CHAPTER I

INTRODUCTION

1.1 Statement and significant of the problem

Dendritic cells (DCs) are the most potent immune stimulating leukocytes that have unique property among antigen presenting cells (APCs) of being able to initiate primary T cell responses. Severals studies indicated that DCs themselves cause the pathogenesis of human immunodeficiency virus-1 (HIV-1) infection. DCs are the first APCs that go to HIV-1 infection site, and become infected [1]. DCs carry HIV-1 and migrate to lymph nodes where HIV-1 can infect T cells in lymph nodes. However, the function of HIV-1-infected DCs in T cell activation is still inconclusive.

DCs infected with virus might directly stimulate cytotoxic T lymphocytes (CTLs) without the help from $CD4^+$ T cells [2-4]. Carbonneil *et al* had reported that DCs, generated from peripheral blood monocytes of HIV-1 infected patients, in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-α (IFN-α) (IFN-DCs) in vitro, expressed high levels of major histocompatibility complex (MHC) and costimulatory molecules and were able to induce IFN-γ production by HIV-1-specific $CD8^+$ T cells. Furthermore, IFN-DCs derived from healthy donors were able to induce a weak proliferation of purified $CD4^+$ T cells and a strong proliferation of purified $CD8^+$ T cells, suggesting that IFN-DCs are able to induce $CD8^+$ T cell-mediated immune responses without $CD4^+$ T cell help. Therefore, an impairment of DC function may induce critical consequences for antiviral cellular immunity [2].

In addition, HIV-1 infection has been associated with a depletion of blood and splenic DCs. DCs were demonstrated to be trapped in lymph nodes and most DCs were found to exhibit an immature phenotype with low expression of CD80 and CD83 in vivo [5, 6]. Donaghy *et al* had reported that blood DCs isolated from HIV-1-infected patients did not induce the proliferation of autologous or allogeneic $CD4^+$ T lymphocytes, in contrast with monocyte or macrophage derived DCs propagated from the same patients [7].

Smed-Sorensen *et al* found that HIV-1–infected dendritic cells up-regulated cell surface markers but failed to produce interleukin-12 p70 (IL-12 p70) in response to CD40 ligand stimulation. They also found that TNF-α could be detected in both $p24^+$ and $p24^-$ DCs, but IL-12 p70 could be found only in the $p24^-$ DCs. Thus, although $p24^+$ DCs showed a mature phenotype similar to $p24^-$ DCs after CD40L stimulation, they appeared to have an impaired cytokine profile [8]. These observations suggested that HIV-1 infection disables DC function, a phenomenon that may be relevant for optimal induction of HIV-1–specific immune responses.

If the infected DCs express IL-12 p70 but decrease in IL-10 production, these cells will be poor stimulator of allogeneic $CD4^+$ T cell proliferation and IL-2 production resulting in defection in T cell immune responses. Granelli-Piperno *et al* found that monocyte-derived dendritic cells (MoDCs) infected with HIV-1 do not express CD83 and DC–lysosome-associated membrane protein maturation markers. Therefore, DCs infected with HIV-1 fail to mature. However, they found that DCs infected with HIV-1 still are able to produce IL-10 and develop immune suppression [9].

Although, HIV-1 infection can adversely affect DC function, however, monocyte-derived DCs may still be good in term of their functions [10]. Chougnet *et al* had reported that MoDCs isolated from HIV-1 infected patients and cultured in GM-CSF and IL-4 for 1 week were mostly uninfected and functionally intact [11]. It has been suggested that ex vivo generated dendritic cells (DCs) might be the most potent cellular adjuvant for a therapeutic HIV-1 vaccine [10, 12]. Several studies had shown the efficacy of using MoDCs from HIV-1 infected patient prime with heat inactivated autologous HIV-1 virus in inducing immune responses [10, 11, 13-16]. Garcia *et al* had assessed the safety of the virological and HIV-1–specific immune responses. The autologous MoDCs were loaded with autologous heat-inactivated HIV-1 in patients with non-advanced chronic HIV-1 infection receiving highly active antiretroviral therapy (HAART). In this study, they found no adverse effect in MoDCs function in these patients [10].

In Thailand, we need more basic knowledge and research in term of using MoDCs as therapeutic vaccines for HIV-1 infected patients. Therefore, we propose to study the function of MoDCs from HIV-1 infected patients.

1.2 Literature reviews

1.2.1 The biology of HIV virus and HIV pathogenesis

1.2.1.1 Biology of HIV virus

Human immunodeficiency virus (HIV) belongs to the lentivirus subfamily of retroviruses that produces chronic infection in the host and gradually damages the host immune system [17, 18]. Three major types of lentiviruses have been characterized in primates: simian immunodeficiency virus (SIV) and among human, HIV-1, the predominant type in the world, and HIV-2, primarily found in West Africa and India [19].

The HIV virus is roughly spherical and about 120 nm in size. Its outer envelope or coat is composed of a double layer of lipid envelope that bears numerous spikes. Each spike is composed of three molecules of gp120 and the same number of gp41 embedded in the membrane. Beneath the envelope is a layer of matrix protein P17 that surrounds the core or capsid. The capsid has a hollow, truncated cone shape and is composed of another protein, p24 that contains the genetic material of the HIV virus. Two strands of RNA consisting of about 9,200 nucleotide bases, integrase P32, a protease P10, ribonuclease, reverse transcriptase P64 and two other proteins, nucleocapsid p6 and p7, fit inside the viral core [20], as shown in Figure 1.1.

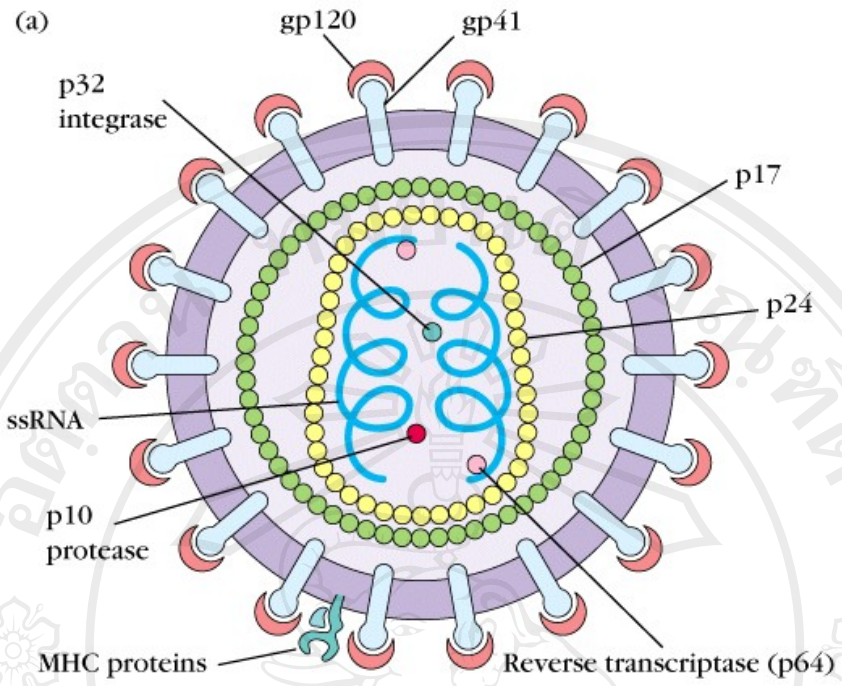

Figure 1.1 Structure of HIV.

http://tutor.lscf.ucsb.edu/instdev/sears/immunology/images/figure19-08a.jpg

HIV-1 has more genes with complex interactions which are *LTR-gag-pol-vif-vpr-vpu-tat-rev-env-nef-LTR* [20] as shown in Figure 1.2.

The *gag* gene codes for the manufacture of the dense cylindrical core proteins. This gene directs the creation of virus-like particles in the absence of *pol* and *env*. The *pol* gene codes for reverse transcriptase, protease, ribonuclease, and integrase that cut the host cell's DNA and inserts the HIV-1 DNA. The *env* gene codes for two envelope proteins gp120 and gp41. This gp41 is a transmembrane protein that holds gp120 to the exterior of HIV-1. The *tat* gene produces a regulatory protein that increases transcription of the HIV-1 provirus. The *nef* gene modifies the host cell to make the cell able to produce HIV-1 virions later. The *rev* gene switches the processing of viral RNA transcripts after the cell has been infected for over 24 hours. The long terminal repeats (LTR) are not part of the 9,749 bases of the HIV-1 genome but contain sequences that help the regulatory genes control *gag-pol-env* gene expression. The *vif* gene is required for a complete reverse transcription of viral RNA into HIV-1 DNA. The *vpu* helps HIV-1 to bud out of the host cell by destroying the $CD4^+$ T cells, and *vpr* is related to the transmission of cytoplasmic viral DNA into the nucleus [21].

HIV-1 viruses can be divided into three major groups: M, N, and O [22, 23]. The worldwide pandermic M group consists of 11 subtypes, from A to K. Multiple strains are found in many countries, but in Thailand the majority is subtype E. Subtype E, B, and C are found in Southeast Asia. Almost exclusively subtype that infects heterosexuals in northern Thailand is subtype E [24], whereas both genotypes B and E are found in injection drug users in Bangkok [24, 25].

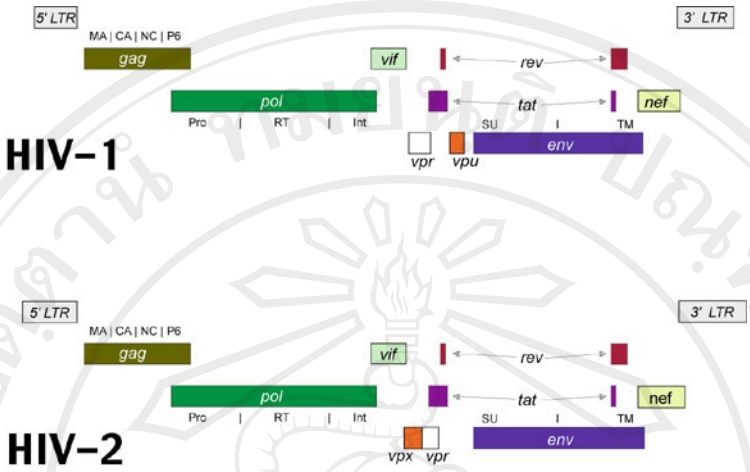

Figure 1.2 HIV genome.

http://www.aids.harvard.edu/images/laboratories/figure_HIV1and2.jpg

The entry of HIV-1 virus to the cells begins with the interaction between virus envelope complex and CD4 together with either CXCR-4 or CCR-5 chemokine receptors on the cell surface. The gp160 spike contains binding domains for both CD4 and chemokine receptors. The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound to CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor. This allows the N-terminal fusion peptide gp41 to penetrate cell membrane. Repeated gp41 interaction, causes the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membrane close together, allowing fusion of the virus to cell membrane and subsequent entry of the viral capsid. After HIV-1 has bound to the target cell, the HIV-1 RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease and protease, are injected into the cell [26, 27].

Once the viral capsid enters the cell, it uses reverse transcriptase enzyme to release the single-stranded RNA from the attached viral proteins and copies it into a complementary DNA. The reverse transcriptase then makes a complementary DNA strand to form a double-stranded viral DNA intermediate. This viral DNA is then transported into nucleus of host cell and integrated into the host cell by using enzyme integrase [28].

To produce a new virus, the transcription factors are needed. The most important transcription factor is NF kappa B (NF-κB). The NF-κB is upregulated when T cells were activated. In this replication process, the integrated provirus is copied to host mRNA which is then spliced into smaller pieces [29].

These small pieces produce the regulatory proteins Tat and Rev. Once Rev accumulates, it gradually starts to inhibit mRNA splicing. At this moment, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome. It binds to the Gag protein and is packaged into new virus particles. The final step of the viral cycle is the assembly of new HIV-1 virons which occurs at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the golgi complex. Then it is cleaved by protease and processed into the two HIV-1 envelope glycoproteins gp41 and gp120. These glycoproteins are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV-1 genomic RNA as the forming virion begins to bud from the host cell. Maturation then occurs either in the forming bud or in the immature virion. During maturation, HIV-1 protease cleaves the polyprotein into individual functional HIV-1 proteins and enzymes. The various structural components then assemble to produce a mature HIV-1 virion. The mature virus is then able to infect other cells. The life cycle of HIV-1 infection are shown in Figure 1.3 [28-30].

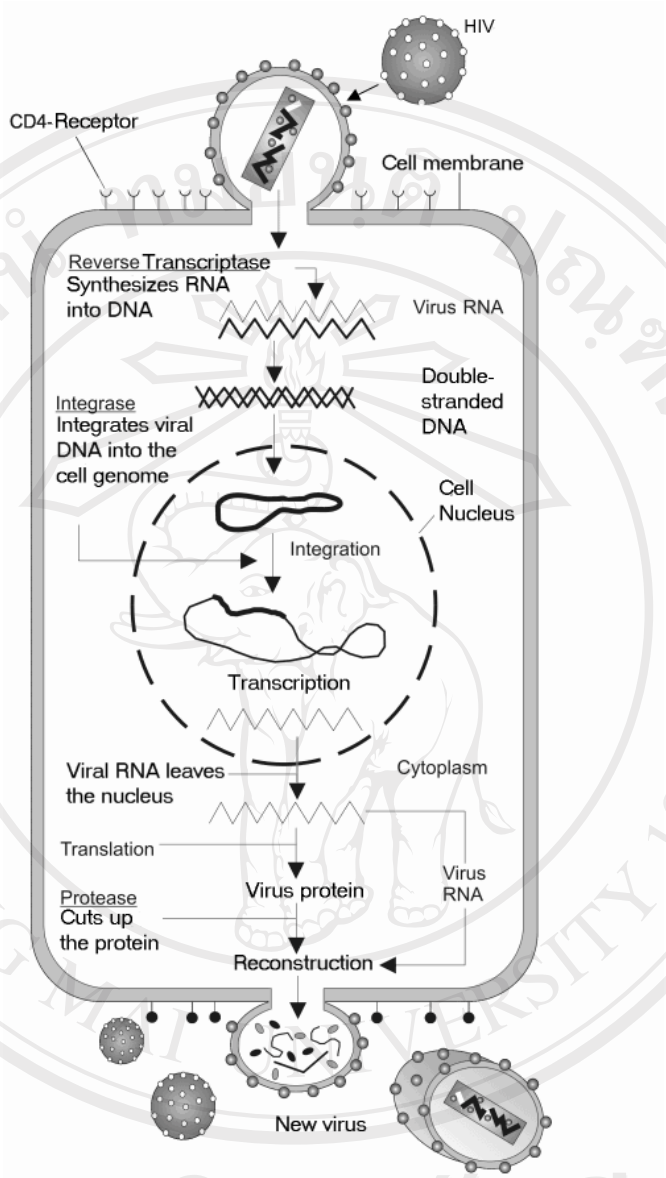

Figure 1.3 The HIV-1 replication cycle.

http://upload.wikimedia.org/wikipedia/en/f/f3/Hiv_gross.png

1.2.1.2 Pathology of HIV-1 infection

There are three phrases of HIV-1 infection, primary infection, clinical latency, and advanced stage disease [31].

At the primary infection stage HIV-1 infected individuals experience an acute viral syndrome. The symptoms generally appear within four weeks after virus exposure. The patients may experience an extremely high level of plasma viremia (viral load level is up to 10^7 HIV-1 RNA copies/ml). Vigorous HIV-1-specific humoral and cell-mediated immune responses are detected very early during primary infection. The role of these responses in the initial downregulation of virus replication has been subjected to considerable debate. However, both humoral and cellular immune responses are likely to contribute to the suppression of the initial burst of virus replication. Due to the complexity of the immune response elicited by HIV-1 infection, the different components of these responses were discussed including HIV-1-specific antibody responses, HIV-1-specific cytotoxicity, and cytokine responses. During this state, the HIV-1-infected individual may experience the acute retroviral syndrome such as fever, rash, lymphadenopathy, headach and diaarhea [31-34].

Clinical latency is characterized by chronic immune activation and persistence viral replication without signs or symptoms of disease. All virologic parameters, including plasma viremia, titer of infectious virus, and virus expression in peripheral blood mononuclear cells (PBMCs), are generally low during the prolonged clinically latent period of HIV-1 infection. However, the replication of the virus during this state is still continued.

HIV-1-specific CTLs directed against either structural (envelope and gag-pol) or regulatory (Nef, Tat) viral proteins are detected in a large percentage of HIV-1-infected individuals during the clinically latent period.

The explanation for this latency stage will be discussed. The first reason may be the higher concentration of HIV-1 within lymphoid tissues cause abnormal trafficking of lymphocytes due to either changes in the expression of adhesion molecules following cellular activation, or slower migration through the lymphoid tissue caused by histopathologic abnormalities. The second reason may be controlled by HIV-1-specific CTLs [31, 35-39].

Advanced stage diseases are characterized by either the level of $CD4^+$ T-cell counts below 200 cells/μl or those with an AIDS-defining clinical illness. HIV-1-specific CTL activity is generally lost at this stage of disease. The titers of neutralizing antibodies against a variety of HIV-1 proteins are significantly decreased. In contrast, the levels of expression and production of certain cytokines such as IL-10 and IFN-γ remain very high. The destruction of lymphoid tissue is the primary mechanism responsible for the severe immunosuppression associated with late-stage HIV-1 disease [31, 39-41].

The patients who develop such a course of infection are called typical progressor. Typical progressors are the majority of HIV-1-infected individuals. These patients experience a long period of clinical latency about six to eight years. Despite the lack of symptoms, HIV-1 disease is active as is indicated by the persistent replication of virus and by the progressive loss of $CD4^+$ T cells with the level of $CD4^+$ T cells counts more than 500 cells/μl. When the $CD4^+$ T cell counts fall below 500 cells/μl, AIDS defining illness generally occurs within eight to ten years. When $CD4^+$

T-cell counts are below 200 cells/μl, the clinical status may be characterized by severe and persistent constitutional signs and symptoms [31, 38, 42-44].

There are three additional subgroups of HIV-1 infected individuals. The first group is rapid progressors who have an unusually rapid progression of disease. About 10-15% of HIV-1 infected individuals developed a rapid progression to AIDS within two to three years of primary infection. The levels of viremia may rise paridly which the immune system cannot control [31, 45].

The second group is long-term nonprogressors. This group does not experience progression of disease for a long time. Long-term nonprogressors have $CD4^+$ T-cell counts that are within the normal range and are stable over time. In addition, they generally have low levels of virologic parameters and preservation of lymphoid tissue architecture and immune function [31, 46-48].

The last group is long-term survivors. These group experience progression of HIV-1 disease similar to typical progressors. Both clinical and laboratory parameters remain stable for an extended period of time. The mechanisms that are responsible for preventing further progression of HIV-1 diseases are unclear. There is a possibility that the viruses have changed in their genotype or phenotype, as well as the possibility that preservation of certain HIV-1-specific immune responses are involved [31]. The progression of HIV-1 infection in each group was shown in Figure 1.4.

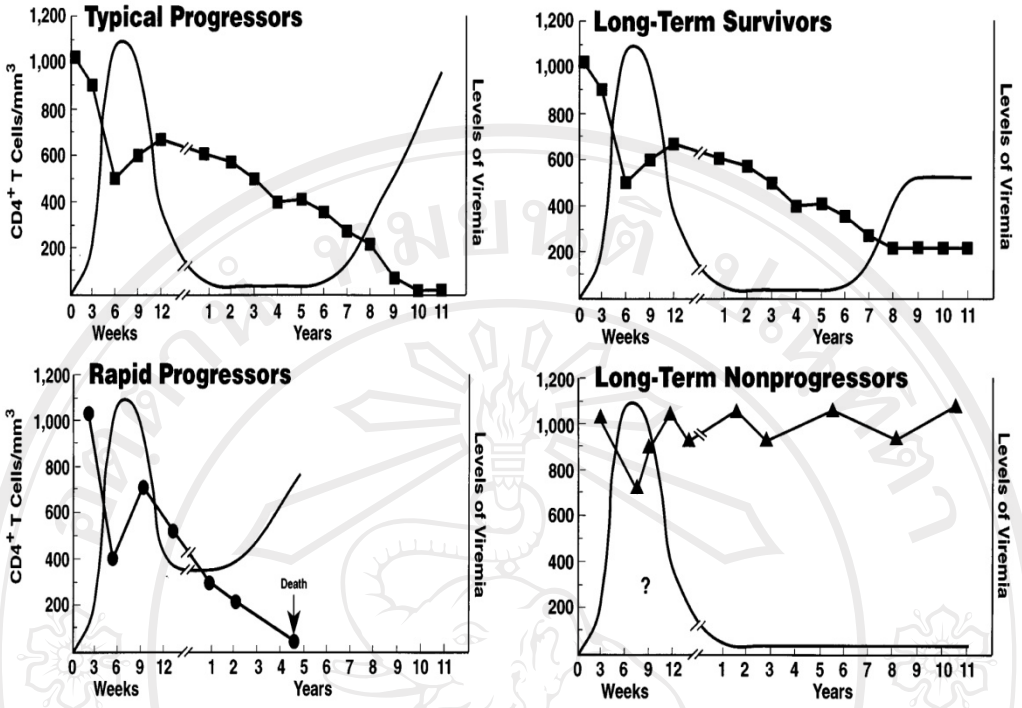

Figure 1.4 Schematic representations of the different courses of HIV-1 infection on the basis of the changes of $CD4^+$ T-cell counts and viremia over time [31].

1.2.2 HIV-1 vaccines and therapeutic dendritic cell based vaccines

1.2.2.1 HIV-1 vaccines

The controlling of HIV-1/AIDS epidemic requires an effective vaccine. However, the development of AIDS vaccine has proven an enormous scientific challenges. The specifications for a safe and effective HIV-1/AIDS vaccine should include the following criteria.

1. The vaccine should be formulated and administered in a manner that assures induction of both mucosal and systemic responses.

2. The vaccine should boost specific antibody of the secretory immunoglobulin (Ig) A class at oral, rectal, and genitourinary mucosal sites. Serum IgG responses should be elicited broadly at the neutralizing sites. The vaccine should not elicit enhancing antibodies and non-neutralizing antibodies to gpl60 portion.

3. The vaccine should elicit strong and durable systemic Thl-like responses. These responses should be directed at the most conserved and broadly class II MHC-restricted epitopes available. Specific Th2-1ike responses should be elicited primarily at mucosal sites to augment IgA production. Elicitation of Thl-like $CD8^+$ T cells response may be desirable.

4. The vaccine should elicit $CD8^+$ CTL responses that include CTL precursors as well as effectors. These responses should be directed to the most conserved HIV-1 proteins broadly class I MHC-presented epitopes [49].

1.2.2.2 Development of HIV-1 vaccine

The main strategy in development HIV-1 vaccine is based on immunization with various forms of antigens. Alternatively the approaches that result in the expression of antigen were used. Therefore, there are two types of strategies in the development of HIV-1 vaccines.

1.2.2.2.1 Non-expression-based agents

Non-expression-based vaccines include all vaccines that do not rely on in vivo expression of an immunogen such as whole killed virus, native-like subunits and non-native like subunits.

The whole killed virus vaccine includes the entire collection of virus proteins in a structural format that mimics the infecting virion. However, in the case of HIV-1 this type of vaccine was not work out because the gpl20 readily dissociates from the gp41 on the virus surface, resulting in a gpl20 deficient vaccine. Such vaccine could not induce potentially critical responses, since the V3 loop and CD4 binding region epitopes are found on gpl20. However, this vaccine has a potential advantage of avoiding induction of T cells or antibodies that kill or inhibit uninfected cells that have passively bound gp120 [49-54].

The native-like subunit is referred to native protein purified from virus or infected cells. Native-like subunits limit the immune response to specific proteins of interest and exclude others. Information of proper folding of subunits can help to elicit responses to conformational epitopes. However, subunits are less likely to accurately represent super-molecular structure that exists on the virion or infected cell. They may retain a substantial element of native conformation. All gpl60

molecules may be distinct in conformation, as the gpl60 precursor is cleaved in infected cells to generate cell surface gpl20 and gp41[49, 55-58].

Non-native like subunits are the subunits that absent or altered in glycosylation. Denaturation also destroys native structure. A denatured immunogen might be used to boost a desired cellular response without eliciting an antibody response to conformational epitopes. The denatured antigen may offer advantages in Thl downregulation of Th2 response. However, studied in mice and guinea pigs showed that the process of reduction, alkylation, and denaturation did not consistently enhance the immunogenicity of gpl60 for T cells or antibody responses when used alone or together with nondenatured antigen [49, 59-62].

1.2.2.2.2 Expression-based agents

Expression-based agents are classified by the ability to replicate a structure or gene transfer that enables transmission to new cells. The example of expression-based agents such as attenuated live virus, recombinant virus, recombinant bacteria, retroviral vector and genetic immunization [49, 63-68].

There are several attempts to develop the vaccine for HIV-1 as show in Table 1.1

Table 1.1 Strategy in development of HIV-1 vaccine [69]

Vaccines strategies	Description
Whole Killed	Chemically inactivated HIV-1 viruses
Live attenuated vaccines	Live attenuated HIV-1 viruses containing deletions of *Nef* or *Vpf* genes
Subunit vaccines	Recombinant viral proteins such as p24
Peptide-based vaccines	Chemically synthesized HIV-1 protein fragments or defined immunogenic epitopes
Pseudovirions	Non-replicating and non-infectious virus like particles such as Gag, GagPol and Env
Recombinant vaccines	Non-HIV-1 viruses/harmless bacteria engineered to carry genes encoding HIV-1 proteins for example Vaccinia-MVA[a]
DNA vaccines	Naked plasmid-DNA containing one or more HIV-1 genes
Combined vaccines	Combination of different vaccines in a mixed modality immunization schedules for example DNA vaccines plus live viral vectors plus recombinant proteins
DNA vaccine delivery systems	Wide variety of constructs using HIV-1 genes such as Nef based etc. Whole HIV-1 genome based non-infectious Incorporating inducers of cytokine production susch as IL-2
Heat shock protein (HSP) adjuvant systems	Based on HSP70
Allogenic vaccines	No report yet of candidates

[a] Modified Vaccinia Ankara virus

1.2.2.3 Therapeutic dendritic cell based vaccines

DC vaccine is defined as DCs loaded with antigens such as tumor associated antigens or HIV-1-Gag proteins. At the present, most DC vaccines have been used to stimulate immune responses against cancers. Therapeutic DC vaccines are also a hope for therapeutic HIV-1 vaccines [70-73].

The generation of DCs can be easily generated from monocytes or $CD34^+$ precursors. Most clinical studies used monocyte-derived dendritic cells (MoDCs). The MoDCs can be generated by cultured monocytes in the presence with GM-CSF and IL-4. Monocytes will develop into immature DCs within 5 days and can be further matured in cultured by using different stimuli such as LPS [74-78].

To become the effective antigen-presenting cells, the MHC molecules of DCs must be loaded with antigens. In case of HIV-1, Gag protein or a short peptide of virus aimed to elicit CTL response in controlling of virus [79].

Leonia Bozzacco *et al* show that DCs primed gag P24 antigen to DEC-205 can cross-present several different peptides from a single protein and elicit $CD8^+$ T cell responses [80].

Most of immunotherapeutics are administered intravenously, but DCs are frequently administered intradermally or even through direct injection to lymph nodes. The data supported that matured DCs can migrate to lymph nodes better than immature DCs. The increasing in migration would enhance the efficacy of the vaccines. The approaches used to stimulate DC migration are the uses of inflammatory cytokines to condition tissues, matrix metalloproteinases and Toll-like receptor (TLR) ligands also enhance DCs migration as shown in Figure 1.5 [81-85].

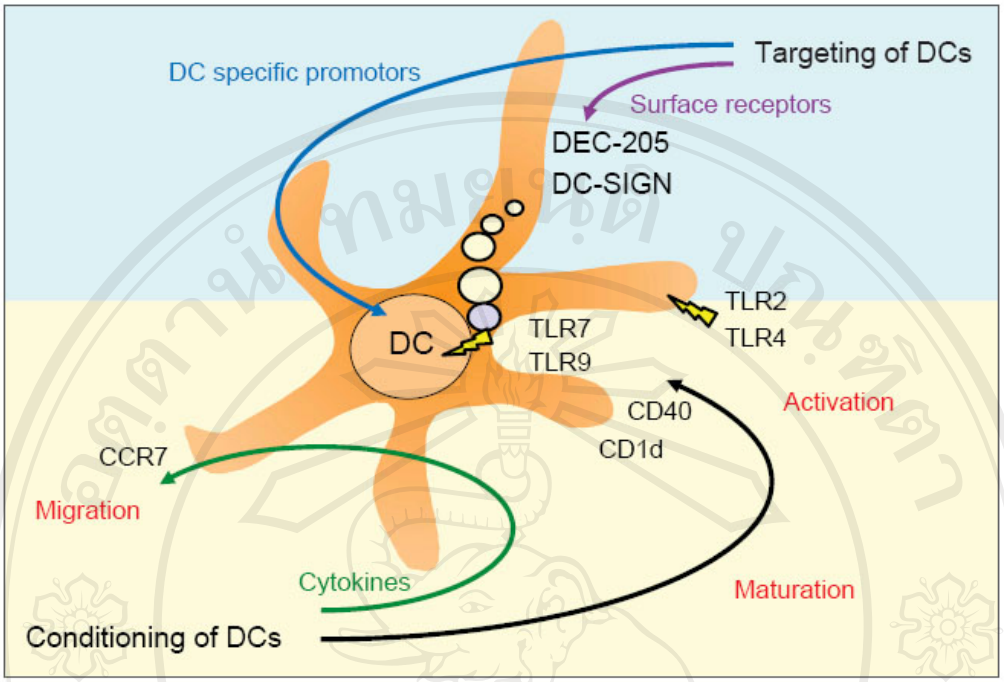

Figure 1.5 DC Immunotherapy. Conditioning of DCs by cytokines, CD40 ligand, TLR ligands or α-galactosylceramide, which binds to CD1d, should be explored to optimize DC maturation and activation, and to improve the migratory capacity of DCs in vivo. In addition, in vivo targeting of antigens to DCs by exploiting either DC-specific surface receptors or promoters of DC-specific genes may eventually replace vaccination with ex vivo-generated, antigen-loaded DCs [71].

The direct injection into lymph nodes can cause the skin migration problem. Although intranodal injection can destroy the architecture of the first node, the migrations to the subsequent node follow the pathway through lymph vessels. The efficacies of intranodal administration compare to intradermal administration remain to be explored [86, 87].

It would be advantageous to be able to directly isolate antigen-loaded DCs from the blood without in vitro culture, or to activate and target DCs in vivo. Flt-3 ligand is a potential candidate because it can expand human DCs in vivo without activating them. However, non activated or improper activated DCs can cause T-cell tolerance rather than productive T-cell immunity [88, 89].

Recently, several DC-associated C-type lectin-like receptors (DEC 205 and DC-SIGN) have been shown to rapidly bind and endocytose materials. The antigen loaded via these receptors could stimulate both of $CD4^+$ and $CD8^+$ T cell. Criteria for DC vaccines are concluded in Table 1.2 [90, 91].

Table 1.2 Quality criterias for DC vaccines [71, 92-94]

Release criteria	**Condition**
Microbiological controls	Negative for bacterial and fungal contamination
Purity	More than 80% as determined by flow cytometry, light scatter
Morphology	Immature: loosly adherent, floating, roundish cells with some extensions Mature: loosly attached, veiled and cluster cells
Phenotype	**Monocyte-derived;** Immatured: $CD14^{-or\ low}$, $CD83^{-}$, $CD80^{-/low}$, $CD86^{low}$, MHC class I^{+}, MHC class II^{+}, $CCR5^{+}$ Mature: $CD83^{+}$, $CD80^{+}$, $CD86^{+}$, MHC class II^{+}, $CCR7^{+}$ **$CD34^{+}$ cell-derived;** Interstitial: $CD14^{+}$, $CD1a^{+/-}$, $CD83^{+}$, $CD80^{+}$, $CD86^{+}$, MHC class I^{+}, MHC class II^{+}, Langerhans cells: $CD14^{-}$, $CD1a^{+}$, $CD83^{+}$, $CD80^{+}$, $CD86^{+}$, MHC class I^{+}, MHC class II^{+}

Table 1.2 (Continued)

Release criteria	Condition
Viability	More than 70% as determined by trypan blue exclusion.
Stability of DC phenotype	Determined after one and two days of subsequent culture in medium either without or with cytokines. DC must remain viable and maintain characteristic and morphologic and phenotype over two days in medium without cytokines.
Induction of immune response	Mixed lymphocyte reaction: T-cell proliferation at DC/PBMC ratio of 1:20 in at least one donor. Recognition of loaded antigen by T cells, as determined by cytotoxicity assay or cytokine production
Antigen-loaded state	Antigen-specific stimulation assay: Tests ability of antigen-loaded DCs to stimulate antigen-specific T cells

1.2.3 Biology of dendritic cells

Dendritic cells (DCs) are unique antigen-presenting cells (APCs) because they are the only APCs that are able to induce primary immune responses. The progenitors of dendritic cells are in the bone marrow that give rise to circulating precursors in tissues where they reside as immature cells with high phagocytic capacity. DCs can also encounter pathogens such as viruses which induce secretion of cytokines such as IFN-α that activate eosinophils, macrophages, and natural killer (NK) cells. Once the immature DCs capture antigens, they migrate to lymphoid organs where they can present antigen in the context of major histocompatibility complexes (MHC) to naïve T cells. At this stage, they become mature DCs that can induce lymphocyte expansion and differentiation. Consequently, helper T cells secrete cytokines that permit the activation of macrophages, NK cells, and eosinophils. Cytotoxic T cells eventually lyse the infected cells. B cells are activated after contact with T cells and DCs and then migrate to various areas where they mature into plasma cells and produce antibodies that neutralize the initial pathogen [95].

1.2.4 Subsets of dendritic cells in human

The progenitors of dendritic cells in human can be distinguished into two groups: $CD34^+$ myeloid progenitors and $CD34^+$ lymphoid progenitors. $CD34^+$ myeloid progenitors can differentiate into two subgroups of dendritic cells: $CD14^+$ $CD11c^+$ $CD1^-$ and $CD14^-$ $CD11c^+$ $CD1^+$. $CD34^+$ lymphoid precursor can differentiate to $CD14^-$ $CD11c^-$ IL-3Rα^+ DC precursors. $CD14^+$ $CD11c^+$ $CD1^-$ dendritic cell precursors will differentiate into either immature DCs in response to GM-CSF and IL-4 or macrophages in response to macrophage colony stimulating factor (M-CSF). $CD14^-$ $CD11c^+$ $CD1^+$ dendritic cell precursors will differentiate into langerhans cells in response to GM-CSF, IL-4 and transforming growth factor (TGF-β). $CD14^-$ $CD11c^-$ IL-3Rα^+ from lymphoid precursor will give rise to lymphoid dendritic cells in response to IL-3. These immature dendritic cells are now ready to capture antigen and then mature and migrate to lymphoid organs. The immature dendritic cells are able to differentiate into mature dendritic cells in response to either cytokines (IL-12 and a IFN-γ) or pathogen products (LPS, DNA and CD40 ligand) [95].

Monocytes and $CD11c^-$ IL-3Rα^+ DC precursors display many phenotypic differences. Monocytes, express significant levels of CD11b, CD13, CD14, CD33, and CD45RO. Moreover, they express high levels of GM-CSFRα and low levels of IL-3Rα. $CD11c^-$ DC precursor displays cytokine receptors, low levels of GM-CSFRα, and high levels of IL-3Rα. In contrast to immature $CD11c^-$ DCs, immature monocyte-derived DCs ($CD11c^+$) also display high endocytic or phagocytic ability [95].

Although, $CD34^+$ progenitors response to the same cytokines in the generation of interstitial dendritic cells and langerhans cells, only the interstitial dendritic cells from monocytes can induce IL-2 secretion that drives naïve B cells to differentiate. Both subsets of $CD34^+$ progenitors express IL-12 upon CD40 ligation. Interstitial dendritic cells also secrete high amount of IL-10 and also have a higher efficiency of antigen capture than langerhans cells. Mommaas AM. *et al* found that LCs lack functional mannose receptors and are poor stimulators of antigen specific $CD4^+$ T-cells or T helper cells [96]. However, Mortarini R. *et al* found that interstitial DCs and LCs are more potent in primming $CD8^+$ T cytotoxic function [97].

1.2.5 Dendritic cell activation and maturation

Immature dendritic cells are very potent in antigen capture with several mechanisms such as macropinocytosis; mannose receptor DEC-205, the dendritic cell receptor for endocytosis, FCγ receptor type 1 (CD64), FCγ receptor type II (CD32) and phagocytosis. Either antigens or pathogens induce the immature DCs to undergo phenotypic and functional changes from antigen capturing cells to antigen presenting cells. Several factors can induce immature dendritic cell maturation such as LPS, bacterial DNA, CD40L, dsRNA and cytokine such as TNF-α, IL-1, IL-6, IL-10, TGF-β, and GM-CSF [98, 99]. Immature dendritic cells with antigen capture capacity express high levels of intracellular MHC class II, endocytosis and phagocytosis. They also express high levels of CD68, CCR-1, CCR-5 and CCR-6; low level of CCR-7, CD54, CD58, CD80, CD86, CD40 and CD83; and do not express DC-lysosome-associated membrane protein (DC-LAMP). After antigen capturing, DCs will become mature dendritic cells that mainly act as antigen presenting cells with low endocytosis

and phagocytosis. The expression of CCR-1, CCR-5 and CCR-6 are decreased. However, the expression of surface MHC class II, CCR-7, CD54, CD58, CD80, CD86, CD68 and DC-lysosome-associated membrane protein (DC-LAMP) are increased [95].

All mediators of dendritic cell maturation susch as LPS, TNF-α, and IL-1 trigger peripheral blood dendritic cells to migrate to T cell area of lymphoid organs. After antigen uptake, the inflammatory stimuli shut down the response of immature DCs to macrophage inflammatory protein-3α and all other chemokines specific for immature DCs. Consequently, mature dendritic cells escape from the local gradient of macrophage inflammatory protein-3α. Then mature DCs leave the tissues and enter the lymph stream, directed by 6Ckine which is chemokine ligand 21 (CCL21) in lymph vessels. Then dendritic cells enter the draining lymph nodes and spread into paracortical areas of T cell zone. These dendritic cells might become source of macrophage inflammatory protein-3β and 6Ckine because of these two chemokines can attract mature DCs and naïve T lymphocytes. These dendritic cells play a role in helping antigen-bearing dendritic cells to encounter specific T cells. The interaction between dendritic cells and specific T cells take place at the site of tissue injury. The maturation signal from CD40 ligand induces the release of chemokines such as IL-8 and macrophage derived chemokines that attract lymphocytes [95, 100].

1.2.6 Antigen processing and presentation

Dendritic cells process and present antigen via MHC class I and MHC class II. Antigenic peptides are presented through MHC class I molecules from both endogenous and exogenous pathways. The classical endogenous pathway process

peptides from self and intracellular pathogen. Dendritic cells can also present exogenous peptides originating from phagocytosed particulate antigens through MHC class I. These exogenous peptides are generated in the proteasome and transferred into endoplasmic reticulum and loaded with MHC class I [95, 100].

Soluble and particulate antigens are mainly captured by immature dendritic cells and presented mainly through MHC class II by macropinocytosis or receptor-mediated endocytosis and phagocytosis. The antigens are degraded in endosome, and generated peptides are transported into MHC class II rich compartment while dendritic cells mature. The loading of antigen through MHC class II was assisted by HLA-DM in lysosome-related intracellular compartments or MHC class II rich compartment. Then dendritic cells are ready to present antigen loaded MHC class II on their surfaces [95, 100].

Due to the ability of dendritic cells to present antigen both of endogenous and exogenous pathways through the MHC class I, the role of cross-priming of class I presentation of exogenous antigen must be discussed. There are two routes for presenting exogenous antigens via MHC class I: 1) Transporter associated with antigen processing independent (TAP-independent) pathway which antigen is most hydrolyzed in endosome. 2) Phagosome to cytosol pathway or TAP-dependent pathway. TAP-dependent pathway involved in the immune responses against transplantation antigens, particulate antigens, tumors and viruses. Some studies found that monocyte-derived dendritic cells (MoDCs) loaded with apoptotic bodies obtained from macrophage infected with influenza virus can stimulate $CD8^+$ CTLs. Thus the exogenous class I presentation pathway is very important [95, 100, 101].

In addition to MHC class I and class II, dendritic cells express the third class of MHC molecules involved in antigen presentation to T cells in response to microbrial lipid and glycolipid-containing antigens. This molecule is CD1 that can present both of exogenous and endogenous lipids. This pathway involved not only microbrial immunity but also autoimmunity and anti-tumor responses. In human, CD1 molecules are expressed by myeloid DCs and functionally heterogeneous. There are two subgroups of CD1 molecules. The first group presents glycolipids to repertoire of T cells which includes human CD1b and CD1c. Another group is human CD1d that binds a limited set of antigens and activates a restricted set of T cells as well as NK T cells [95, 101-103].

1.2.7 Antigen presentation and T cell activation

The critical function of dendritic cells both in vitro and in vivo is that these cells can prime naïve $CD4^+$ T cells. T-helper cells primed by DCs can interact with B cells and stimulate antigen specific antibody production. DCs are also important in priming naïve $CD8^+$ T cells directly without T-helper leading to the generation of antigen specific CTLs from naïve precursors.

In human, plasmacytoid DCs are unresponsive to GM-CSF but response to IL-3 and express a specific combination of surface inhibitory immunoglobulin-like transcript (ILT) such as ILT-1 and ILT-3^+ [100, 104]. These cells mainly activate Th2 cells, whereas MoDCs mainly activate Th1 cells [100, 105]. The recognition of MHC-peptide complexes on DCs by antigen specific T cell receptors constitutes DC-T cell interaction. The cluster of DC-T cell interaction is mediated by several adhesion molecules such as integrin β1 and β2 and other types immunoglobulin super

family such as CD2, CD50, CD54 and CD58 [106, 107]. Recently, high-affinity receptor for intracellular adhesion molecules 3 was found specifically expressed on MoDCs. The main factor on T cell activation is the interaction between costimulatory molecules expressed on DCs and their ligands expressed by T cells. CD83 and CD86 on DCs are the most critical molecules for T cell responses [108, 109].

T cells can also activate DCs via CD40 ligand (CD40L-CD40 signaling) leading to increased expression of CD80 and CD86 and the release cytokines such as IL-1, TNF-α, chemokines and IL-12. Triggering of CD40 on DCs results in upregulation of OX40 ligand which signals naïve T cells to express IL-4 and upregulate CXCR-5, that directs B lymphocytes into follicles. Mature DCs also express 4-1BB ligand of OX40-L which is a costimulator expressed primarily on activated $CD4^+$ and $CD8^+$ T cells and induces $CD8^+$ T cell proliferation and IFN-γ production resulting in cytotoxic T cells responses [95, 100].

DC subsets may provide T cells different cytokines that determine the classes of immune responses, type I and type II $CD4^+$ helper cell profiles. In human, monocyte-derived $CD11c^+$ DCs polarize naïve T cells predominantly towards Th1 profile such as IFN-γ, IL-12 and IL-2, whereas the $CD11c^-$ DC subset induces T cells to predominantly produce Th2 cytokines such as IL-4, IL-6, and IL-10 [100, 110]. The extent of T cell polarization by $CD11c^-$ DCs may be related to their differentiation and maturation stages. Thus, $CD11c^-$ DC precursors may be prone to elicit more of the Th0 cytokine profile which produce both of Th1 and Th2 cytokine profiles [100, 111], whereas their mature progeny may induce Th2 differentiation. The induced pattern of T cell cytokine secretion is dependent on the production of IL-12. Indeed, $CD11c^+$, but not the $CD11c^-$ DC subset, can be induced to secrete IL-12 [95, 100].

1.2.8 Dendritic cells and HIV-1 infection

Previous studies indicated all types of changes in peripheral blood DCs including decrease, increase, and no change in the numbers of DCs during HIV-1 infection. No change has been found in the percentage of LCs when infected individuals were compared to uninfected individuals or when HIV-1-infected individuals at various stages of disease were compared [1, 3, 11, 112]. Study of lymphoid organs showed that there has no selective loss in the number of DCs in paracortical regions of the lymph nodes in HIV-1 positive non-AIDS patients [113].

In term of DC function, the ability of DCs in activating T cells had been studied, and the results were conflicted. Several studies found that DCs from HIV-1-infected individuals or DCs infected in vitro were much less efficient in activating T cells [2, 5-9, 112], whereas another study found no differences in the abilities of peripheral blood DCs to activate allogeneic $CD4^+$ T cells in HIV-1-infected versus uninfected individuals [11].

DCs from HIV-1-infected patients showed no defects in their abilities to present antigens to the uninfected T cells. The only defect was that DCs from HIV-1-infected patients had a decreased ability to activate allogeneic T-cells. However, most of these studies were performed by using peripheral blood DCs [6].

Similar to the difficulties inherent in studies on infection of DCs, analysis of depletion and dysfunction of DCs in peripheral blood yielded conflicting results. In study using purified LCs, there may not be significant DC dysfunction or depletion until the late stage of AIDS [114].

DCs are involved in the initiation of HIV-1 infection following exposure to the virus. At mucous membranes, which are the major sites of the initiation of HIV-1

infection, DCs are the first immune competent cells to encounter antigens. As described before, DCs are the most potent APCs in initiating immune responses of T cells. Dendritic cells also express $CD4^+$, CCR-4, CXCR-5 and mannose receptor. DCs migrate to lymph nodes and interact with T cells in lymph nodes. All of these leading to the spreading of viruses [1, 2, 5-9, 95, 100]. These data suggested that a DC, whether it simply carries HIV-1 or is infected with the virus, is able to bring the virus to T cell and establish a productive infection [1].

DCs have been identified in all components of the human lymphoid system including lymph nodes, spleen, tonsil, thymus, blood afferent lymph, and progeny that develops from $CD34^+$ progenitor [115-119].

There are several lineages of dendritic cells that can be infected with HIV-1 virus as shown in Table1.3.

Table 1.3 In vivo infection of components of the dendritic cell lineage [120]

Cell	Isolation method	Detection method	Results/frequency
Bone marrow	$CD34^+$ (positive selection)	Coculture and PCR	Up to 1/500 of $CD34^+$ cells. Infected cells in 14% US patients, 36.5% Zaire, (n=76) hemopoesis suppressed in all seropositive
	$CD34^+$ immunomagnetic bead selection	PCR	Rarely infected (AIDS n= 0/4) (HIV-1^+ n = 1/7)
	Nonadherent non-T cells	PCR on E-BFU and GM-CFU after culture	No HIV-1 infection (AIDS) n=6)
	$CD34^+$	Culture and PCR	No infection of $CD34^+$ cells (AIDS n=6)
	FACS sorted $CD34^+$ controls = $CD4^+$ $CD14^+$ cells	PCR	No infection in $CD34^+$ all CD4 infected (n=14)
	$CD34^+$	PCR on CFU	No infection of colonies increased colony formation with antisense RNA Accessory cell defect (n=27)

Table 1.3 (continued)

Cell	Isolation method	Detection method	Results/frequency
Blood DCs	Cultured, LD, nonadherent, FcR cell	ISH[a], IHC[b]	2-23% postive ISH <1% by p24 IHC
	Cultured LD nonadherent cocktail- (panning)	Antigen Presentation	Impairment of APC function. Function of DC< <macs
	Cultured, LD Er-, macrophage depleted	PCR	4/9 PCR^+ copies in DC ≤ T cells
	Er-, cultured, non-adherent, FcR, LD	ISH, PCR	By PCR <1/1000 infected, 10x < $CD4^+$ T cells. (n=25)
	Cultured, nonadherent, Phagocytosis, negative for CD4, 3, 14, 15, 16, 19	PCR	No infection detected (n=4)
	Cultured LD, cocktail negative	Limiting dilution PCR	Frequency of infection of DC = lymph (n=19)

Table 1.3 (continued)

Cell	Isolation method	Detection method	Results/frequency
Langerhans cell	Skin biopsy	IHC (p17) EMp17$^+$ cells in 7/40	Virions by EM in 1 patient.
	Skin biopsy	EM	Productively infected Cells
	Blister sheets	p24 IHC	0/8 p24$^+$ (seropostive) 1/3 p24$^+$ AIDS
	Oral mucosal biopsy	IHC (p24, 41, 120) IHC (p24) IHC, ISH	2/26 virus no infection 0/66
	EC	Coculture P24 IHC	3/3 p24$^+$
	Trypsinized epidermis, U. enriched ECs Epidermal sheets (EDTA heat)	Coculture with macrophage	12/23 HIV-1$^+$
		PCR (*gag, env,pol*)	5/11 PCR$^+$
		PCR, ISH. IHC	LC rarely in,fected 26/28 PCR$^+$ on dermis (n=28)
		PCR	EC and LC PCR$^+$
	MACS sorted CD1a$^+$ from EC	PCR (*env. gag*)	5/11 PCR$^+$
	CD1a EC, MACS sorted (CD1a) from sheets	Competitive PCR *gag*	107-3645/100,000
	Macs sorted CD1a$^+$ CD1a$^+$ CD1a$^+$	RT-PCR *tat* RT-PCR (*tat, nef, rev*, TcR)	6/9$^+$ tat RNA HIV-1 RNA in CD1a TcR in 1 CD1a No HIV-1 in CD1a

Table 1.3 (continued)

Cell	Isolation method	Detection method	Results/frequency
Lymph node	Suction blister	PCR, sequencing	PCR1 (n=1)
		IHC, IS-PCR	100 cells ISH negative
		ISH, IHC	Correlation between CD1a cells and $gp41^+$ cells in paracortex
		ISH, IHC	Virus expression in lymphocytes
		IS-PCR	Latent infection of T cells and Macrophages
Synovial DCs	Low-density non-adherent	ISH, IHC	DCs infected (n=3)

[a] In situ hybridization
[b] Immunohistochemistry

There are several routs and sites that DCs can be infected with HIV-1 viruses. The first one is sexual HIV-1 transmission. The probability of sexual HIV-1 transmission for each encounter with the virus is rather small. HIV-1 virus can cross genital mucosal epithelium for their transmission [121, 122]. It can cross this barrier in several ways as described in upper panel of Figure 1.6. The transmission models are shown in Figure 1.6.

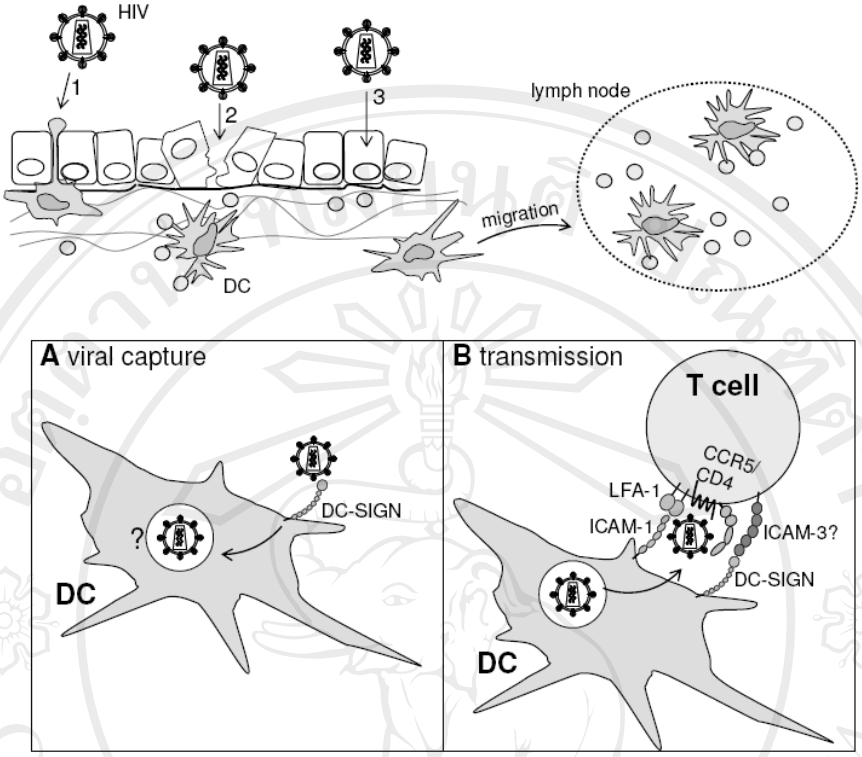

Figure 1.6 HIV-1 transmission models [123]. In sexual transmission, there are 3 routs that HIV-1 can cross the barrier (upper panel). Virus can be captured by intraepithelial mDCs (1). Virus can also enter through breaches or lesions caused by hormones, microbicides or other sexually transmitted diseases (2). Lastly, virus can be captured by epithelial cells that transfer the virus to target cells underneath the epithelia (3). After the virus was captured, it dissociates from DC-SIGN and resides in an unidentified non-lysosomal compartment as shown in A. After T cell encounter, HIV-1 is recruited to the site of T cell interaction as shown in B.

HIV-1 can cross a mucosal barrier where they were captured by intraepithelial myeloid dendritic cells (mDCs). DCs can capture HIV-1 through C-type lectin receptors (DC-SIGN). HIV-1 resides in unidentified lysosomal compartment of DC until DC encounters with T cell and HIV-1 is then transferred to T cells. This interaction is called infectious synapse. The infectious synapse between DC and T cell depends on DC-SIGN expression and strong cell-cell adhesion mediated by intracellular adhesion molecules-1 (ICAM-1) to lymphocyte function-associated antigen-1 (LFA-1) interaction. mDCs migrate to lymph nodes where they interact tightly with T cells. mDCs play a crucial role in sexual HIV-1 transmission through viral capture in the mucosa and subsequent transfer to T cells in the lymph nodes [124]. In addition to HIV-1 transmission in the lymph nodes, mDCs may also facilitate HIV-1 replication in mucosal T cells at the portal of viral entry (Figure 1.6). HIV-1 can also be bound and primarily uptaked via other surface molecules such as adhesion molecules, complement receptors and Fc-receptors. On the other hand, pDCs are not located in high amounts at sites of pathogen entry, they do not capture, endocytose and process antigens as effectively as mDCs, and their role in T cell proliferation is less pronounced [124-126].

1.2.9 DC-SIGN, the mediator of HIV-1 infection

DC-SIGN or CD209 is a C-type lectin receptor present on both macrophages and dendritic cells. DC-SIGN is the best studied example of a C-type lectin receptor on DC that mediates the infection of HIV-1. HIV-1 attachment to DC-SIGN is mediated by the highly glycosylated HIV-1 envelope protein gp120. After binding to DC-SIGN, HIV-1 is internalized. The virus will survive intracellularly for a long period of time by residing in an acidic, non-lysosomal compartment. The mysterious about this evading is still unknown. When HIV-1-loaded DC encounters with T cell, DC externalizes the virus to the site of cell-cell contact where CD4 and co-receptors on the T cell are recruited to the same region. Furthermore, HIV-1 affects the transmission process by interfering with intracellular trafficking of DC-SIGN. The result of this event is mDCs express more DC-SIGN on the surface, thus enhancing DC-T cell clustering. However, this is trans-infection because DC itself did not infect by HIV-1 virus. DC-SIGN can also facilitate HIV-1 virus infection to DC itself as well. Other C-type lectins may also be involved in HIV-1 capture and transmission. $CD1a^{++}$ langerhans cells lack DC-SIGN and bind HIV-1 via langerin. Blood mDC and pDC subsets that lack DC-SIGN can bind HIV-1 through CD4 independently. It is unclear that HIV-1 can bind to MoDCs and langerhans cells via C-type lectins. These studies suggested that not a single receptor is responsible for HIV-1 binding to all DC subsets [127-134]. For pDCs, they are not located in the pathogen entry site. These cells do not capture, endocytose and process antigens as effectively as mDCs. pDCs are involved in the innate immune responses against many viruses, including HIV-1. They play a different role in the controlling of virus. Since some in vitro studies have shown that pDCs are susceptible to HIV-1 infection and they are able to

transmit HIV-1 to T cells as well, their role in HIV-1 pathogenesis remains to be pursued [135-140].

1.2.10 Dysfunction of DCs infected with HIV-1

DC is the first of cellular targets of HIV-1 infection. Its migratory nature makes it a strong candidate for viral spreading and transmission. Sometime, DCs in lymphoid tissues may serve as reservoirs of HIV-1 that continually contribute to infection of newly recruited T cells. HIV-1 infects DC through many ways such as CCR-4, CXCR-5, $CD4^+$ and DC-SIGN. HIV-1 can attach to and infect DCs by using HIV-1 envelope protein, gp120 bind to the target cells. There are several mechanisms that gp120 may dysregulate DC functions as show in Figure 1.7.

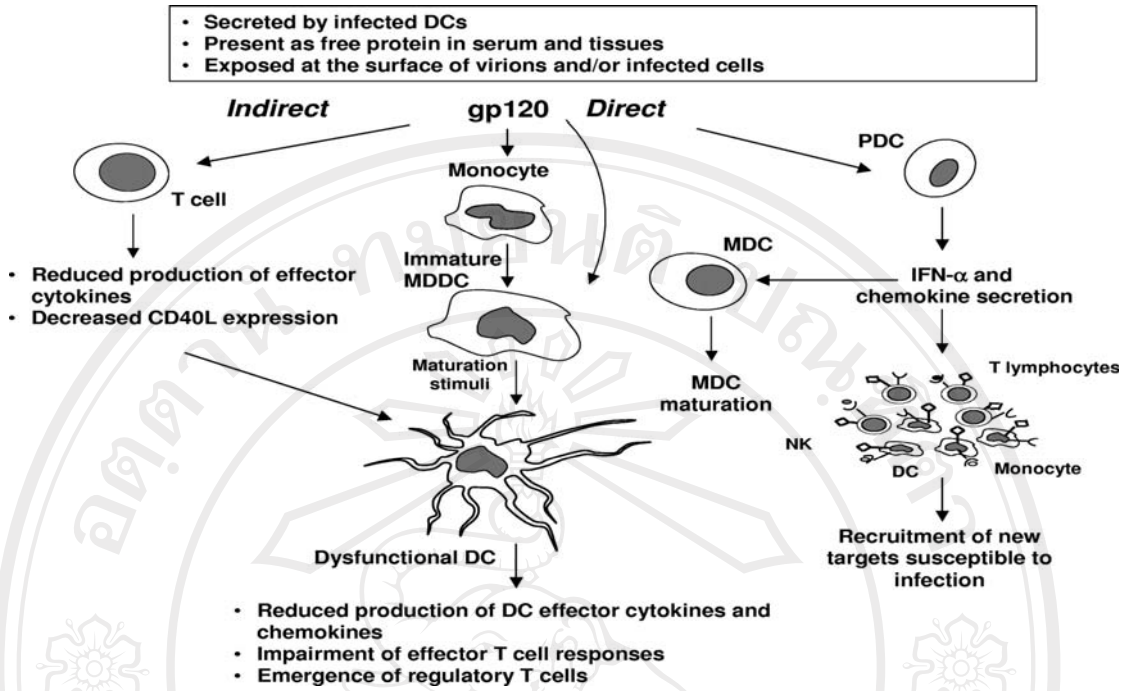

Figure 1.7 Model showing how HIV-1 may succeed in evading the host immune response through gp120-mediated direct and indirect bystander effects [141]. In this model, gp120 affects the biology and function of uninfected DCs by acting at several levels. Gp120 induces DC precursors (blood monocytes) to differentiate into DCs that exhibit a canonical phenotype but do not undergo full activation upon maturation induction. For immature DCs, gp120 induces a partially mature phenotype. pDCs can also be induced with gp120 to produce IFN-α and CC-chemokine, thus favoring the recruitment of new targets susceptible to viral infection and to immune dysregulation. On the level of T cell activation, consequently impairing the process of DC maturation induced by their interactions with activated T cells.

The first mechanism is that after DCs were exposed to HIV-1 envelope protein gp120, they changed in their differentiation or maturation programs [141]. Some studies found that in blood DCs isolated from HIV-1-infected patients, plasmacytoid (pDCs) and myeloid (mDCs) dendritic cells expressed high levels of maturation markers, CD86 and CD40 [142, 143]. pDCs in chronically-infected individuals exhibit an impairment of virus-induced production of type I IFN, possibly because they have been previously activated [144, 145]. The decreased expression of costimulatory molecules is expected to have negative effects on antigen presentation and activation of T cell responses [146]. Smed-Sorensen *et al* reported that while exposure of mDCs and pDCs to HIV-1 alone resulted in only weak maturation of both DC subsets, TLR7/8 ligation induced full maturation in both infected and non infected cells [139]. Granelli-Piperno *et al* also showed that infected DCs specifically fail to mature in response to different stimuli [9]. Fantuzzi *et al* reported that functional but not phenotypical impairment of DCs is also observed when immature DCs were generated from monocytes in the presence of gp120 and then stimulated with LPS or CD40L [147]. The major functional defects observed in DC subsets upon their exposure to HIV-1 apart from infection are summarized in Table 1.4.

Table 1.4 Recombinant gp120 and virion inactivated-induced, functional defects in DC subsets [141]

Conditions		**DC response**	**DC subset**
gp120	Recombinant protein (R5 and X4)	—Up-regulation of CD80, CD86, CD40, CD83 and MHC class I and II	iMoDC
		—No induction of IL-12, TNF-α, CCL3, CCL4, CCL5	iMoDC
		—No stimulation of T cell response	iMoDC
		—Impaired maturation of DC promoted by classical stimuli	iMoDC
	Recombinant protein (X4)	—Up-regulation of CD80, CD86, CD40, MHC class II, CD54, CXCR4, CCR7	iMoDC
		—Down-regulation of mannose receptor, CCR6	iMoDC
		—Induction of IL-10, IL-12, IL-18, TNF- α	mMoDC
	Recombinant protein (R5)	—Induction of migration	iMoDC
	Recombinant protein (R5 and X4)	—Induction of type I IFN	pDC
		—Up-regulation of CCL2, CCL3, CCL4	
Inactivated virion	AT2 inactivated (X4 and R5)	—Up-regulation of CD80, CD86, CD40, CD83 and MHC class II	iMoDC
	AT2 inactivated (X4 and R5)	—Induction of IFN- α	pDC
	AT2 inactivated (X4 and R5)	—Induction of type I IFN-α	pDC
		—Up-regulation of CCL2, CCL3, CCL4	
	AT2 inactivated (X4 and R5)	—Up-regulation of CD80, CD86, CD83, CCR7	pDC
		—Induction of IFN- α and TNF- α	
	Heat-inactivated HIV-1	—Induction of IFN- α	pDC
		—Up-regulation of CD80, CD86	

It has been suggested that different effects of HIV-1 exposure on DC maturation appear to be a consequence of virus dose and duration of virus exposure. HIV-1 affects DC migration by triggering cell-specific signaling machinery or it did not induce their full maturation [148].

The second posibility is the altered production of cytokines or chemokines in HIV-1 gp120-exposed DC subsets. pDCs have been extensively studied in the context of HIV-1 infection. They directly recognize and respond to HIV-1 infection by producing large quantities of IFN-α [149-151], where as neither gp120 nor gp41 can induce IFN-α production by mDCs or MoDCs. However, in other studies, gp120 and gp160 prepared from different strains of viruses failed to induce any detectable IFN production by pDCs [150, 152, 153].

It has been reported that IFN-α production by pDCs in response to HIV-1 requires at least two interactions between the virus and the cell. Initially, envelope-CD4 interaction mediates endocytosis of HIV-1. Subsequently, endosomally delivered viral nucleic acids stimulate pDCs through Toll-like receptor TLR7 [150, 152].

Del Corno *et al* found that gp120 up-regulated the production of some inflammatory chemokines such as CCL2, CCL3 and CCL4 from pDCs, whereas mDCs seemed to be completely refractory. However, the respective effect of gp120-chemokine receptor in the altered production of cytokines or chemokines has only been partially explored [141, 154].

The third possibility is that induction of DC chemotaxis by gp120. This recruits both T cells and monocytes. Some studies show that pre-exposure of MoDCs to R5 HIV-1 or its recombinant gp120 protein prevents DC migration toward CCR5 ligand.

This may due to gp120-mediated internalization of chemoattractant receptors, including HIV-1 fusion co-receptors. These results suggested that APC-dependent inflammatory reactions can be interfered by the virus [141, 155-157].

The last possibility is that the exposure to HIV-1 gp120 may affect DC survival. However, the reasons are not yet clearified [141, 150].

Objectives:

1. To set up the method for generation of monocyte-derived dendritic cells.
2. To study T cell responses to generated monocyte derived dendritic cells.
3. To compare T cell responses activated by monocyte-derived dendritic cells from either HIV-1 infected patients or HIV-1 negative volunteers.