinical latency. For example, the continuous replication of HIV that has been shown to occur in the lymphoid tissue during the asymptomatic phase could result in single cell lysis of syncytia formation in infected CD4+ T cells. In addition to direct HIV-mediated cell killing, indirect mechanisms may also play a role (Table 1).

**Table I. Potential Mechanisms of HIV-Induced Cytopathicity.**

| |
|---|
| **Direct** |
| Accumulation of unintegrated viral DNA |
| High levels of viral RNA and aberrant host cell RNAs |
| Virion budding from the cell membrane |
| Intracellular complexing of HIV envelope and CD4 |
| **Indirect** |
| Syncytia formation |
| Autoimmune phenomenon |
| Autoantibodies |
| Innocent bystander killing |
| Superantigens |
| Apoptosis |

**Clinical Manifestations of HIV Infection**

The classification system for HIV infection has been revised to include the $CD4^+$ T-lymphocyte count as a marker for HIV-related immunosuppresion. This revision establishes mutually exclusive subgroups for which the spectrum of clinical conditions is integrated with the $CD4^+$ T-lymphocyte count. The objectives of these changes are to simplify the classification of HIV infection, to reflect current standards of medical care for HIV-infected persons, and to categorize more accurately HIV-related morbidity.

The revised CDC classification system for HIV-infected adolescents and adults catagorize persons on the basis of clinical conditions associated with HIV infection and $CD4^+$ T-lymphocyte counts. The system is based on three ranges of $CD4^+$ T-lymphocyte counts and three clinical categories and is represented by a matrix of nine mutually exclusive categories (Table2)

**Table II. 1993 revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults**

| | Clinical categories | | |
|---|---|---|---|
| $CD4^+$ T-cell categories | (A) Asymptomatic acute (primary) HIV or PGL | (B) Symptomatic not (A) or (C) Conditions | (C) AIDS-indicator conditions |
| (1) ≥500/μl | A1 | B1 | C1 |
| (2) 200-499/μl | A2 | B2 | C2 |
| (3) <200/μl AIDS-indicator T- cell count | A3 | B3 | C3 |

PGL= persistent generalized lymphadenopathy

$CD4^+$ T-lymphocyte Categories

The three CD4+ T-lymphocyte categories are defined as follows:

- category 1 : ≥500 cells/μl
- category 2 : 200-499 cells/μl
- category 3 : <200 cells/μl

These catogories correspond to $CD4^+$ T-lymphocyte counts per microliter of blood and guide clinical and therapeutic actions in the management of HIV-infected adolescents and adults.[108-114]

**Clinical Categories**

The clinical categories of HIV infection are defined as follows:

**Category A**

Category A consists of one or more of the conditions listed below in an adolescent or adult (≥13 years) with documented HIV infection. Conditions listed in categories B and C must not have occured.

- Asymptomatic HIV infection
- Persistent generalized lymphadenopathy
- Acute (primary) HIV infection with accompanying illness or history of acute HIV infection.[114,115]

**Category B**

Category B consists of 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: a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated

immunity; or b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection.

**Category C**

Category C includes the clinical conditions listed in the AIDS surveillence case definition (Appendix III). For classification purposes, once a Category C condition has occured, the person will remain in Category C.

**Therapeutic Strategies**

Zidovudine, a nucleoside analogue; becomes active as an antiviral agent when it is phosphorylated inside the infected cell. In the phosphorylated (zidovudine triphosphate) form it competes with one of the natural DNA building blocks, deoxythymidine triphosphate, for incorporation into the nucleic acid chain. Incorporation of nucleotides is catalysed by viral RT, which recognizes zidovudine triphosphate in preference to deoxythymidine triphosphate. When phosphorylated zidovudine is added, DNA replication is effectively terminated. The azido group on the 3' position prevents further addition of new bases to the chain.

Zidovudine is a very potent and selective inhibitor of acute HIV infection in vitro. Its marked anti-HIV activity has been confirmed in several cell systems, including peripheral blood lymphocytes, monocytes and macrophages.

In the clinical setting, zidovudine significantly slows the progression of HIV infection and can reduce the frequency and severity of opportunistic-infections. It increases length and quality of life, is effective against HIV-associated neurological dysfunction, increases or maintains CD4 cells counts

and immune function, and has been shown to reduce the amount of HIV in the plasma of AIDS patients. However, significant bone marrow toxicity may require monitoring.[110,117]

Dideoxycytidine (ddC) and dideoxyinosine (ddI) are another 2 nucleoside analogues with considerable activity against HIV. Clinical studies, however, have shown that both are associated with peripheral neuropathy in a high percentage of patients. Moreover ddI can cause pancreatitis, which is generally unpredictable and may be fatal in a minority of patients. Both agents have been approved for clinical use either singly for those who fail or cannot tolerate zidovudine or in combination with zidovudine to active a higher antiviral effect.[118,119,120]

HIV protease Inhibitors

The HIV-1 protease enzyme has a well-documented three-dimensional structure that is distinctive enough to allow for specificity. In addition, mutations on the active site of the protease molecule have been found to prevent complete processing of the viral precursor proteins, so that immature, non-infectious virions are formed. All of these factors have made the HIV protease an important target for the design of anti-HIV drugs.

HIV protease inhibitors have been successfully synthesized and early tests show that the most active compounds inhibit protease and HIV replication. Although oral bioavailability may be limited with these compounds, the concentrations necessary to significantly inhibit protease are very low compared to those that may cause unwanted toxicities, suggesting that these may be highly selective agents.[121,122]

**Combination therapy**

A large number of compounds, both nucleosides and non-nucleosides, are now being studied as inhibitors of the viral reverse transcriptase.The use of these compounds as single agents has led to the emergence of resistant virus, and in the case of the non-nucleoside RT (NNRT) inhibitors, this is particularly rapid and has been linked to clinical failure. These findings have indicated that combinations of drugs, rather than monotherapy, will be required for the successful treatment of HIV disease. Such strategies include regimens of drugs directed both at the same and at different viral enzyme targets, The simultaneous co-administration of reverse transcriptase inhibitors may have potential clinical benefits. The presence of multiple binding sites on the RT suggests that it may be practical to use combinations of compounds that have a different mutational basis of resistance.

A important rationale for targeting HIV-1 RT with drug combinations is the synergistic activity against the virus exhibited by zidovudine in combination with other RT inhibitors. For example, zidovudine and the NNRT inhibitor nevirapine have been shown to inhibit HIV-1 synergistically at all concentrations tested.[123] A second NNRT inhibitor, u-90152 is synergistic when used in combination with either zidovudine or ddC.[124,125]

**Laboratory diagnosis of HIV infection**

The diagnosis of HIV infection is usually made on the basis of the detection of antibodies to HIV. Serological tests for detecting antibodies to HIV are generally classified as initial tests (sometimes referred to as screening tests) or supplemental tests (sometimes referred to as confirmatory tests). Initial tests provide the presumptive identification of antibody-positive specimens,

and supplemental tests are used to determine whether specimens found reactive by an initial test contain antibodies specific to HIV.[126]

The most widely used initial tests are ELISAs and particle agglutination assays. The earliest assays used purified HIV lysates, and deficiencies in sensitivity and specificity were identified and rapidly corrected. The sensitivity and specificity of initial assays have since improved dramatically as a result of new methods of viral purification, different test formats, and the greater use of recombinant and synthetic peptide antigens.[126-129]

A number of rapid/simple initial tests are now available. Most of them use an immunodot format in which specimen and reagents are added by means of a dropper to an absorbent membrane. A positive result is indicated by the appearance of a colored dot or line. These tests require no instrumentation, can generally be performed in less than two minutes, and the results are interpret visually. These tests are most suitable for use in laboratories with limited resources and small number of specimens.

When a single initial assay is used for testing in a population with a very low prevalence of HIV infection, the probability that a person is infected when a positive test result is obtained is very low, since the majority of people with positive results are not infected. This problem occurs even with a very high specificity assayed. Accuracy can be improved by re-testing the sample with a supplemental test.[130]

The most commonly used supplemental test is the western blot(WB).[131] Studies have shown that combinations of ELISAs or rapid/simple assays can provide a positive predictive value similar to that of the WB at a much lower

cost. WHO therefore recommends that countries consider testing strategies that maximize the use of ELISAs and rapid/simple assays as an alternative to the WB.[130]

A number of other assays have been introduced in recent years which assist in the establishment of the diagnosis of HIV infection and may also be used to monitor the progress of the infection and the response to therapy. These include assays that detect HIV p24 antigen [132,133] and the presence of viral nucleic acid by means of a process called polymerase chain reaction (PCR).[134] Circulating p24 antigen appears early in the course of HIV infection, is detecteble for several weeks, and then disappears or falls to very low levels until the onset of clinical illness. Rising titres of HIV antigen late in the illness are correlated with a poor prognosis. The presence of circulating p24 antigen is also associated with increased levels of infectious virus particles, as the probability of isolating HIV from an infected person is highest when p24 antigen is detected.

PCR is an enzymatic method of amplifying the amount of viral nucleic acid in a specimen until it can be detected by conventional techniques. In theory, as little as a single viral genome can be detected; in practice, the technique can have limited specificity. It is also time-consuming, labour intensive, expensive, and remains largely a research tool. At this time, a diagnosis of HIV infection should not be made on a single positive PCR test result, in the absence of any other detectable markers.

**Antigenic variability**

As in lentivirus infection, millions of genetic variants can exit at the same time within one infected host. This heterogenicity of the viruses is described by various terms such as 'quasispecies' or 'population polymorphism' both of which indicate that the variability of HIV in a single individual can be very considerable.[9,10]

Aspects of the genetic variability of HIV could influence its ability as a pathogenetic agent. Strains that differ in replication rate and killing capacity, may affect the severity of the disease and the rate of progression.[11,12,13,14] The importance of the appearance of syncytia inducing (SI) strains for disease progression is well known.[16,18] Antigenic variation can lead to strains able to escape from specific humoral and cell-mediated immune controls, and the development of differential tropisms for various cellular types will affect the course of the disease.[19,20] Genetic variations can also influence the sensitivity of different strains to different strains to different antiviral drugs.

This evolutionary process of the viral population during the course of infection is perhaps one of the most challenging aspects of HIV which therapeutic strategies have to overcome. A major aim of antiretroviral therapy is to inhibit the evolution of the virus inside every infected individuals.

After initial infection, viable strains of HIV will start to expand and produce new variants dictated by then ability to escape the immune response. The source of this variation-expansion of the viral population in a single patient , very similar to a micro-evolutive phenomenon is the

low accuracy of RT.[135] Once the quasispecies has reached a sufficient degree of internal variability, however, a second potent genetic mechanism for the generation of variability is offered by genetic recombination. This process can be very efficient, especially in the presence of SI variants. Phenotypic variants are viruses exhibiting altered characteristics as a result of changes in the viral genome. Of particular interest is the region of the genome coding for the envelope proteins.

The V3 loop is one of five hypervariable regions in the gp120 portion of the envelope gene (hypervariable regions being defined as areas exhibiting less than 30 percent amino acid conservation.[21,22,23] Research has shown that substitutions, particularly in the V3 domain, are a key factor in the determination of cellular tropisms and in the antigenicity of gp120.[19,20]

The mutations in the envelope gene which cause transition from NSI (non syncytia inducing variants) to SI strains are still not fully understood. Recent observations, however, have indicated that the substitution of basic amino acid residues at positions 11, 24, 25 and 32, in the 35-amino acid V3 loop sequence, contributes to an SI phenotype.[19,20]

The generation of antigenic variants of HIV-1 has proved to be the major obstacle in the development of a vaccine against AIDS. It is in the best interest of the virus to select the least immunogenic variant so as to elicit a minimal immune response, and the infidelity of HIV-1 RT result in the presentation of a continually evolving antigenic profile for the host immune system to contend with Phylogenetic analysis of HIV DNA sequence is being widely used to examine lineages of different viral

strains as they evolve and spread throughout the globe.[136] Refering to the next lower stratum of HIV taxonomy to describe a distinct cluster of genetically-related variants within subtype, in one study the term genotype was used by Ou et.al.[37] Louwagie et al., however, used the term genotype as a third synonym along with clade (form the Greek Klados, meaning branch) and subtype to describe the major worldwide genetic groupings of HIV-1.[136] To refer to closely related genetic variants that have been identified within a subtype, genotype will be used when determination is made by genetic sequencing or probe hybridization, while serotype will apply when identification is by serologic method.

**Genetic Diversity and Worldwide subtypes**

Several methods can be used to quantify the degree of genetic difference and often referred as genetic diversity, divergence or variation within or between subtypes, genotypes, and isolates.[38,39] The similarity and differences are by first matching up all possible pairs among a sample of nucleic acid sequences and determining the percentage of nucleotides at corresponding positions with in each pair for which mutations such as substitutions or deletions have occurred. Then a mean is determined among all these percentages within all possible pairs to determine the overall diversity among the isolates compared. Sometimes this percent of genetic divergence is subtracted from 100% representing exact 'homology' between isolates to express the degree of nucleotide similarity. The degree of genetic difference between isolates is often illustrated in graphic trees produced by various types of phylogenetic analyses.

Rapid heteroduplex mobility assays[41] have recently been developed to estimate genetic diversity without expensive and labor-intensive DNA sequencing. Consensus sequence, representing the most common nucleotides sequence at each specific position in the genome among a group of isolates, is often generated for comparison.

Serotyping was developed to provide an alternative technology to genetic sequencing for monitoring HIV-1 molecular epidemiology. This method is performed by Enzyme immuno assay (EIA) using specific synthetic peptides derived from the concensus sequences of 14-16 amino acids on the V3 loop coated into microtiter plates. Peptide serotyping continues to provide a highly accurate, simple and inexpensive alternative method for monitoring HIV-1 subtypes in Thailand.[42,43,44,45]

Nine subtypes, designated A, B, C, D, E, F, H, I and O, have been described for HIV-1 based on genetic similarities and differences in the env gene, which codes for the viral envelope and is the most common gene region for HIV phylogenetic analysis.[30,31,32,33,34,35] Subtypes A and D have been found primarily in central and western Africa, while, subtype B is the predominant subtype in North America, Europe, Japan and Australia. Subtype C has been found mostly in southern Africa, the Central African Republic, and India. Subtype E can be found in Thailand and recently in the Central African Republic. Subtype F has been found in Romania[33] and is a rare variant in Brazil.[31] Isolates from Gabon and the Russian Federation were designated Subtype H.[30,35] An 'outlayer' subtype O contains two human and two chimpanzee isolates.[30]

Two distinct env genotypes of HIV-1, designated 'Thai A' and 'Thai B' were first identified in Thailand.[36,37] Thai genotype A differed from all previously identified subtypes and was thus classified into a new subtype E.[137] Although Thai genotype B falls within subtype B, it is distinctly different from the other subtype B variants that are predominant in the Americas and Europe, Such as reference strains HIV-$1_{LAI}$, HIV-$1_{MN}$.[30] Subtype B sequences usually have a GPGR tetrapeptide sequence at the tip of the V3 loop on the virus envelope, which is the principal determinant responsible for eliciting neutralizing antibodies to HIV-1. However, Ou et al. found a GPGQ motif in 88% of the Thai genotype B isolates.[137] The GPGQ motif was also found in all of Thai genotype B sequences from another set of specimens collected in Bangkok by Okuda et al.[45] The GPGQ tetrapeptide motif is commonly found in subtypes A, C, and D identified principally from Africas, as well as in the new subtype E from Thailand and the Central African Republic.

**Segregation by Mode of Transmission.**

One of the most remarkable findings of the molecular epidemiology of HIV-1 in Thailand has been the marked segregation of the two Thai genotypes by mode of transmission. Nationwide, approximately nine of ten persons presumed infected by sexual transmission such as female and male sexworkers and male STD patients are infected with genotype A, while about three out of four injecting drug users are infected Thai genotype B.

This segregation was first observed among a geographically diverse sample of about 60 specimens of known genotype determined by direct sequencing, cloning or hybridization.[37] A peptide enzyme immunoassay (EIA),[42,45,138] confirmed the segregation among a larger

number of specimens from the original samples in 1991.[37] The reasons for the observed partial segregation of Thai HIV-1 subtypes by transmission mode remain unclear, and the causes may by multiple. A 'founder effect' involving kinetics of population genetics may be involved : Chance introductions of different strains into different risk groups will lead to the continued predominance within each group of the first strain introduced, and the equilibrium of the proportions of strains within each population thus may never occur.