CHAPTER II

LITERATURE REVIEW

HISTORY

Acquired immune deficiency syndrome (AIDS) was first described in homosexual men by Dr. Gottlieb and colleagues in 1981. The first cases were recognized based on a unique common parasitic infection, namely, Pneumocystis carinii that never causes diseases in healthy persons[34]. After June 1981, there were additional reports of opportunistic infections in intravenous drug abusers and hemophiliacs[35]. Kaposi's sarcoma, which is mainly found in patients with cell-mediated immune defects, was also reported in these groups of patients[36]. In 1982, Centers for Disease Control (CDC) of the United States of America named this kind of immune deficiency as Acquired Immune Deficiency Syndrome or AIDS[37].

ETIOLOGY

The high incidence af AIDS in homosexual men, intravenous drug abusers and hemophiliacs made scientists suspect that AIDS might be caused by a transmissible agent, probably through blood or blood products. In 1983, Dr. Luc Montagnier and colleagues from Pasteur Institute, Paris reported an isolation of virus from generalized lymphadenopathy homosexual men, and the virus was called Lymphadenopathy Associated Virus (LAV)[38].

One year later, Dr. Robert Gallo and colleagues from the National Cancer Institute of the United States of America also

isolated a virus from the blood of AIDS patients. He named the virus as Human T Lymphotropic Virus type III (HTLV-III)[39] because it is very similar to other viruses in the same group which can infect human T lymphocytes.

After more detailed studies of the virus, it was found that LAV and HTLV-III were the same. To eliminate confusion caused by the two names, an international commission had changed its name to Human Immunodeficiency Virus (HIV)[40]. Now, the virus can be further classified into HIV-1 and HIV-2. There are evidences that HIV-2 is 50-60% different from HIV-1, which is the original HIV as described above, in terms of genetic informations. Preliminary information, however, suggests that HIV-2 may not be quite as pathogenic as HIV-1. HIV-2 has not yet spread extensively in the developed world but has a high prevalence in West Africa[41,42].

HIV-I Biology

HIV-1 is classified in the Family Retroviridae , Subfamily Lentiviridae, Genus HIV. It causes the death of infected cells, distinguishing it from other retrovirus in the Subfamily Oncovirinae such as HTLV-I and HTLV-II which immortalize the infected cells.

Like all retroviruses, HIV-I is a single - stranded plus - sense RNA virus slightly larger than 100 nm in diameter. On electron microscopy, it has a characteristic dense, cylindrical nucleoid containing core proteins known as protein p24 (molecular weight 24,000 daltons), genomic RNA, and reverse transcriptase surrounded by a glycoprotein envelope. The HIV genome codes for a number of proteins, both structural and

nonstructural proteins which can be described as shown in Table II[43,44,45,46].

Table II : Summary of HIV proteins

Gene and gene product	Description
:structural proteins	
- env (envelope)	
gp160	Precursor of Env glycoprotein
gp120	Outer Env glycoprotein
gp41	Transmembrane glycoprotein
- gag (group-specific antigens or core)	
p55	Precursor of Gag proteins
p24	Gag protein
p17	Gag protein
p15, p9, p7	Gag protein
- pol (polymerase)	
p66	Reverse transcriptase of pol gene
p51	Reverse transcriptase of pol gene
p31	Endonuclease of pol gene
:nonstructural proteins	
- tat (transactivator)	
p14	Transactivator of viral proteins (formerly tat-3)
- rev (regulator)	
p19/20	Regulates expression of virus proteins (formerly art or trs)

Table II : Summary of HIV proteins

Gene and gene product	Description
- vif (infectivity factor)	
p23	Determines virus infectivity (formerly sor)
- vpr (R)	Unknown (formerly R)
- nef (negative factor)	
p27	Reduces virus expression; GTP-binding
- vpu (only in HIV-1)	
p14/p16	Unknown
- vpx (only in HIV-2)	
p14/16	Unknown

In summary, the basic structure of HIV is a spherical virus of 100 nm in diameter with a surface glycoprotein envelope. The envelope composes of 2 parts : a transmembrane glycoprotein (gp41) which is embedded in the lipid bilayer and the gp120 glycoprotein which projects externally[41]. The inner part of the virus is core. It consists of two major proteins : p18 and p24 , within which are two sets of single stranded ribonucleic acid (RNA) and reverse transcriptase enzyme. By using reverse transcriptase, HIV can convert its RNA into deoxyribonucleic acid (DNA) copy and this DNA copy will become incorporated into the DNA of the host cell.

One prominent biologic features of HIV infection in vitro and in vivo is the cytopathic properties of the virus. Since HIV is T_4-lymphotropic viruses and can infect T_4 lymphocytes by attaching its gp120 to the T_4 (CD_4) - receptors on the T_4 (CD_4)

lymphocytes, the infected persons will have progressive depletion of T_4 (CD_4) - bearing lymphocytes which eventually leading to immunodeficiency and secondary infections and neoplasms [47]. Furthermore , HIV can infect other T_4 (CD_4) bearing cells such as macrophage, monocyte, B lymphocyte, neuron and endothelial cell[3,48,49].

Life cycle of HIV

When HIV infects target cells via binding with T_4 (CD_4) receptors, the virus' RNA then passes into the target cells (most of which are helper T-cells). Reverse transcriptase enzyme converts the RNA into DNA and then circular double-stranded DNA is made by host cell DNA-dependent DNA polymerase[50]. The circular viral DNA (or plasmid) can integrate into host DNA and can replicate simultaneously with host DNA. Cells with integrated viral DNA (or provirus) are designated as latently infected cells and this will do no harm to the cells.

On the other hand, by unknown reasons or probably by some cofactors (such as other concomitant infections), viral DNA can be stimulated and released from host genome. In this case, viral messenger RNA (mRNA) can be transcribed and then translated to viral structural and non-structural proteins. After assembly of viral genome and its proteins, the full viral particle buds out of infected cells and thereby killing the cells[51].

MODES OF TRANSMISSION

From the epidemiologic evidences, HIV can be transmitted by contaminated blood or blood products such as Factor VIII concentrates. In whole blood from AIDS patients, only 1/10,000

PBMCS have been found to contain viral DNA[52] whereas in cell-free plasma or in semen, there are only 10-50 infectious particles per milliliter[53,54]. Besides blood, HIV could be found in cerebrospinal fluid which is an unlikely source of contagion[1]. There were also reports of HIV isolation from saliva, tears, nasal discharge, urine and feces from some cases of AIDS patients but the viral concentrations in these excreta were usually very small. In other reports, there was no detectable free virus in bronchial fluid or saliva[53,54,55,56,57].

In summary, HIV can be transmitted through three primary routes[41,58]:

1. sexual contact with an infected person
   - : male-to-male
   - : male-to-female
   - : female-to-male
2. parenteral exposure to infected blood or blood products such as
   - : blood transfusion including Factor VIII in hemophiliacs
   - : sharing blood-contaminated needles and syringes in intravenous drug abusers
3. perinatally from an infected mother to her child (there were also few documented cases of HIV-transmission by breast-feeding)

PATHOGENESIS

After viral infections, both the virus and the immune responses of infected host determine the extent of pathogenesis as shown in Table III[1]

Table III : Factors that influence HIV pathogenesis

- Virus characteristics	
Replication : host range	
Cytopathology	
CD_4 antigen modulation	
Latency	
- Host immunologic response	
Humoral immunity against virus	: neutralizing antibodies , ADCC and enhancing antibodies
Autoantibody production	
Cellular immunity against virus	: cytotoxic and suppressive responses
Hyporesponse	: carcinoma and infection
Hyperresponse	: B-cell lymphoma and Kaposi's sarcoma

From many reported studies, the host immune response can modulate, reduce, or enhance viral pathogenesis as will be discussed later.

Mechanisms of T_4 cell depletion

1. Burst of infected cells

Since we know that CD_4^+ cells are the main target of HIV infection, once the cell is infected, the virus can replicate itself by taking advantage from the host cell. When a certain amount of viruses is reached, the infected cell will then burst as a consequence of explosive viral budding[59].

2. Syncytial formation of HIV-infected T cell

The appearance of HIV antigen (gp120) on the

surface of CD_4^+ infected T cell can act as a bridge for uninfected CD_4^+ cell. When uninfected CD_4^+ cells come in contact with infected cell via gp120-CD_4 receptors, these cells may fuse together to form multinucleated giant cells. Eventually these multinucleated giant cells will lose their function and be destroyed by immune surveillance[60,61].

3. **Autoimmune destruction of helper T cell[50,53,62]**

This is mediated by cytotoxic T cell which recognizes self CD_4 antigen that is altered by HIV infection thus resulting in destruction of helper T cell. The destroyed helper (CD_4^+) T cell may be HIV-infected T cell or may be uninfected CD_4^+ T cell but bound with free gp120.

4. **Antibody Dependent Cell-mediated Cytotoxicity (ADCC)[63,64,65]**

This is mediated by antibodies to HIV such as anti-gp120 which bind to infected T cells as well as uninfected CD_4^+ T cells bound with free gp120. These antiboby-coated CD_4^+ T cells will then be destroyed by a unique set of mononuclear cells that posses Fc receptor on their surfaces but having no phagocytic activity, a process called ADCC.

: HIV infection of other cell types of the immune system

Other cell types such as monocytes/macrophages , which also present CD_4 antigen on their surfaces , can also be infected by HIV[2,3]. Another proposed mechanism of infection is via the Fc receptors on the monocytes / macrophages. HIV-anti-HIV immune complexes can bind to monocytes / macrophages via Fc and complement receptors, then the virus can enter into these cells by endocytosis. This anti-HIV which facilitates HIV infection is called enhancing antibody[4,5,6,]. These cells might be important

as a reservoir for infection and in the pathogenesis of some of the clinical disorders associated with AIDS. For example, HIV has been detected primarily in monocytes, macrophages, and microglial cells in the brains of patients with encephalopathy, suggesting a key role of these cells in the encephalopathic process[66].

CLINICAL MANIFESTATIONS

Staging of HIV infection

Precise staging of patients with HIV infection is important for several reasons[67]:

1. It gives patients a realistic assessment of life expectancy.
2. It can help guiding physicians to follow up patients for the development of complications.
3. It provides data for therapeutic decisions and for measuring response to therapy. Since HIV infection may cause many clinical manifestations, CDC of the United States of America has classified stages of HIV infection based on the clinical manifestations into 4 stages[68] :

- Stage 1 : Acute HIV infection

HIV-infected patient at this stage will have very mild symptoms such as flu-like syndrome and lymphadenopathy. Some might have encephalitis, meningitis, myelopathy and neuropathy. Patients will recover from these symptoms within 1-2 weeks without any medications. Anti-HIV is usually negative at this stage by the conventional tests but seroconversion will soon follow.

- **Stage 2 : Asymptomatic infection**

HIV-infected persons will be found anti-HIV positive without any clinical symptoms. Anti-HIV can be detected as early as 6-8 weeks after HIV infection but almost all will be positive by 3-6 months.

- **Stage 3 : Persistent generalized lymphadenopathy (PGL)**

Patients will have generalized lymphadenopathy without other symptoms. The 2 or more enlarged lymph nodes should not be on the same draining chain and the size should be 1 cm or more and persist for more than 1 month. By lymph node biopsy , there is no any pathology except reactive hyperplasia. Lymph nodes at the inguinal regions are not considered as PGL.

- **Stage 4 : Symptomatic HIV infection**

This stage is subclassified into

Stage 4-A : Constitutional disease

This is the same as the original AIDS related complex or ARC. Patients might suffer from

- unknown cause of weight loss (more than 10 kilograms or 15 pounds or more than 10% of baseline weight)
- fever (more than 38°C) more than 4 weeks
- diarrhea for more than 1 month without obvious cause
- night sweating
- oral candidiasis
- herpes zoster

Stage 4-B : Neurological disease

This stage involves both central and peripheral nervous systems manifesting as encephalitis, meningitis, myelopathy and neuropathy. Some may have progressive dementia, cognitive or affective changes or full-blown psychosis. This is called HIV encephalopathy or AIDS dementia complex.

Stage 4-C : Secondary infectious disease

This is caused by microorganisms which are harmless to healthy persons such as candidiasis , pneumocystis carinii pneumonia, toxoplasmosis, etc

Stage 4-D : Secondary cancers

Kaposi's sarcoma is the most frequently found cancer. Some might present as primary central nervous system lymphoma and non-hodgkin's lymphoma.

Stage 4-E : Other conditions

There are also other classification systems for HIV infection such as

- The Walter Reed staging system which combines clinical and immunologic data such as anti-HIV,lymphadenopathy, T helper cells number, delayed type hypersensitivity response, oral thrush and other opportunistic infections[69].

- WHO classification which consists of 4 stages of HIV infection :-Asymptomatic, Progressive Generalized Lymphadenopathy (PGL),AIDS related complex (ARC) and full-blown AIDS.

IMMUNOLOGIC ABNORMALITIES

Since the main target of HIV is helper T cell which is the co-ordinating center of the immune response, the destruction and depletion of helper T cells will be the cause of several immunologic abnormalities observed in HIV infection. The immunologic abnormalities involve both humoral and cellular immunities as described in Table IV.

Table IV : Immunologic abnormalities in AIDS[70,71]

: T cells
-CD_4^+ cell depletion*
-Decreased proliferation to soluble antigens*
-Decreased helper response for PWM-induced immunoglobulin synthesis*
-Impaired delayed - type hypersensitivity to both recall and new antigens*
-Decreased γ-interferon production in response to antigens*
-Decreased proliferation to T-cell mitogens, alloantigens and to anti-CD_3
-Decreased Autologous Mixed Lymphocyte Reaction (AMLR) response
-Decreased cell-mediated cytotoxicity to virally infected cells
-Decreased IL-2 production
-Lymphopenia

Table IV : Immunologic abnormalities in AIDS[70,71]

: B cells
-Polyclonal activation with hypergammaglobulinemia (IgG, IgA, and IgD), increased spontaneous plaque forming cells, and proliferation*
-Decreased humoral response to immunization*
-Circulating autoantibodies
: Macrophage / monocytes
-Decreased chemotaxis*
: Natural killer cells
-Decreased cytotoxicity*
: Other humoral responses
-Increased acid-labile γ-interferon production
-Increased soluble immune complexes
-Decreased α_1 - thymosin

*=characteristic disorders

The immunologic abnormalities that can be used to categorize HIV-infected patients into different disease stages include T lymphocyte count, T_4/T_8 ratio, proliferative response to T-cell mitogen and delayed type hypersensitivity response. Full-blown AIDS patients represent the worst in response while asymptomatic carriers may respond nearly like the healthy individuals[69].

LABORATORY DIAGNOSIS

For the detection of HIV infection, a sensitive and specific test for anti-HIV or HIV identification is necessary. Since the first discovery of HIV in 1983 , many tests have been

developed for this purpose. Now there are many types of tests which can be used to detect HIV infection.

The tests can be divided as[72] :

1. Antibody tests
2. Tests for virus and viral antigens
3. Tests for proviral DNA

Antibody tests

a) Enzyme-linked immunoassays (EIA) :

This is the most widely used serologic test for HIV-1 antibody detection. It is used primarily as a screening test for blood donors and individuals at risk for HIV infection. The repeatedly reactive serum or plasma should be retested with a supplemental test, such as Western blot[73,74]. Most of the EIA used now are based on the principle of indirect EIA with HIV antigen-coated plate. The antigens used may be whole viral lysate, recombinant protein or synthetic peptide antigens[73,75].

b) Rapid immunoassays :

This takes shorter time than the conventional EIA. HIV-coated inert particles can be crosslinked by anti-HIV if present in the sample. These assays include particle agglutination, hemagglutination and latex bead agglutination [76,77]. Furthermore, immunoblot can also be used for rapid immunoassay[78]. The greater advantages of rapid immunoassays over the conventional EIA are no need of sophisticated equipments, not too expensive for use in developing countries and tolerable to extremes of climate[79].

c) Supplemental tests :

These are used for confirmation of anti-HIV positive

by EIA or other screening tests. These tests include :

- **Immunoblotting (Western blotting) :**
  The antigen used is partially purified whole virus which is electrophoresed in a polyacrylamide gel to separate the individual viral proteins, transferred onto nitrocellulose, and then can be used to react with patient serum[80].
- **Immunofluorescence assay (IFA) :**
  This method is used in some reference centers, hospitals or medical schools[81,82]. HIV-infected cell lines such as H9 are inactivated and fixed on glass slides and used to incubate with patient serum and then followed by the same procedure as indirect immunofluorescent technique.
- **Radioimmunoprecipitation assay (RIPA) :**
  This technique is rather cumbersome and expensive and requires the maintenance of infected cell lines for preparation of radiolabeled viral proteins[72]. After patient serum incubation, anti-HIV, if present, binds with radiolabeled viral proteins. The radioactive antigen-antibody complexes can be separated by SDS-PAGE to determine specific viral patterns. The banding patterns are similar to those of Western blot except that RIPA is more sensitive for the detection of gp120 and its precursor, gp160[72].

<u>Tests for virus and viral antigens</u>

HIV can be detected by viral culture technique or antigen capture EIA. HIV antigen (p24) capture EIA is used for early diagnosis of HIV-1 infection in neonates and in seronegative

persons belonging to the high risk groups. The technique is also useful in predicting disease progression in asymptomatic seropositive persons and in monitoring antiviral therapeutic interventions[72]. The limitation of HIV antigen detection is that in the presence of high titer of anti-p24, HIV antigen may not be detected.

HIV culture needs coculturing of patient's lymphocytes with peripheral blood mononuclear cells (PBMC) from healthy seronegative donor. The presence of HIV in the culture is usually assessed by reverse transcriptase or p24 antigen assays. Although the sensitivity and specificity of various culture methods vary (between 10 and 95% or approach 100% now), viral culture is still the reference method for identifying HIV infection. The disadvantages of viral culture are costly, time consuming, labour intensive and increased risk of laboratory personnel for exposure to high concentrations of HIV[83].

Tests for viral DNA

The techniques used are Southern blot, dot blot, in situ hybridization and the most novel DNA amplification or polymerase chain reaction (PCR)[72]. Basic principle of these techniques bases on the hybridization between two complementary DNA strands. Since the number of PBMC infected with HIV in a patient is extremely low, as few as 1 in 10^4 cells[84,85], it is necessary to amplify HIV proviral DNA to detectable levels by PCR[86]. PCR can be applied to detect HIV DNA or RNA directly from cells of infected persons[84,85].

Viral culture and PCR can be used for early detection of HIV infection. There was evidence of HIV-1 isolation in blood

samples from 31 out of 133 gay men (23%), 27 of whom have remained seronegative for up to 36 months after the positive culture[87].

PROGNOSTIC MARKERS

Besides the detection of HIV specific antigen and antibody, there are some additional immunologic markers which can be used to predict disease progression. The mostly used prognostic markers are CD_4 cell count, β_2-microglobulin, neopterin, anti-p24, p24 antigen, elevated level of soluble interleukin-2 receptors and IgA[88].

In summary, the prognostic markers which are used to follow severity and progression of HIV infection are

- decreasing CD_4^+ T cells[17,18]
- increasing β_2-microglobulin level[19,20]
- increasing neopterin level[21,22,23,24]
- decreasing anti-p24 titer simultaneously with increasing p24 antigen level[25,26,27,28]
- Impaired delayed - type hypersensitivity reactions[36,37]

One can also divide prognostic markers into specific and non-specific markers. The specific prognostic markers are p24 antigen and anti-p24. The non-specific markers are CD_4^+ T cells, β_2-microglobulin and neopterin.

: p24 antigen and anti-p24, the correlation between antigen and antibody can be depicted as in Figure 1

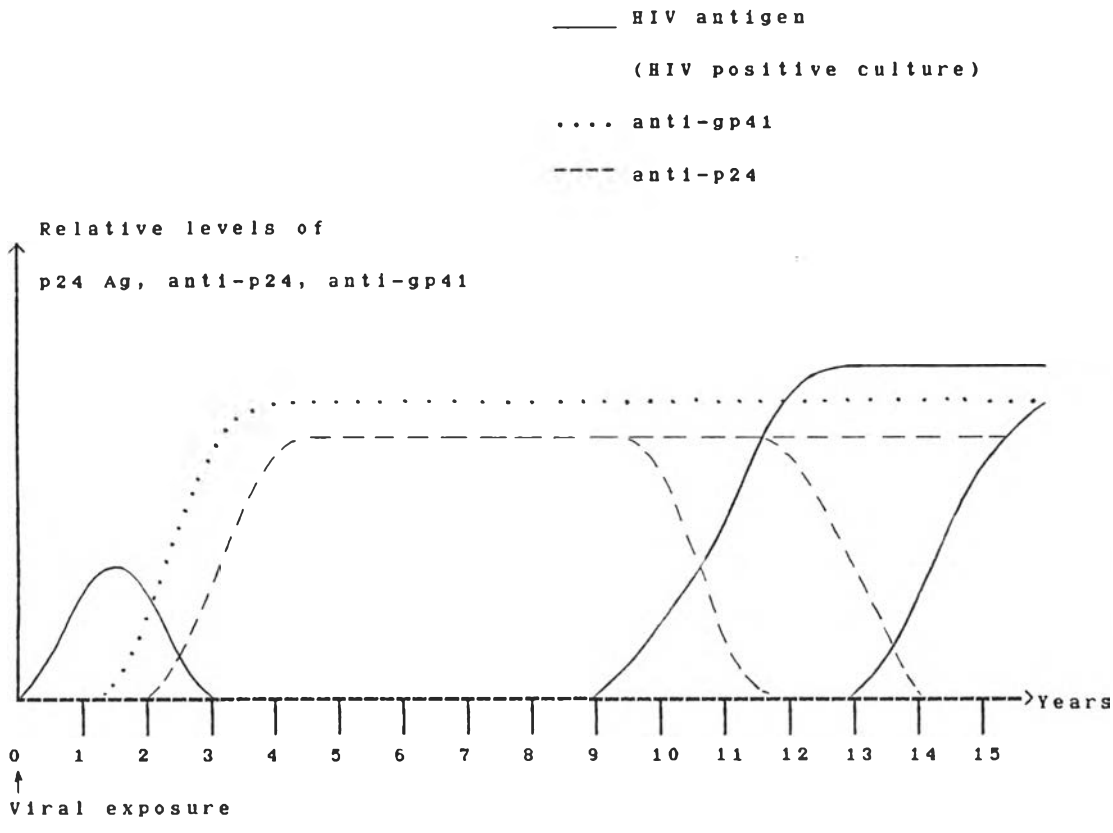

Figure 1. Correlation between antigen and antibody of HIV infection

When anti-p24 is positive, HIV antigen (or p24 antigen) is usually undetectable. Therefore HIV antigen can be detected in early HIV infection or when infected patients severely progress to AIDS[32,89,90]. It has been proved that the development of AIDS is usually preceded by a decline in anti-p24 titer with HIV antigenemia[32,33].

: CD_4^+ T cells, the alteration in the percentage and total number of CD_4^+ T cells can be found in many diseases such as autoimmune diseases and some viral infections[91]. Since CD_4^+ T cells are the main target of HIV infection, the CD_4^+ T cells decrease progressively from asymptomatic, PGL, ARC and to the lowest level in full-blown AIDS patients[30,31].

: β_2 - microglobulin, it is a low molecular weight protein (approximately 11,500 daltons) which forms the light chain of MHC class I (Major Histocompatibility Complex). MHC class I is present on the surface membrane of nucleated cells such as T and B lymphocytes, macrophages.[92,93] Elevated concentrations of serum β_2-microglobulin have been reported in patients with viral infections (including HIV infection) and hematologic malignancies [19,94,95]. Increased level of β_2-microglobulin correlates with increased cellular turnover which is the consequence of cell activation[29].

: neopterin, it is a low molecular weight compound which is derived from GTP (Guanosine triphosphate)[96]. Neopterin is secreted by monocytes/macrophages which have been stimulated by gamma interferon[97]. There is reported evidence that cellular activation will increase the multiplication of HIV as well. For this reason, the higher neopterin levels were found in patients with more advanced HIV infection as a result of cellular activation[21]. In addition, neopterin can be used to predict susceptibility to HIV infection in high risk individuals. Ones who have higher level of neopterin will be infected by HIV easier than those with lower level of neopterin[22,23].