

CHAPTER II

LITERATURE REVIEW

1. Human immunodeficiency virus (HIV)

The AIDS virus was discovered by Luc Montagnier (Pasteur Institute, France) and Robert Gallo (National Cancer Institute, USA) around 1983. The human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), belongs to the Lentivirus subfamily of Retroviruses. In 1990, less than 5% of the world's population was seropositive, but by the end of 2001 an estimated 5 million people were infected (UNAIDS, 2002). HIV affects the immune system, causing acquired immunodeficiency. The structure and replication properties of the virus may be the reason why the immune system is unable to dominate the infection.

1.1 HIV subtypes

There are two types of human AIDS viruses, HIV-1 and HIV-2, distinguished according to their genome organization and phylogenetic relationships. Both HIV-1 and HIV-2 are further divided into subtypes. HIV-1 has three distinct groups M, N, and O. HIV-2 has six distinct groups A to F. Most infected individuals in the world are in the HIV-1 group M. Group M includes 10 different subtypes, identified as A, B, C, D, E, F, G, H, J, and K, and they are unevenly distributed around the world (Novitsky et al., 2001).

In Thailand, HIV-1 subtype B was first detected in intravenous drug users (IDUs) in the Bangkok area in late 1987. During the late 1980s, HIV-1 subtype E, the Thai subtype, had spread very rapidly among heterosexuals in Thailand, with the highest rates occurring in the northern regions of the country. Although Thailand has both subtypes B and E, 90% of HIV transmission in the country involves subtype E through heterosexual sex. While HIV subtype A and D have been found primarily in central and western Africa, subtype B is predominant in Europe, the Western hemisphere, Japan, and Australia. Subtype C has been found mostly in southern Africa, the Central African Republic, and India (Weniger et al., 1994).

1.2 The virus: its genes and proteins, and its replication cycle

1.2.1 Structure and molecular features of HIV (Pavlakis et al., 1997, Burke et al., 1997)

The virion of HIV is composed of two identical copies of single stranded RNA, about 9,200 bases long. **Figure 1 A.** gives an overview of the genes and corresponding proteins for HIV-1.

The structure of HIV, which is a retrovirus, is enveloped, positive-strand RNA viruses in a cone-shaped core surrounded by a membrane envelope that relies on a unique enzyme, reverse transcriptase, to convert their RNA genome into a DNA 'provirus' that is integrated into the cellular genome. The core viral particle is composed of the p24 (CA) protein. The viral envelope is a lipid bilayer that is produced by the cellular plasma membrane, which consists of gp41, a transmembrane protein, and gp120; a surface molecule required for attachment to host cell receptors. The structure of HIV-1 is shown in **Figure 1 A**.

The genomic organization of HIV-1 is shown in **Figure 1 B**. The full HIV genome is encoded on one long strand of RNA. When the virus is integrated into the host's DNA genome (as a provirus) then its information is also encoded in the DNA. The HIV-1 genome has three coding regions in common, *gag*, *pol*, and *env*, which encode the capsid proteins or internal structural proteins (Gag), the viral enzymes necessary for replication (Pol), and the external glycoprotein (Env). HIV has one promoter and one polyadenylation site within the long terminal repeats (LTRs) and expresses one primary transcript. However, HIV-1 also contains six accessory gene products (*tat*, *rev*, *vif*, *vpu*, *vpr*, and *nef*), some of which are essential for HIV replication and reproduction. This is its form when it is a free virus particle.

Gag protein

The *gag* gene gives rise to the 55-kilodalton (kD) Gag precursor protein, also called p55, which is expressed from the unspliced viral mRNA. The p55 is cleaved during the viral maturation process into four smaller protein designated MA (matrix [p17]), CA (capsid [p24]), NC (nucleocapsid [p9]), and p6 by virally encoded protease (a product of the *pol* gene).

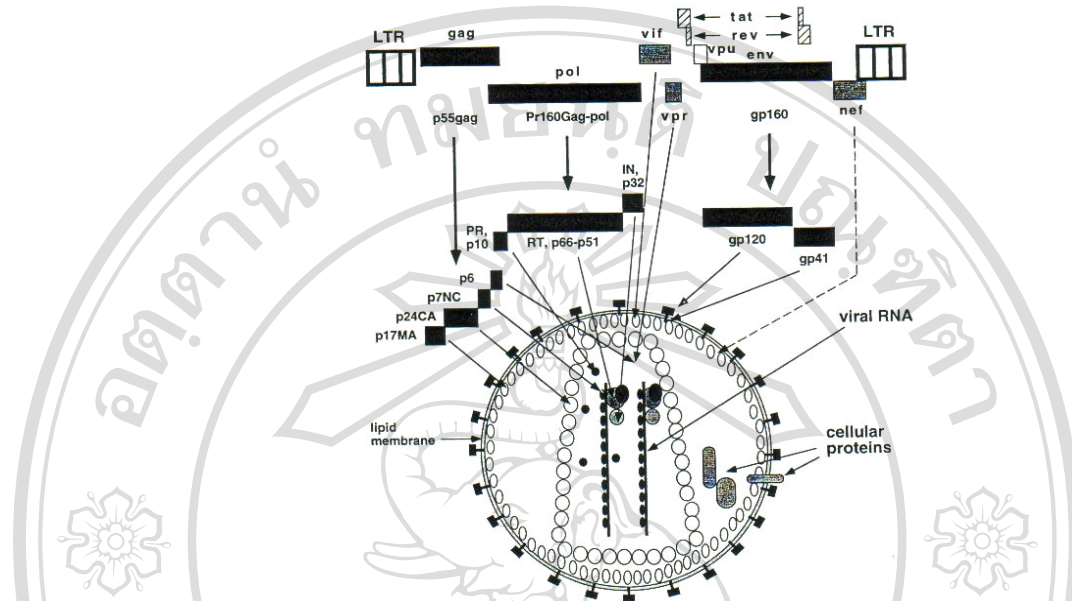
Gag-Pol Precursor

The viral protease (PR), integrase (IN), RNase H, and reverse transcriptase (RT) are always expressed within the context of a Gag-Pol fusion protein. During viral maturation, the virally encoded protease cleaves the Pol polypeptide away from the Gag and digests it further to separate the protease (p10), RT (p50), RNase H (p15), and integrase (p31) activities.

Env

The 160 kD Env (gp160) is expressed from singly spliced mRNA. A cellular protease cleaves gp160 to generate gp41 and gp120. The gp41 moiety contains the transmembrane domain of Env, while gp120 is located on the surface of the infected cell and virion through noncovalent interactions with gp41. The proteins expressed by HIV are shown in **Table 1**.

A.



B.

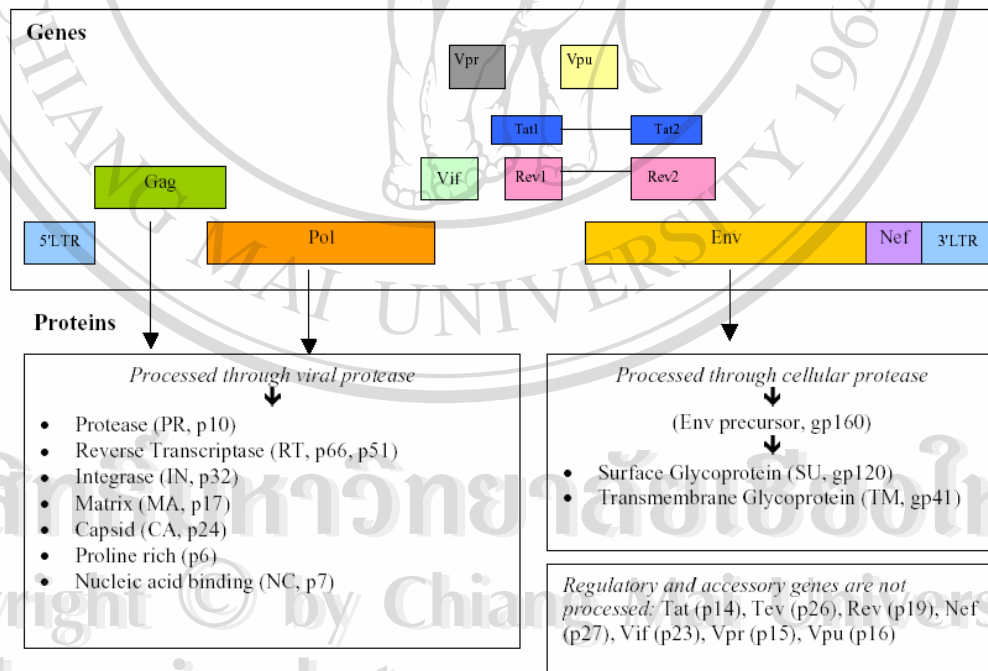


Figure 1. A. Virion structure and genomic organization of HIV-1 (Pavlakis GN., 1997) B. HIV-1 genes and proteins. The figure gives the approximate position of the genes in the HIV-1 genome, and the corresponding proteins (Levy, 1994 (a)).

Table 1. HIV genes, HIV proteins and their functions

Gene	Gene Product		Description	Localization
	HIV-1	HIV-2		
Structure				
<i>Env</i> (envelope)	gp160	gp140	Precursor envelope glycoprotein	Virion
	gp120	gp105	Outer or surface glycoprotein	Plasma membrane
	gp41	gp36	Transmembrane glycoprotein	
Gag (Group antigen; core)	p55	p55	Precursor core protein	Virion
	p24	p26	Core protein	
	p17	p15	Matrix protein	
	p9, p9	p6, p9	Nucleocapsid protein	
Pol (Polymerase)	p66	p64	Reverse transcriptase	Virion
	p51	p53	Reverse transcriptase	
	p31	p34	Integrase	
	p15	p15	Protease	
Accessory				
<i>Tat</i> (trans-activator)	p14, p16	p14, p16	Transactivator of viral RNA Synthesis	Nucleus
<i>Rev</i> (regulator)	p19/20	p16	Regulated viral mRNA expression	Nucleus
<i>Vif</i> (viral infectivity factor)	p23	p23	Increased viral infectivity	Cytoplasm, virion
<i>Vpr</i>	p18	p18	Assisted viral replication	Virion, nucleus
<i>vpu</i> (only in HIV-1)	p15	–	Involved virus release	Membrane protein
<i>vpx</i> (only in HIV-2)	–	p16	Involved virus infectivity	Virion
<i>Nef</i>	p27	p27	Pleiotropic, including suppression of virus, CD4 down regulation	Cytoplasm, plasma Membrane, virion

1.2.2 Life Cycle of HIV Infection (Pavlakis, 1997, Burke, 1992).

The life cycle of HIV can be described in six steps: binding to the target cell, fusion into the cell, reverse transcription, integration into the host's genome, replication, and budding of new virions.

Step 1: Binding to the target cell

HIV camouflages itself with host cell proteins, preventing the immune system from recognizing it as a foreign-body. The envelope that encases the viral core (nucleocapsid) is mostly covered with host-derived proteins, including major histocompatibility complex (MHC) proteins. Hidden amongst the host cells integrated on the envelope surface are two viral glycoproteins, gp 120 and gp41. Glycoprotein 120 facilitates binding, and glycoprotein 41 enables fusion. The HIV-1 surface glycoprotein, gp120, attaches to the surface receptor (usually CD4) of the host cell, and the resulting conformational change in gp120 allows binding of HIV to a second cell surface receptor, usually CXCR4 or CCR5. CD4 receptors are most highly expressed on T-helper (Th) cells making them especially susceptible to HIV infection. However, macrophages, monocytes, dendritic cells, langerhans cells, hematopoietic stem cells, certain rectal-lining cells, and microglial cells are also susceptible. Following the initial binding of gp120, a co-receptor and gp41 allow fusion of the virus into the target cell.

Step 2: Reverse Transcription and DNA synthesis

HIV genes are carried in two strands of RNA, while the genetic material of human cells is found in DNA. In order for the virus to infect the cell, a process called "reverse transcription" makes a DNA copy of the virus's RNA.

After the binding process, the viral capsid (the inside of the virus which contains the RNA and important enzymes) is released into the host cell. A viral enzyme called reverse transcriptase makes a DNA copy of the RNA. This new DNA is called "proviral DNA".

Step 3: Integration

The "proviral DNA" then enters the host-cell nucleus, and with the help of an enzyme integrase becomes integrated into the host cell's genome, forming what is referred to as a provirus. Here, the virus can remain dormant for an undetermined amount of time. As long as the provirus remains in the latent state the viral genes are not expressed, however, each time the target cell replicates the viral DNA is passed along to daughter cells.

Step 4: Transcription

When the cell tries to make new proteins, it can accidentally make new HIV viruses, since their genetic material is inside the cell's nucleus. The strands of viral DNA in the nucleus separate, and special enzymes create a complementary strand of genetic material called messenger RNA or mRNA (instructions for making new HIV).

Step 5: Translation

The mRNA carries instructions for making new viral proteins from the nucleus to a kind of workshop in the cell. Each section of the mRNA corresponds to a protein building block for making a part of HIV. This complex step in viral replication is regulated by the viral accessory proteins, Rev, Tat, and Nef. Gag and Gag-Pol polyprotein precursors associate with the viral RNA genome close to the host cell membrane, and together with the Env-gp precursor, are processed by HIV protease (PR) to yield single proteins. As each mRNA strand is processed, a corresponding string of proteins is made. This process continues until the mRNA strand has been transformed or "translated" into new viral proteins needed to make a new virus.

Step 6: Viral Assembly

Finally, The virus assembles into a new virion. Long strings of proteins are cut up by a viral enzyme called protease into smaller proteins. These proteins serve a variety of functions; some become structural elements of new HIV, while others become enzymes, such as reverse transcriptase. Once the new viral particles are assembled, they bud off the host cell, and create a new virus. The host cell's membrane is modified by the insertion of gp41 and gp120. This virus is then able to infect new cells. Each infected cell can produce a lot of new viruses. Frequent budding results in lyses of infected T-cells. T-cells also tend to bud in a greater number of virions than other cells. Macrophages have lower levels of budding, but usually do not lyse and therefore continue producing low levels of the virus.

1.3 Features of HIV transmission

The transmission of a virus can be greatly influenced by the amount of infectious virus in a body fluid and the extent of contact an individual has with that body fluid. The appearance of AIDS in diverse populations implicated several routes of transmission such as sexual transmission, blood and blood product transmission and mother to fetus and infant transmission.

1.3.1 Sexual transmission

Transmission by a sexual route is responsible for the majority of infection worldwide, including male homosexual and bisexual men relationships and heterosexual couples, which account for 75 to 85 percent of HIV infection (Stoneburner et al., 1996). The risk of becoming infected through sexual transmission is associated with several factors: nature of sex (e.g. number of sexual partners, number of sexual exposures and likelihood of the sexual partner being infected), viral

variations in tropism and infectivity, host factors (other infectious disease, stage of infection, blood during sex such as menses, immunogenetic profile, etc.), vaginal douches, astringents, abrasives or trauma, the use of barriers to block HIV such as condoms.

In Thailand, meanwhile, recent modelling suggests that the main modes of transmission have been changing. Where most HIV transmission in the 1990s occurred through commercial sex, half of the new HIV infections now appear to be occurring among the wives and sexual partners of men who were infected several years ago (UNAIDS, 2002).

Individuals repeatedly exposed to HIV through unprotected sexual contact, but remain uninfected, form a population of persons likely to have either natural or acquired resistance to the virus. Since Rowland-Jones and collaborators (Rowland-Jones et al., 1995(a)) reported the detection of HIV-1-specific CTL in a cohort of highly exposed but apparently HIV-1 uninfected Gambian female prostitutes, there has thus been intense interest in understanding mechanisms responsible for protection against HIV-1 infection, and a search for models of naturally acquired immunity to HIV-1.

1.3.2 Mother to child transmission

HIV-1 may be transmitted from an infected woman to her infant during gestation (in utero), delivery (intrapartum), or post partum through breast-feeding. Transmission of the virus from mother to child has correlated with several factors, dependent mainly on the characteristics of the mother (e.g. extent of immunodeficiency, the absence of neutralizing antibodies to HIV, low CD4+ cell counts, co-infections, risk behaviour, nutritional status, immune response, genetical make-up), but also on the virus (e.g. phenotype, tropism, high virus levels in the plasma) and possibly, the child (genetical make-up), or exposure to infected breast milk (Levy, 1993). However, exposed but uninfected infants were reported. Several investigators detected cellular immune response, including lymphocyte proliferation, to HIV-1 peptides or HIV-1-specific CTL in exposed, apparently uninfected individuals and suggested that these responses are markers or perhaps even responsible for clearance of infection. Thus, exposed but uninfected infants are included in HEPS persons.

1.3.3 Transmission by blood and blood products

Epidemiologic analyses of transfusion-associated AIDS cases have made unique contributions to the understanding of HIV transmission and the natural history of the disease. For those individuals who have been infected by blood transfusion, plasma, or blood cell products (e.g. platelets), the transmission of HIV would have occurred via either free virus or virus-infected cells. Sharing needles among injecting drug users (IDUs) is a major route of HIV-1 transmission by blood, while blood transfusion is the most efficient mode of HIV-1 transmission. An inability to implement screening of blood donors and other blood supplies has led to transmission of HIV-1 through contaminated blood products.

2. HIV receptors and chemokines

HIV-1 uses the CD4 molecule as the primary receptor, but a coreceptor is also required to enter into the target cells. The chemokine receptors, CCR5 and CXCR4, are considered the major coreceptors in HIV-1 infection. The CCR5 molecule is used for fusion and entry by macrophage-tropic (M-tropic) or non-syncytium-inducing (NSI) HIV-1 strains, which are the predominant form of transmission and frequently found during the asymptomatic stages of infection. Higher cellular expression of CCR5 on CD4+ T cells correlates with activation and progression of the disease. The CXCR4 protein is mainly used by T lymphocyte-tropic (T-tropic) or syncytium-inducing (SI) HIV-1 strains, which emerge later in the disease in temporal association with the rapid decline of CD4+ T cells and the progression to AIDS.

The β -chemokines RANTES (regulated upon activation, normal T-cell expressed and secreted), and MIP-1 β (macrophage inflammatory protein-1 β), are ligands for CCR5 and thus provide the basis for discovery that these CC chemokines suppress HIV-1 infection in vitro (Cochi et al., 1995). CCR5 receptor binding by these chemokines inhibited M-tropic virus infection of CD4 cells (Cohn, 1997). RANTES is the most active inhibitor of HIV replication. Moreover, the absence of CCR5 on a cell has been associated with resistance to HIV infection. The role of CCR5 as a coreceptor for HIV in vivo is supported further by a report of resistance to HIV infection with R5 viruses by individuals who are homozygous for a 32-bpCCR5 gene deletion (Δ 32 CCR5) (Dean et al., 1996). The CXCR4 ligand is the stromal cell-derived factor 1 (SDF-1) chemokine. A CXC chemokine, SDF-1, a ligand for CXCR4, was shown to suppress replication of T-tropic HIV-1 isolates, thus confirming the connection between chemokines and HIV suppression. Those viruses that use only CXCR4 are now referred to as X4. Those that use CCR5 are referred to as R5, whereas viruses that use both receptors with comparable efficiency are referred to as R5X4. Although CCR5 and CXCR4 are believed to be the primary co-receptors for entry of HIV-1, several additional chemokine receptors (e.g., CCR2B, CCR3, CCR8, CCR9, CX₃CR1, GPR1, GPR15/BOB, STRL33/Bonzo, US28, and V28) have been shown to serve as co-receptors for HIV.

3. Immunopathogenesis of HIV infection

The immunopathogenesis of human immunodeficiency virus (HIV) infection is extremely complex. A variety of virologic and immunologic mechanism contributes to the progressive deterioration of immune function and progression of HIV disease to the acquired immunodeficiency syndrome (AIDS) (Pantaleo, 1993).

3.1 The course of HIV infection

3.1.1 Typical Progressors (Burke and McCutchan, 1997)

The typical course of HIV infection, for about 80% to 90% of HIV-infected persons, and a course of HIV disease with median survival times of approximately 10 years, has three discernible phases (**Figure 2**).

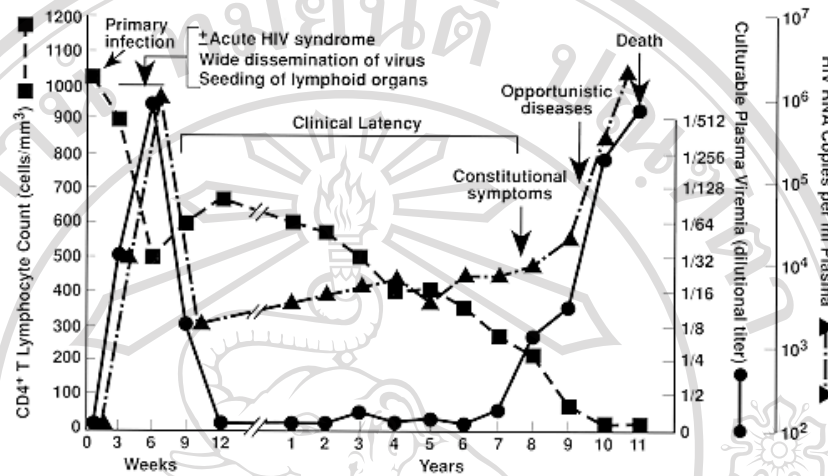


Figure 2. Typical course of HIV infection. During the period following primary infection, HIV disseminates widely in the body; an abrupt decrease in CD4+T cells in the peripheral circulation is often seen. An immune response to HIV ensues, with a decrease in detectable viremia. A period of clinical latency follows, during which CD4+ T cell counts continue to decrease, until they fall to a critical level below which there is a substantial risk of opportunistic infections (Pantaleo et al., 1993).

Primary infection is characterized by a peak in viral load, followed by the appearance of a cytotoxic T lymphocyte (CTL) and neutralizing antibody response. The viral load at the end of primary infection (viral set point) and associated CD4 count are strong predictors of subsequent disease progression. Furthermore, the diagnosis of acute HIV-1 infection cannot rely on standard tests such as the enzyme-linked immunosorbent assays (ELISA), which, typically, only becomes positive several weeks after infection (Busch et al., 1995). Currently, the detection of high plasma viral load (viral RNA) or p24 antigenemia represents the only valid laboratory approach for the diagnosis of primary infection, in the absence of serologic evidence for HIV-1 antibody (von Sydow et al., 1988, Rosenberg et al., 1997). The viral RNA assay is considered the more sensitive and can detect infection earlier than the p24

antigen test (Busch et al.). Subsequent evidence of seroconversion is necessary to confirm HIV-1 infection. Diversity and dynamics of the interaction between HIV and the immune system in early HIV infection suggest that the quality of the immune response is one factor influencing the set-point of the viral load. During primary HIV infection, subsets of CD8+ T cells that manifest cytolytic function are mobilized and expand to variable degrees. The number of CTL precursors directed against the viral Gag, Pol, and envelope proteins correlates with a decrease in the burst of plasma viremia, suggesting that CTLs play a fundamental role in the control of HIV infection. The cellular immune response appears to be more important than the humoral response in controlling HIV replication during acute infection, because neutralizing antibodies against HIV are not detected for at least 30 to 60 days after the resolution of the peak viremia.

This first stage is followed by a period of apparent clinical stasis, called asymptomatic infection. This period is variable in length, averaging 8 to 10 years in the absence of therapy (Pantaleo et al., 1993).

The last stage corresponds to the acquired immune deficiency syndrome (AIDS). The progression to AIDS in typical progressors results from the continuous replication of virus in the lymphoid tissue, which is associated with progressive destruction of this tissue and severe impairment of immune function. (Pantaleo, 1993). CD4 counts are then markedly reduced (below 200 cells/mm³) and CD8+ T cell responses compromised, leading to a generalized immunodeficiency. The patient suffers from opportunistic infections and/or unusual forms of cancer.

3.1.2 Rapid Progressors (RPs)

A small proportion of persons infected with the virus, approximately 5% of HIV-infected individuals, develop AIDS and die within months. Following primary infection, CD4+ T-cell levels rapidly decline within 2 to 3 years and full-blown AIDS develops within 3 years after primary HIV infection (Pantaleo et al., 1995 (a)). Immune responses are usually defective in these rapid progressors, with low levels or no detectable anti envelope-neutralizing antibodies to the autologous virus. Although it is unclear whether HIV-specific cytotoxicity is defective in rapid progressors, it has been shown that the CD8+ T-cell-mediated suppression of HIV replication is severely impaired. Rapid progressors uniformly exhibit higher HIV RNA levels in the plasma as well as higher HIV DNA load in peripheral blood mononuclear cells (PBMC) when compared with nonprogressors. The viral load is usually higher soon after seroconversion and persists throughout the course of the disease. Host factors such as age or genetic differences among individuals, the level of virulence of the individual strain of virus, as well as influences such as co-infection with other microbes may determine the rate and severity of HIV disease expression in different people.

3.1.3 Long-Term Nonprogressors (LTNPs)

A small percentage of infected persons (5%) have stable CD4 T-cell counts, and show no progression of disease for many (>10) years after HIV infection, despite lack of therapy (Pantaleo et al., 1995 (b)). Lack of exposure to STD or recreational drugs does not appear to explain the delayed course of disease. The criteria used for

nonprogression include documented HIV infection for more than 7 years, stable CD4+ T-cell counts higher than 600 cells/ μ l, absence of symptoms, and no anti-retroviral therapy (Buchbinder et al., 1994). The persistent immune competence seen in a small percentage of HIV-infected individuals reflects the heterogeneity of both the virus and the host. Some HIV strains, for example, are more cytopathic than others. Host factors that promote longer survival lack "enhancing" antibodies, an appropriate balance of two crucial CD4+ cell subsets and their cytokines, and a strong CD8+ cell antiviral response (Levy, 1994). From an immunologic standpoint, immune functions are conserved in LTNPs, and both HIV-specific humoral and cell-mediated immune responses are very strong. In addition to normal and stable CD4+ T-cell counts, the absolute number of CD8+ T lymphocytes is significantly and consistently higher in most LTNPs (Pantaleo, 1995 (b)). Studies of the immune response in LTNPs have not revealed any single immune response uniformly associated with nonprogression. In vitro tests show strong HIV-specific CD8 T-cell and neutralizing antibody responses. Many investigators noted a high frequency of anti-HIV-specific memory CTLs against the envelope (Pantaleo, 1995 (a)); and memory CTLs against Gag, Pol and the envelope were also present at high levels compared with levels in patients with intermediate and advanced stage disease. The demonstration of strong CTL responses and low viral load is consistent with a potential role of CTL as a protection host defense. However, LTNPs appear to be a heterogeneous group of individuals in which multiple factors may contribute to the state of nonprogression. The level of antibodies in LTNPs have also proved inconsistent. In some studies, LTNPs exhibit potent and broad neutralizing antibody responses against laboratory-adapted isolates as well as a diverse panel of primary HIV isolates (Pantaleo, 1995 (b)). Furthermore, involvement of genetic factors (i.e. major histocompatibility complex [MHC] class I and II molecules) has been proposed as an explanation for the lack of disease progression in LTNPs.

3.1.4. Highly- exposed persistently seronegative (HEPS) persons

Since Rowland-Jones and collaborators (Rowland-Jones et al., 1995 (a)) were reported the detection of HIV-1-specific CTL in a cohort of highly exposed, but apparently HIV-1 uninfected commercial sex workers in the Gambia, there has thus been intense interest in understanding mechanisms responsible for protection against HIV-1 infection and a search for models of naturally acquired immunity to HIV-1. Today, it is known that exposure to HIV does not always lead to overt infection and its conventional manifestations. Various groups of individuals, who have almost certainly been exposed to HIV, such as sexual partners of HIV-infected individuals (heterosexuals and male homosexuals) (Mazzoli et al., 1997), commercial sex workers (CSWs) (Rowland-Jones et al., 1995 (a)), infants born to HIV-1 infected mothers (Clerici et al., 1993 (a)), recipients of HIV-1 infected blood products, injecting drug users (IDU) who have shared needles with HIV-1 infected persons (Barcellini et al., 1995), HIV-1 exposed health care workers (Clerici et al., 1994, Pinto et al., 1995), and recipients of needle stick injuries have particularly high incidence rates of infection by HIV-1. These individuals are referred to as highly exposed persistently seronegative (HEPS), exposed uninfected (EU), or exposed

seronegative (ES). In this study, the term HEPS is used. Moreover, the standard criteria of HEPS individuals are HIV-1 specific IgG negative by all standard serologic tests, lack of detectable HIV-1 proviral DNA and HIV-1 RNA by polymerase chain reaction (PCR) and reverse transcriptase (RT) PCR, and they have no clinical or laboratory signs of immunodeficiency. Meanwhile, all their sex partners are positive to all the tests. The importance of the CCR5 receptor demonstrated that individuals homozygous for a 32 base-pair deletion in the CCR5 coding sequence do not express the protein at the cells surface, and, consequently, their CD4 T cells resist in vitro infection with M-tropic isolates of HIV (Liu, 1996, Dean, 1996). Individuals that are homozygous for this defect are highly, but not completely resistant to HIV infection or in vivo exposure. Notably, cells from homozygous individuals were infectable by T-tropic HIV-1 isolates (using CXCR4 for entry) as well as by dual-tropic isolates. Thus, HIV-specific immune response may be detected in individuals with multiple exposure to HIV, despite the fact that they are seronegative, support the concept that HIV infection can be effectively controlled or even prevented by the immune response.

Immune responses to HIV have been detected in at least some such persons, suggesting the possibility that abortive infection may have occurred or there is infection below the limits of detection. Acquired factors may be other mechanisms that play a role in resistance to HIV infection in HEPS persons. They include production of interleukin-2 (IL-2) and interferon-gamma (IFN- γ) from T helper type 1 (Th1) after stimulation with HIV peptides (De Maria et al., 1994, Cheynier et al. 1992), HIV-specific IgA antibody at the mucosal surface (Kaul et al., 1999), CD16+ natural killer (NK) cells, HIV-specific cytotoxic T cell (CTL) response (Bernard et al., 1999), and other roles of immunity against HIV in HEPS persons.

Upon HIV infection, imbalance of cytokine patterns between Th1 and Th2 are generated with disease progression. These are important in the control of viral replication. Several studies have shown that peripheral-blood mononuclear cells from presumably HIV-1-exposed, but apparently uninfected individuals, proliferate (Borkowsky et al., 1990, Clerici et al., 1992) and secrete interleukin-2 (Barcellini et al., 1995, Clerici et al., 1992, Clerici et al., 1993 (a), Salk, 1993), on exposure to T-helper-cell-epitopes. This observation led Clerici, Shearer, and others to postulate that some exposed but uninfected individuals had had a Th1 immune response to HIV-1, which eliminated the virus and provided subsequent protection.

It has been postulated that repeated exposure to HIV through continued contact is responsible for the maintenance of these responses, and that these somehow prevent establishment of infection. A recent report suggests that commercial sex workers who decrease the frequency of exposure have an increased risk of becoming infected, presumably due to a decline in CTL responses (Kaul et al., 2001 (a)). Because the majority of HIV transmission through is sexual intercourse, CSWs are likely to experience a variety of HIV-1 variants, whereas the serodiscordant couples are a single event. In Thailand, many investigators reported several factors in the HEPS group (CSWs or HIV infected sex partners) (Beyrer et al . 1999, Sriwanthana et al . 2001, Louisirotnchanakul et al., 2002) while Th1 and Th2 cytokine patterns have not yet been studied in this group.

In this study, the Th1 and Th2 cytokine patterns of HEPS persons were investigated with a flow cytometric intracellular cytokine assay and compared with those of their HIV-seropositive sex partners and normal subjects. In addition, humoral immune responses (antibody response; IgA) were studied in HEPS, in which HIV-1 proviral DNA was detected and confirmed that all HEPS persons are uninfected with HIV-1 and their sexual partners are HIV-1 infected.

3.1.5 Immunologic features of HIV pathogenesis

Long-term survival (or nonprogression) with HIV infection appears to depend on dominant type 1 responses of CD4+ cells. Cytokines, such as IL-2, support cell-mediated immunity and enhance CD8+ cell antiviral responses and their production of cell antiviral factor (CAF). Likewise, the production of Th1-type cytokines (e.g. IL-2 and IFN- γ) in long-term survivors helps maintain CD8+ cell anti-HIV activity. In contrast, in progressors, Th-2 type cytokines (e.g. IL-4 and IL-10) express suppression of CD8+ cell antiviral responses. Thus, individuals progressing to the disease could reflect a shift from a Th1- to Th2-type cell response (Clerici et al., 1993 (b)). The type 2 cytokines subsequently produced, not only turn off the type 1 cytokines needed for strong cell-mediated immune responses, but can also directly affect the antiviral responses of CD8+ cells.

When sufficient numbers of CD4+ cells are lost by direct or indirect mechanisms, the levels of cytokines such as IL-2 needed for maintaining the function of CD8+ cells will be reduced. The resultant loss of CD8+ cell antiviral activity and CAF production then leads to the emergence of HIV from many 'latently' infected cells in both lymph nodes and peripheral blood. The noncytopathic strains will induce further CD4+ cell loss by indirect mechanisms. With increased HIV production, the mutations associated with formation or emergence of the 'virulent' strains can eventually take place (Levy, 1994 (b)).

4. Immune response to Human Immunodeficiency Virus Infection

The immune response to the Human Immunodeficiency Virus (HIV) is determined by many complex factors. Prominent forms of HIV-induced immune dysfunction include defects in T- and B-cell responses to specific antigens, polyclonal hypergamma-globulinemia, enhanced autoantibody and immune complex formation, dysregulated cytokine production, decreased natural killer cell activity, and defective monocyte and dendritic cell function. In the route of HIV infection, the amount of HIV in the inoculum, the pathogenic potential of a given HIV strain, and host genetics may modify the host response to HIV. Some components of an immune response to HIV may enhance HIV infectivity or be directly responsible for clinical manifestations of the disease.

4.1 Innate Immune System (Burke et al., 1997)

Various different cell types, cytokines, and other soluble factors mediate the responses that occur generally within minutes or hours after an infection is initiated. The cell types include neutrophils, natural killer (NK) cells, macrophages/

monocytes, eosinophils and mast cells. Cytokines include the interferons (IFNs)- α , β and γ , which can be induced early in the response.

4.2 Adaptive Immune System

The adaptive responses are characterized by two features: specificity and memory. The only cell type that displays both these characteristics is the lymphocytes, of which two classes are known. B cells make antibodies; a human effector response, which once secreted, becomes widely distributed around the body. T cells not only have regulatory roles through helper (Th) and suppressor (Ts) activities, but also they may have effector activity, mediating delayed type-hypersensitivity (DTH) or cytotoxicity (cytotoxic T lymphocytes or CTLs), that is, lysis of infected cells.

4.2.1 Humoral Mediated Immune Responses to HIV Infection

The importance of the neutralizing antibody response in the control of HIV infection is not clear. Some reports correlate antibody levels with clinical stage, but others show that neutralizing antibodies do not influence viral load (Harrer et al., 1996), or do not correlate with protection. However, the antibody response only is not sufficient to fight an intracellular pathogen, and requires the assistance of the cellular immune response.

Following HIV infection, antibody responses are mounted rapidly; the main neutralizing antibody epitopes are located in at least five regions of the viral envelope: four sites in gp120, and one in gp41. Antibodies that neutralize HIV-1 recognize one of three distinct neutralizing domains of the HIV-1 envelope: the third hypervariable (V3) loop of the envelope and the transmembrane gp41 protein. Given the importance of the V3 loop in the interactions of the HIV-1 envelope with chemokine receptors, antibodies that bind to this domain of envelope can inhibit viral infection of cells. Antibodies specific for the V3 loop are the first neutralizing antibodies that arise in HIV-1-infected individuals.

Enhancing antibodies promote infection, but their clinical relevance in HIV-infection has not been fully resolved. The natural route of infection by many viruses, including HIV, is through a mucosal surface. IgA antibodies are a key element in the mucosal immune system. However, humoral immune responses include anti-cell antibodies and HIV-specific urinary, cervicovaginal and serum IgA (Mazzoli et al., 1999) that can neutralize primary HIV isolates found in some highly exposed persistently seronegative (HEPS) individuals (Rowland-Jones, 1995 (b), Shearer et al., 1996 (b), Fowke et al., 1996). HIV-1-specific cell-mediated immunity in HEPS may be present in the absence of detectable HIV-1-specific serum IgG antibodies, and can coexist with IgA humoral immunity [Mazzoli et al., 1997, Kaul et al., 1999, Beyrer et al., 1999]. The ability of mucosal and plasma IgA to inhibit HIV-1 transcytosis across the mucosal epithelium may represent an important mechanism for protection against the sexual acquisition of HIV-1 infection in HEPS individuals (Devito et al., 2000 (a), Devito C et al., 2000 (b)). Secretory IgA that can neutralize viral infectivity extracellularly is well established. IgA antibodies can neutralize viruses intracellularly if the virus is infecting an epithelial cell through which the IgA

is passing en route to the lumen. IgA demonstrates cross-clade neutralizing activity and is able to inhibit HIV mucosal transcytosis. IgA in plasma can neutralize primary HIV-1 isolates and this systemic humoral response may thus form a second line of defense and also contribute to the eradication of the virus in HEPS individuals.

4.2.2 Cell-Mediated Immune Responses (CMIR) to HIV Infection

In most viral infections, the cell-mediated immune response plays a vital role in arresting or eradicating the infection's agent. The activities include cytolytic responses of NK cells as well as CD8+ and CD4+ cells. CD4+ helper T-cell responses are required for the induction of B-cell antibody production and other T-cell responses. In reference to CTL activity, CD8+ and CD4+ lymphocytes generally respond to the presentation of epitopes in association with class I and class II MHC molecules, respectively.

4.2.2.1 Cytotoxic NK cells

Natural killer (NK) cells recognize and kill the virus-infected cells through ADCC. In this process, HIV-infected cells are killed through recognition by NK cells of antibodies bound to the viral envelope proteins on the infected cell surface. NK cells target virally infected cells with altered MHC class I molecule expression on their cell surface. NK cells are also targets of HIV infection. The level of active effector cells in the infected individuals is considered, and a reduction in ADCC can be demonstrated with disease progression. NK cells produce IFN- γ , known to stimulate the CD8+ T cell response. As a consequence, reduced NK function resulting from HIV infection may affect CTL activity.

4.2.2.2 T cell responses

There are two main types of T cells, T helper (Th) and T cytotoxic (Tc) cells. It has been suggested that there may also be a third type, T suppressor cells (Ts), but they are currently not well defined. Both Th and CTL cells make up the effector cells of the cell-mediated immune response.

Foreign antigen is presented as short peptides on the surface of antigen presenting cells (APC) by the MHC class I and II molecules, after processing by cellular proteasomes and degradation by phagolysosomes respectively. These are then recognized by means of the T cell receptor (TCR), a heterodimeric cell surface molecule that interacts with the peptides. CD4+ T cells respond via MHC class II molecules, and the CD8+ T cells via MHC class I molecules.

Cytotoxic CD8+ Cells

The virus-specific CD8+ cytotoxic T lymphocytes (CTLs) have been implicated in the control of HIV replication. A strong CTL response correlates with low plasma viremia, and a prolonged asymptomatic stage. Multiple mechanisms have been associated with this antiviral effect. CTL can lyse HIV-1-infected cells in vitro and block propagation of the infection.

McMichael et al. (McMichael et al., 2001) suggest that CD4+ T cells are damaged early in primary infection and this results in a suboptimal response by the

CD8+ T cells (CTLs). CTLs have multiple antiviral mechanisms, ability to kill infected cells and produce cytokines. Cytokines affect viral replication through their influence on Th cell activation and proliferation. The production of CC chemokines (a type of cytokine secreted by Th cells) (Cocchi et al., 1995) such as MIP-1 β , MIP-1 α and RANTES has activity against HIV. CC chemokines suppress HIV replication by competing for or down-regulating CCR-5, a primary co-receptor for CD4, and they are necessary for fusion of the viron into the cell. CTLs secrete these antiviral factors at sites of viral replication. Perforin is another weapon of CTL cells. It is a protein made by CD8+ T- cells and, together with granzymes, is an important trigger of cell death.

Consistent with the importance of CTLs in controlling HIV-1, the major histocompatibility complex (MHC) class I haplotypes of infected individuals has a significant predictive value for the rate of clinical disease progression. Since MHC class I molecules bind fragments of viral proteins and present those fragments to immune cells to initiate immune responses, the particular fragment of a virus that is immunologic for CTLs and the magnitude of virus specific CTL responses are determined in part by the MHC class I molecules expressed in an individual.

However, at some point during infection CTLs become incapable of controlling the virus. A possible contributing factor to this failure is the inability of other T-cells to help. The immune system is able to diminish the number of viruses initially, however, in effect it is positively selecting for CTL resistant HIV mutants. These mutants are not recognized by HLA molecules, since only the virions capable of escape survive to eventually reproduce the entire viral population that are CTL escaped mutants. At this point the immune system is completely defenseless against the virus, and HIV is free to reproduce and kill the immune system, thereby leaving the infected individual susceptible to opportunistic infections.

It is noteworthy that a decrease in HIV-specific CTL activity may occur without a reduction in other cytolytic functions of CD8+ cells in AIDS patients. In addition, the CTL response to some viral peptides (e.g., Gag) may decrease with progression to disease, while this activity against other proteins (e.g., Env) does not. These results suggest a selective loss of the anti-HIV CTLs that could have clinical relevance.

CD4+ Helper Cell Responses

The role of CD4 helper T cells in HIV-specific immunity is more complicated, since these cells are also a primary target for the virus. CD4 help is important for the generation and persistence of a successful CD8 response to viral infections, (Whitmire et al., 1998, Zajac et al., 1998) and mediate different immune responses depending on their type. Humoral CD4+ T cells can be separated into Th1 and Th2 subsets (Lucey et al. 1996). Th1 cells secrete IL-2, TNF- β , and IFN- γ , which are important for strong cell-mediated immunity; Th2 cells produce IL-4, IL-5, IL-6, and IL-10, which increase antibody production. The cytokines produced by Th1 and Th2 subsets also cross-regulate one another. Most recently, because similar cytokines can be produced by other cells in the body, the terminology of type 1 and type 2

responses has been used when the specific cells producing the cytokines are not identified (Romagnani, 2000).

CD4⁺ cell function that may influence anti-HIV responses have been found to reduce early in HIV infection before a substantial decrease in CD4⁺ number. Clerici and Shearer have categorized these activities that decrease over time by measuring cell proliferation and IL-2 production after exposure to recall antigens (e.g., tetanus, influenza A), irradiated human-leukocyte-antigen (HLA)-disparate white blood cells (allogeneic response), and PHA (mitogen response) stimulation. Many asymptomatic individuals have responses to all three stimuli; those who have no response to any are known to progress more rapidly to disease.

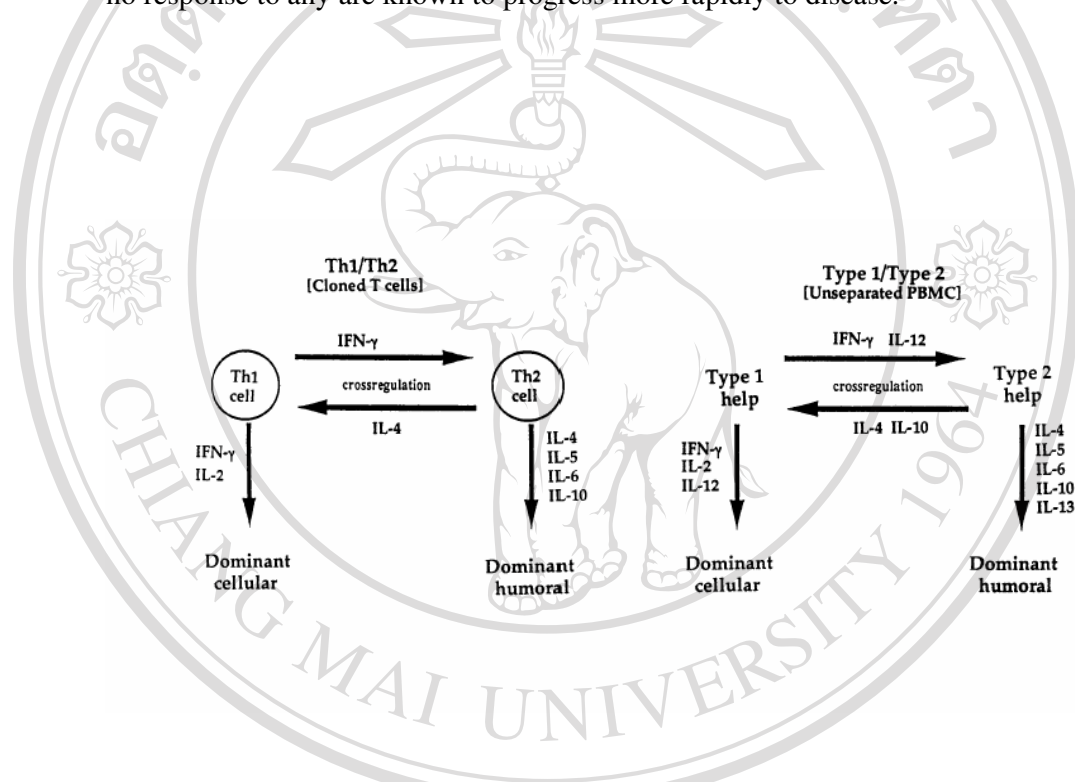


Figure 3. Comparison of nomenclature for Th1 and Th2 cytokines produced by cloned CD4⁺ Th cells and type 1 and type 2 cytokines produced by primary cultures of leukocytes of all types (Lucey et al., 1996).

The emphasis on the newer type 1-type 2 nomenclature is on the functional effects of the cytokines, independent of their cells of origin, and does not rely on cloned cells. Both Th1 and type 1 cytokines elicit predominantly CMI, whereas Th2 and type 2 cytokines elicit predominantly humoral immunity. At the same time, some Th1 (IFN- γ) and type 1 (IFN- γ , IL-12) cytokines downregulate humoral immunity by decreasing the levels of Th2 and type 2 cytokines (horizontal arrows pointing to the right). Conversely, some Th2 (IL-4) and type 2 (IL-4, IL-10) cytokines downregulate

CMI by decreasing the levels of Th1 and type 1 cytokines (horizontal arrows pointing to the left) as shown in **Figure 3** (Lucey et al., 1996).

The separation of T helper CD4+ cells into Th1 and Th2 cells and the interactions of type 1 and type 2 cytokines provide a helpful concept, but the system is more complex. While Th1-like cytokines (e.g., IFN- γ , IL-2) may increase cellular immune anti-HIV responses, these same cellular factors can augment HIV-1 production. In contrast, the Th2-like cytokines (e.g., IL-4 and IL-10) suppress HIV expression, but reduce CD8+ cell response (Romagnani, 2000).

Table 2. Leukocyte sources of type 1 and type 2 cytokines (Lucey et al., 1996)

Cell source	Cytokine(s)	
	Type 1	Type 2
CD4 T cell	IL-2, IFN- γ , IL-12, TNF- β	IL-4, IL-5, IL-6, IL-10, IL-13
CD8 T cell	IL-2, IFN- γ	IL-4, IL-5, IL-10
NK cell	IFN- γ , TNF- β	
Monocyte/macrophage	IL-12	IL-6, IL-10
B cell	IL-12, TNF- β	IL-6, IL-10
Dendritic cell	IL-12	
Neutrophil	IL-12	
Mast cell		IL-4, IL-5, IL-6
Eosinophil		IL-4, IL-5, IL-6

With regard to CD4+ cells, the Th subsets appear to represent terminally differentiated cells derived from a precursor (or Th0-type) cell capable of expressing many different cytokines. Differentiation of Th0 into Th1 and Th2 cells can occur in response to antigens or the type of cytokine present at the time of differentiation. The induction process cannot change on an already committed Th1 or Th2 cell. IL-12, for example, induces Th1 cell development from Th0 cells while down-regulating Th2 cell cytokine expression (e.g. IL-10). IL-1, produced by macrophages, selectively stimulates Th1 cells; IL4 stimulates development of Th2 cells, which in turn produce IL-4 that can down-regulate Th1-type cytokine expression.

Type 1 and type 2 cytokines can play an immune regulatory role in HIV infection and affect progression to disease. Th1-type responses are primarily found in healthy, asymptomatic HIV-infected individuals, whereas a predominance of the Th2-type subset response occurs during the symptomatic stage of disease. PBMC from asymptomatic individuals produce type 1 cytokines (e.g. IL-2, IFN- γ) early in disease, while those progressing to disease show increased production of type 2

cytokines (e.g. IL-4, IL-10). Th1-type response is protective against disease in an individual (Clerici and Shearer, 1993 (b)). Several reports have supported this conclusion in uninfected high-risk individuals (Clerici, 1992., Clerici, 1994 (a)). The disease progression is associated with type 2 cytokine production that may be responsible for suppressing the type 1 cytokine response (Romagnani, 2000). Increased production of type 2 cytokines, such as IL-4 and IL-10, can not only affect the production of type 1 cytokine necessary for maintaining cellular immune cell function, but it can also directly influence the antiviral responses of CD8+ effector cells. These results suggest that inducing a type 1 response directly or indirectly can be beneficial to cellular immune response to HIV.

CD4+ cytotoxic cells

Some CD4+ cells can have cytotoxic activity directed at either infected or uninfected CD4+ cells, or cell expressing HIV peptides in association with the class II MHC molecule. This response is usually found with Th1-type CD4+ cells, and it is virus-strain specific. Therefore, while these types of CD4+ cells may enhance cell-mediated response against HIV, through their CTL activities, they could also have a detrimental effect on the infection.

4.2.2.3 Non-lytic cellular responses

The non-lytic cellular response consists of antiviral activity that does not involve killing infected cells, but it suppresses viral replication through a variety of mechanisms. A subset of CD8+ T cells has the capacity to inhibit viral transcription. This is due to a soluble factor released by these cells, the CD8+ cell antiviral factor CAF, and possibly another mechanism associated with cell-to-cell contact. The extent of CAF production correlates with the stage of disease: less CAF is produced later on infection.

CD8+ and CD4+ T cells as well as NK cells and macrophages also produce a set of chemoattractant cytokines, called chemokines, which suppress viral replication. These comprise three β -chemokines, RANTES, MIP-1 α and MIP-1 β (Cocchi et al., 1995), which interfere with viral attachment to the β -chemokine receptor, CCR5. Consequently, macrophage-tropic NSI viruses, exclusively using the CCR5 coreceptor for viral entry, are sensitive to these antiviral responses, whereas T-cell-line tropic SI viruses are not. Non-lytic antiviral activity induced by combined CAF and β -chemokines have been associated with protection from infection (Stranford et al., 1999).

4.2.2.4 Cell-Mediated antiviral immune responses in HIV exposed persistently seronegative (HEPS) persons

Many previous reports suggested that some individuals were exposed to an infectious virus or viral antigens, but have not had an established infection. These people include sexual partners of infected individuals, children born of infected mothers, and health care workers with needle-stick injuries (Clerici, 1992, Clerici, 1993 (a), Clerici, 1994 (a)). The reason for this phenomenon is under study. Antigen-specific production of IL-2 by PBMC can be demonstrated in some of these subjects.

These observations on the subjects studied have been considered potential evidence that a predominant type 1 response occurred after exposure to HIV, and that an infectious virus was eliminated by cellular immune responses before infection was established and prior to production of antiviral antibodies. Alternatively, perhaps a noninfectious virus or insufficient viral antigens were presented to the host so that an antibody response was not elicited. One hypothesis under investigation is that the Th1-like response observed actually reflects protection of the individual from infection. In this case, strong cellular immune responses effectively block the infection.

However, a combination of factors, including cellular immunity, viral characteristics, and coreceptor integrity, may be involved in the persistent non-transmission of HIV.

5. Cytokines (Oppenheim JJ., 2001, Vilcek and Le, 1994)

Cytokines, soluble mediators, control many critical interactions among cells of the immune system. They form a diverse group of intracellular signaling peptides and glycoproteins with molecular weights (MW) of between 6,000 and 60,000, and most of them are genetically and structurally unrelated to one another. Cytokines are produced locally by a variety of tissues and cells. Only a few cytokines are normally present in detectable amounts in the blood and are able to influence distant target cells. Most cytokines, unless produced in excess, act only locally over short distances, in either a paracrine or an autocrine manner.

5.1 Type 1-Type 2 cytokine nomenclature

Th1 and Th2 clones were identified by mRNA expression and production of IFN- γ and IL-4, respectively. In a broader context, Th1 cytokines include IFN- γ , IL-2, and tumor necrosis factor beta (TNF- β) (lymphotoxin), whereas Th2 cytokines include IL-4, IL-5, IL-6, IL-10, IL-13, and possibly IL-9. However, it is now realized that other cytokines not produced by Th1 or Th2 clones, such as IL-12, also make important contributions to the regulation of the immune system. In addition, several Th1 and Th2 cytokines (e.g. IL-4, IL-5, IL-6, IL-10, and IFN- γ) are now recognized as not only deriving from CD4+ T cells, but also from other multiple leukocytes and even non-hematopoietic cells (Table 1). For this reason, the terminology, "type 1" or "Th1-like" is used instead of Th1, and "type 2" or "Th2-like" is used instead of Th2 in the characterization of in vivo immune-dysregulated diseases and conditions. The type 1-type 2 cytokine nomenclature emphasizes the function of a cytokine rather than the CD4+ T cell as the sole source of the cytokine. In this nomenclature, a type 1 response is defined as a strong cellular immune response with normal or increased levels of IL-2, IFN- γ , TNF- β , and/or IL-12, while a type 2 response is defined as an impaired cellular response with an increase in one or more B-cell activities (e.g. hypergammaglobulinemia, autoantibody production, or hyper-IgE) and an increase in the level of IL-4, IL-5, IL-6, IL-10, and/or IL-13 (Clerici and Shearer, 1994).

5.1.1 Interleukin-2 (Vilcek and Le, 1994)

Interleukin-2 (IL-2), formerly referred to as T-cell growth factor, is a powerfully immunoregulatory lymphokine. IL-2 is an autocrine and paracrine growth factor secreted by activated T lymphocytes and is essential for clonal T-cell proliferation. Its role in promoting T-cell proliferation, cytokine production, and the functional properties of B cells, macrophages, and NK cells, make IL-2 critical for activating all types of acquired immune responses.

Resting T lymphocytes neither synthesize nor secrete IL-2, but can be induced to do both by the appropriate combinations of antigen and co-stimulatory factors or by exposure to polyclonal mitogens. Although CD4 Th cells are the main source of IL-2, CD8 T cells and NK cells can also be induced to secrete it under certain conditions. Several signaling pathways regulate the IL-2 gene including the NF κ B pathway. When human lymphocytes are exposed to a T-cell mitogen, IL-2 mRNA expression becomes detectable after 4 hours, reaches peak concentration at 12 hours, and thereafter declines rapidly. Because IL-2 has a very short half-life in the circulation, it primarily acts as an autocrine or paracrine mediator.

5.1.2 Interleukin-4

IL-4 is a glycoprotein cytokine secreted by activated Th cells, mast cells, and a subset of NK cells. Secretion of IL-4 is a hallmark, as well as an inducer, of Th2 differentiation in T cells.

IL-4 and the closely related cytokine, IL-13, are produced in the same cell types and are regulated in similar ways. Both IL-4 and IL-13 favor Th2-cell development while suppressing the development and function of Th1 cells. They promote CTL activity, growth of mast cells and other hematopoietic cells, and expression of vascular cell adhesion molecule 1 (VCAM)-1 on endothelial cells. They also have multiple effects on macrophages, activating cytotoxic functions and increasing expression of class II MHC proteins, but suppressing the synthesis of proinflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF- α .

5.1.3 Interferons- γ

IFN- γ (also called type II IFN or immune IFN) arises from a single gene and differs in virtually all respects from the type I IFNs. Although IFN- γ has some antiviral activity, it is much less active in this regard than the type I IFNs. IFN- γ expression is not directly inducible by infection or double-stranded RNA. It is involved in the regulation of nearly all phases of the immune and inflammatory responses, including the activation and differentiation of T cells, B cells, NK cells, macrophages, and others. It is therefore best regarded as a distinct immunoregulatory cytokine.

IFN secretion is a hallmark of the Th1 lymphocyte. It is also secreted by nearly all CD8 T cells, by some Th0 cells, and NK cells. Each of these cell types secretes IFN- γ only when activated, usually as part of an immune response and especially in response to IL-2 and IL-12. IFN- γ production is inhibited by IL-4, IL-

10, TGF- β , glucocorticoids, cyclosporine A and FK506. Nearly all cell types express the heterodimeric receptor for IFN- γ and respond to this cytokine by increasing the surface expression of class I MHC proteins. As a result, virtually any cell in the vicinity of an IFN- γ -secreting cell becomes more efficient at presenting endogenous antigens and hence a better target for cytotoxic killing if it harbors an intracellular pathogen. Unlike type I IFNs, IFN- γ also increases the expression of class II MHC proteins on professional APCs, and so promotes antigen presentation to helper T cells as well.

IFN- γ is also a potent activator of macrophages. Although IFN- γ tends to promote the differentiation of B cells and CD8 T cells into immunologically active effectors, it does not promote lymphocyte proliferation. It enhances the activity of Th1 cells, but inhibits the production of Th2 cells. IFN- γ not only decreases the production of IL-4 by Th2 cells, but also potently blocks the effects of IL-4 on B cells, promoting IgG1 production at the expense of IgE production.

5.2 Effect of Cytokines on Immune Function and HIV Replication (Levy JA., 1994 (b))

Macrophages produce IL-1, IL-12, and other cytokines that permit CD4+ cells to reach a level of maturation that produces IL-2. IL-12 is needed for self-replication of the CD4+ cell population and to permit growth and function of CD8+ cells. Certain cytokines can induce the differential production of others by CD4+ cells that have led to their classification into Th1 and Th2 types (Shearer, 1993).

Other cytokines, such as IL-6 and IL-10 for B-cell growth and function, and IL-4 for T-cell maturation, are also important in maintaining an effective immune response. Type 1 cytokines can protect against apoptosis of CD4+ cells, whereas type 2 cytokines encourage this process (Clerici and Shearer, 1994).

Although HIV infection is associated with progressive disease in the vast majority of untreated persons, emerging data suggest that such a state of protective immunity can be induced in some infected persons. While the percentage of who remains in this category continues to decline with prolonged follow-up, long-term nonprogressors have been identified, and they can remain asymptomatic after 20 years of infection. Attenuated viruses and host genetic factors may influence disease progression. However, host immune responses to HIV -- including cytotoxic T lymphocytes, T-helper-cell responses, neutralizing antibodies, and other factors -- appear to play a key role in containing viremia among the majority of long-term nonprogressors, and to a lesser extent chronically infected progressors. To begin to understand the immunologic deficits observed in the vast majority of progressors, a range of factors must be considered. These include immune exhaustion, lack of adequate T-helper-cell function, immune escape, ineffective CTL responses in vivo, viral reservoirs, and a number of other factors.

5.3 Cellular activation and endogeneous cytokines in the pathogenesis of HIV disease

A general state of immune activation is associated with all stages of HIV infection. Although a state of immune activation is necessary in order to maintain HIV-specific immune responses, at the same time it may indirectly enhance virus replication, by either leading to the secretion of HIV inducing cytokines or generating a large pool of activated target cells that efficiently support virus replication. This activation is intimately linked to a change in the profile of detectable cytokines in the blood and lymph nodes of HIV-infected individuals (Rosenberg, 1997).

5.3.1 Effect of HIV on cytokine production

Since the initial observation that certain cytokines induce HIV expression from a state of latest or chronic infection to that of active virus expression, the effects of several cytokines on virus expression and replication have been extensively investigated.

Binding of the HIV envelope protein to the CD4 receptor on monocytes enhances the secretion of granulocyte-macrophage(GM)-colony-stimulating factor (CSF). TNF- α , IL-6, and IL-1 β . IL-2 levels are decreased in both blood and lymph nodes of HIV-infected individuals, and in vitro production of IL-2 from PBMCs obtained from HIV-infected individuals is decreased compared with uninfected individuals.

5.3.2 Cytokine induction of HIV Expression

Cytokines that induce HIV expression include IL-1, IL-2, IL-3, IL-6, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), TNF- α , and TNF- β . IL-2 and IL-12 induce virus expression only in T cells, TNF- α , and TNF- β induce expression in both T cells and macrophages, and the remaining cytokines induce expression only in macrophages.

5.3.3 Cytokine Suppression of HIV Expression

In contrast to the cytokines that induce HIV replication, IFN- α , and IFN- β exert a predominantly suppressive effect. IFN- α inhibits the activation of the HIV provirus by indirectly interfering with the NF- κ B-specific interaction.

A third category of cytokines can induce or suppress HIV, depending on the ex vivo culture condition. These include TGF- β , IL-4, IL-10, IL-13, and IFN- γ . IL-4 is active only on macrophages, and the others are active on both T cells and macrophages. Most cytokines induce HIV expression mechanisms acting at both the transcriptional and posttranscriptional levels; TNF- α , TNF- β , and IL-1 induce HIV expression by activation of the transcription factor, NF- κ B (Pantaleo, 1997).

Although the regulatory effect of cytokines on HIV replication is limited to in vitro observation, the fact that several of these cytokines may be found at increased levels in plasma, cerebrospinal fluid, and lymphoid tissue of HIV-infected individuals suggests that they probably mediate similar effects in vivo. It is possible that

cytokines play an important role in maintaining the constant levels of virus expression and replication, particularly in lymphoid tissue.

5.3.4 Th1/Th2 Cytokines Patterns

A paradigm involving differential activation of two subsets of CD4+ T-helper (Th) cell clones, Th1 and Th2, with different patterns of cytokine production has been proposed to explain, at least in part, the T-cell dysfunction observed in progressive HIV-related disease. Differential patterns of cytokines have been found to associate with protective or non-protective immune responses in several pathologic conditions in animal models. In the mouse, Th1-type responses favor strong cellular immunity with normal or increased levels of IL-2, IL-12, and IFN- γ production. Th2 responses result in increased IL-4, IL-5, IL-6, and IL-10 cytokines and promote B-cell differentiation and expansion. This strict Th1/Th2 dichotomy is not observed in humans because other cell types secrete these cytokines, further complicating the issue. The major cytokine facilitator of a Th1 response is IL-12, whereas that of a Th2 response is IL-4. The major cytokine suppressor of a Th1 response is IL-10; the major suppressor of a Th2 response is IFN- γ . Previous studies have suggested that a switch from a Th1 cytokine phenotype to a Th2 phenotype is a critical step in the progression of HIV disease (Clerici and Shearer, 1994). The dominant in vivo cytokine responses to HIV infection appear to be an increase in levels of TNF- α , IFN- γ , and IL-6 concomitant with reduced levels of IL-2, as determined by plasma levels and lymph node cytokine patterns.

Constitutive expression of IL-2 and IL-4 was rarely observed throughout the entire course of HIV infection. In contrast, high levels of constitutive expression of IFN- γ and IL-10 was expressed by non-T-cell subsets (i.e. macrophages).

In general, the Th1 pattern of cytokine secretion (i.e. IL-12, IFN- γ , TNF- γ , and TNF- α , and TNF- β) is associated with protective immune responses. The Th2 pattern of cytokine secretion (i.e. IL-4, IL-5, and IL-10) is associated with nonprotective immune responses (Romagnani, 2000). On the basis of these observations, an imbalance in the pattern of cytokine production may induce nonprotective immune responses, resulting in defective control of virus replication and disease progression. It was initially proposed that a predominance of the Th2 pattern of cytokine secretion was associated with HIV infection and that expression of this pattern was a critical step in the progression of HIV disease (Clerici, 1993 (b)) as shown in **Figure 4**.

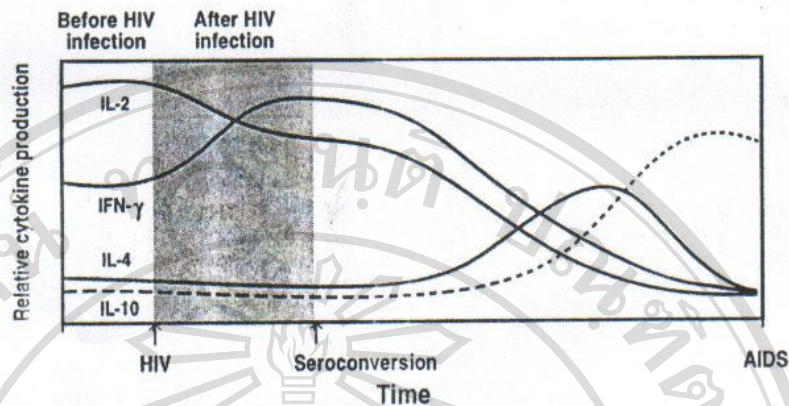


Figure 4. CD4+ cell cytokine production before and after HIV infection. Studies of cultured PBMC suggest that, prior to HIV infection, the dominant T helper cell subtype is Th1 with production of such cytokines as IL-2 and IFN- γ . During the early stages of HIV infection, the Th1 subset predominates, but with advancement to disease it is replaced by the TH2 subset, reflected in the production of cytokines IL-4 and IL-10 (Clerici M. and Shearer GM., 1993).

5.3.5 Intracellular Cytokine Staining (ICCS)

Several methods have been developed that allow cytokine expression to be measured. There are ELISA, RT-PCR, ELISPOT, LDA, ISH, immunohistochemistry and intracellular cytokine staining (ICCS). All have advantages and drawbacks (Pala, 2000). This provides a useful but an incomplete picture, since conventional cytokine assays measure only their bulk release by large numbers of cells and give no indication of the identity or frequency of producer cells. Moreover, since cytokines act as mediators of cell-cell communication, relevance is assigned to the local production of cytokines by individual cell populations. There has been a great deal of interest in developing single cell assays that can detect intracellular cytokine expression. Although LDA and ELISPOT are appropriate ways to estimate the frequency of cytokine producing cells, they are time consuming and labor intensive.

Intracellular cytokine staining was pioneered in the 1980s by Sander et al. (1991), initially to immunostain tissue sections. They demonstrated that it is possible to detect intracellular cytokines by fixation with paraformaldehyde, permeabilization with saponin and subsequent indirect immunofluorescent staining using fluorescence microscopy (Pala, 2000).

The multiparameter capacity of flow cytometry has also been adapted to measure two cytokines simultaneously by single cells, and this has the advantage of rapidly determining the cytokine production of a large number of individual cells. Jung et al. (Jung et al., 1993) and Picker et al. (Picker et al., 1995) described a modified method to increase specific intracellular staining, which allowed the detection of IFN- γ , IL-2 and IL-4 producing cells by single laser flow cytometry.

Monensin, the lipophilic metabolite of *Streptomyces cinnamonensis*, is a Na⁺ ionophore that inhibits trans-Golgi transport by collapsing intracellular Na⁺ and H⁺ gradients, leading to an accumulation of the cytokine in the Golgi complex after 4-hours stimulation with phorbol ester (PMA) and ionomycin. By this result, the signal/noise ratio was increased leading to the detection of weakly fluorescent cells such as the IL-4 producing cell. While IL-4 was detected in approximately 1-3% of peripheral mononuclear cells from healthy donors, up to 30% of the cells produced IFN- γ and nearly 50% IL-2.

Besides monensin, Brefeldin A (BFA) is the better alternative (Picker et al., 1995). BFA is a lactone synthesized from palmitate (C₁₄) by a number of different fungi. It blocks protein secretion at an earlier stage than monensin, by blocking the transport from the ER to the Golgi (Fujiwara et al, 1988; Klausner et al, 1992).

Formaldehyde fixation best preserves cytokine antigenicity often and scatters characteristics of cells without causing too great an increase in autofluorescence. Thus, it is the most common fixative used in ICCS methods, although other fixatives have also been used. Cell permeabilization is necessary for examination of cytoplasmic molecules, which are usually damaged cell surface antigen; caused by autofluorescence, nonspecific staining, and/or cell aggregation; and affected cell size and density. Initial success in minimizing these problems was achieved with the use of saponin, which is thought to act by reversibly solubilizing cholesterol in the cell membrane. Saponin irreversibly permeabilizes cell membranes, while minimizing autofluorescence and nonspecific staining. In addition, several reagents lyse red blood cells and contain fixatives, permitting rapid and simple assessment of peripheral blood specimens without cell separation. It may sometimes be desirable to fix and preserve fixed cells for sometime before permeabilisation and staining, for instance when running large experiments or time courses.

The next development was improvement of the signal to noise ratio and sensitivity of the assay by use of large panels of monoclonal antibodies. The multiparameter capacity of flow cytometry allows simultaneous determination of an individual T-cell's ability to produce multiple cytokines and its phenotype after only short (4 to 8 hours) in vitro incubation with activating stimulus and the secretion inhibitor without cell separation. Direct staining of cytokines developed instance indirect staining. Couple fixation and the permeabilization method with the use of directly labeled monoclonal anti-cytokine antibodies, provided both an improved signal and simpler staining (Prussin et al., 1995; Picker et al., 1995). Formaldehyde fixation and saponin permeabilization are compatible with both indirect and direct staining, but the latter usually produces a lower background, which is simpler and faster, and the co-expression of different cytokines is more easily demonstrated. If possible, PE-conjugated antibodies should be reserved for the weakest signals, such as IL-4 (Pala, 2000). Resting cells do not normally make cytokines, so ICCS requires cells to be activated.

Classical T cell surface phenotype markers such as CD3, CD4 and CD8 correlate at various degrees with cytokine production. Surface staining is best performed before fixation, as epitopes in surface markers may be destroyed by fixation and permeabilisation. Some markers are downregulated by the activation

stimulus used to induce cytokine secretion. This limitation is particularly severe with CD4 in PMA and ionomycin-stimulated human T cells (Pala, 2000), but other markers such as CD3, TCR and CD8 are also affected.

In this study, ICCS was used to detect cytokine levels in CD4+ T cells and CD8+ T cells in HEPS persons, their sex partners and a normal control group. Flow cytometry provides a unique window to gather and analyze data at the level of the individual cell. Moreover, multicolor flow cytometry permits the high-resolution analysis of particular cell types within heterogeneous cell populations without the need for laborious cell-separation procedures. Multicolor flow cytometric analysis enables the simultaneous detection of the light-scattering characteristics (forward and side-scattered light signals) of cells as well as their expressed levels of two or more intracellular and/or cell surface antigens that are defined by immunofluorescent staining.

6. Assay for the diagnosis of HIV infection

The laboratory diagnosis of HIV-1 infection is most often accomplished by the analysis of a blood sample. This may include testing for antibodies, p24 antigen, or culturable virus or gene amplification. Specific serologic assays for the diagnosis of HIV in urine and saliva samples are also available.

6.1 Antibody Testing (Ada, 1999)

Approximate 2 weeks after infection, the presence of viral RNA encapsulated in viral particles can be detected in serum or plasma. A few days later, HIV DNA associated with infected peripheral blood mononuclear cells (PBMC), as well as HIV p24 antigen, become detectable. Approximately 1 week after IgM antibody detection, IgG antibody levels rise significantly and reach a plateau within a few months. HIV RNA and DNA can be detected in almost all infected persons before IgM and IgG antibodies become detectable. Similarly, p24 antigen can be detected before HIV antibodies in more than 90% of subjects.

The easiest and most widely used test for identification of HIV infection is the enzyme immunoassay (EIA), the standard screening test for HIV-1 infection. The positive results of EIA were tested by Western Blot as a supplemental test. Antibodies can be identified to six major HIV proteins; gp41 and gp 120 (envelope), p24 and p17 (core), and p66 and p31 (reverse transcriptase). The EIA test can not detect the class immunoglobulin of anti-HIV in one performance, whereas HIV-1 infected or HEPS person have several patterns of immune response (e.g. HIV-1 specific IgA antibodies found in HIV-1 exposed but persistently IgG seronegative female sex workers). In HEPS persons, a high level of IgA was investigated. Sligh et al. compared the flowcytometric immunofluorescence assay (FIFA) with Western Blot and found that FIFA was a highly sensitive and specific test. Hu et.al. reported that in detection of the anti-HIV class (IgG, IgA and IgM) in the HIV seroconversion panel, IgM was the first marker was detected followed by p24 antigen and IgA, subsequently. In this study, FIFA was used to detect the anti-HIV class (IgG, IgA and IgM).

6.2 Testing for p24 antigen and viral nucleic acids

Although the levels of serum p24 antigen may vary from individual to individual, this antigen can be detected relatively soon after HIV-1 exposure in many patients, and detection often precedes the process of seroconversion by several weeks. This rise in measurable p24 antigen presumably correlates with the burst in viral replication which occurs shortly after primary infection. However, because the timing of this p24 elevation and its rate of increase are not predictable, the p24 antigen assay is not useful typically as a primary tool in the early diagnosis of HIV-1 infection, but it is useful in blood donor screening.

The DNA PCR is a method for detecting and amplifying proviral DNA from the cells of an infected host. Although HIV is an RNA virus, DNA copies of the RNA genome are rapidly produced by the RT enzyme after entry of HIV into a cell. The pairs of primers are selected, short oligonucleotide regions complementary to specific regions of the viral genome, such as segments of the *gag*, *ltr*, or *env* genes for recognition and binding to specific sequences along the viral DNA strand. The detection of the plasma virion RNA by genomic amplification is more sensitive than p24 antigen EIA. Furthermore, plasma HIV RNA is always present when p24 antigen is detected during seroconversion.

RT PCR provides a means for measuring viral RNA, which allows quantitation of the viral load in plasma and differentiation of cells that contain replication genomic RNA (intact virions) from cells producing regulatory messages. This technique is used to determine the viral burden in plasma. By virtue of its extreme degree of sensitivity, the DNA PCR and RT PCR technique is highly subject to false positivity by means of contamination or other common errors in the laboratory processing of specimens.

Because HIV-1 epidemiology is worldwide with several subtypes, HIV-1 subtype E, has spread among heterosexual in Thailand, with the highest rates occurring in the northern regions of the country. Although Thailand has both subtypes B and E, 90% of HIV transmission in the country involves subtype E through heterosexual sex. In this study, the DNA PCR kit (Multiplex HIV-1 PCR from National HIV Repository and Bioinformatic Center –Thailand, Microbiology Department, Faculty of Medicine Mahidol University), was a special kit used for the detection of HIV-1 subtype E, in HEPS persons and their sex partners to confirmed HIV-1 infection.