II. LITERATURE REVIEWS

A. History of *P. marneffei*

Penicillium marneffei is the only thermal dimorphic pathogenic Penicillium among several hundred species of Penicillium, that can cause mycosis in both human and animal. The organism was first isolated in 1956 by Capponi and colleagues from the captive bamboo rat (*R. sinensis*) rodents native to the highlands of central Vietnam (Capponi et al., 1956). These rats had been maintained at the Pasteur Institute of Indochina at Dalat, South Vietnam. Capponi and Sureau first observed the spontaneous death of three bamboo rats due to a reticuloendothelial mycosis. The fungus was isolated from a rat and an experimental infected animal to be studies at the Pasteur Institute, Paris. This fungus was identified and gave a name as a new species by Segretain in honor of Hubert Marneffe, director of the Pasteur Institute of Indochina (Segretain, 1959). This fungus was experimentally highly pathogenic for hamsters, mice and rats, but not for rabbits and guinea pigs, producing a reticulosis similar to histoplasmosis or leishmaniasis.

Segretain was accidentally pricked his finger during experimental studies, with a needle used to inoculate hamsters. He developed a small nodule at the site of inoculation a days later, followed by lymphangitis and axillary lymphnode hypertrophy. He was treated with nystatin, this demonstrated a high *in vitro* sensitivity of this fungus (Segretain, 1959). This accidental infection, emphasized the possibility of human

pathogenicity. There has been no additional report of the isolation of this fungus in the literature, until 17 years later. In 1973 Disalvo and his colleagues reported the first natural human infection in the USA, in a 61-year-old American minister with the Hodgkin 's disease who had travelled in Southeast Asia (Di Savo et al., 1973). In 1984, Pautler and his colleagues also reported the *P. marneffei* infection in a 59-year-old man who had travelled extensively in Far East and recurrently episoded of hemoptysis thought to be related to bronchitis and bronchiectasis. A pneumonectomy revealed granuloma; tissue sections of the lung showed unicellular, yeast-liked cells of *P. marneffei* multiplying by fission and the tissue culture identified the *P. marneffei* (Pautler et al., 1984). The natural human infection of *P. marneffei* were reported as the imported disease in the USA because of the history of their history records of previous traveling in Southeast Asia where *P. marneffei* is endemic. Among the natives of Southeast Asia, the primary cases of penicilliosis marneffei were reported in the patients without HIV infection. In 1984, the first five cases were reported in Bangkok, Thailand by Jayanetra and her collaborators (Jayanetra et al., 1984). The patients came from various parts of the country. In the same year the other two cases were reported (Tanphaichitra, 1984). The additional 5 cases from Chiang Mai province in northern Thailand, were reported during the year 1987-1989 (Supparatpinyo et al., 1992). The first eight Chinese cases of *P. marneffei* infection from Guangxi Zhuang autonomous region, the southern China, were described in 1985 by Deng and his colleages (Deng, 1985). These patients had no predisposing illness or evidence of altered immunity and

were initially misdiagnosed as histoplasmosis. The additional 4 cases from the same region were published in the last three years (Deng et al., 1988). A few cases were also reported in 1985 from Hong Kong, which belongs geographically to southern continental China situated at the same latitude as Guangxi region (So et al., 1985; Chan, 1989).

In 1988, numerous cases of systemic penicilliosis due to *P. marneffei* were reported for the first time among AIDS patients who had travelled in the Southeast Asia, including patients from USA (Piehl et al., 1988), UK (Peto et al., 1988), Netherlands (Hulshof et al., 1990), Australia (Jones, 1992), France (Hilmarsdottir et al., 1993), and Italy (Viviani et al., 1993). Among the natives of the Southeast Asia, the first case of penicilliosis caused by *P. marneffei* in AIDS patients was reported in 1989 (Sathapatayavongs et al., 1989). During the year 1990 to 1992, 86 adults (Supparatpinyo et al., 1992) and 5 children (Sirisanthana, 1993) of AIDS patients infected *P. marneffei* were diagnosed from Chiang Mai, Thailand, and the total number of case reached to approximately 500 cases in 1994 (Chariyalertsak et al., 1996). Among opportunistic infections affecting AIDS patients in Thailand, systemic penicilliosis marneffei is ranked fourth opportunistic infections after tuberculosis, cryptococcal meningitis and pneumocystic carinii pneumonia (Drouhet, 1993).

B. Epidemiology: animal and soil studies.

Thirty years after the discovery of *P. marneffei* in bamboo rat (*R. sinensis*) in Vietnam, a survey of the wild rats in the Guangxi Zhuang region of Southern China was shown that bamboo rats, *R. sinensis*, is less frequent than *R. pruinosus* (Deng et al., 1988). No such study was performed in Thailand, until recently when another member of the family Rhizomydae, *Cnanomys badius*, was identified in eastern and southern Thailand and Burma, these animals were reported as hosts of *P. marneffei*, because this fungi could be isolated from the internal organs of 75% of *R. pruinosus* (Ajello et al., 1995) 92.8% of *R. sumatrensis*, and 30% of *C. badius* (Chariyalertsak et al., 1996).

Furthermore, *P. marneffei* could be isolated from the soil from 3 burrows of *R. pruinosus* in China. Therefore, Deng and his colleagues suggested that contaminated soil was a common environmental source of infection for bamboo rats and people via respiratory route (Deng et al., 1988). Only few patients eat bamboo rats, therefore contamination is probably via respiratory rather than digestive route. Moreover, many yeast forms of *P. marneffei* could be demonstrated from nasal smear and sputum of the HIV patients infected with this fungus (Vithayasai, 1994). Is this suggestive of human to human transmission is not known.

C. Morphological and cultural aspects

1. Mycelial phase

P. marneffei grows as mold, on Sabouraud's dextrose agar at 25 °C, and young colonies became visible within 3 days. In its mycelial form, the colony was downy and grayish pink and produced a soluble red pigment that diffused into the medium. The conidiophores consisted of basal stipes bearing terminal verticils of three to five metulae. Some metulae bore four to seven phialides that produced ellipsoidal, smooth-walled conidia in chains. (Drouhet, 1993).

2. Dimorphism and yeast-like phase

Within 2 or 3 days of incubation at at 37 °C on brain-heart infusion agar, the fungus grew as a yeast, forming white-to-tan, soft colonies. Microscopic examination of this growth revealed unicellular, pleomorphic, ellipsoidal-to-rectangular cells (2 X 6 μm.) that divide by fission and not by budding (Drouhet, 1993).

3. Histopathology

P. marneffei has been identified in skin smear, oral mucosa, nasal smear, sputum, pleural fluid, blood, bone marrow and cerebrospinal fluid. The yeast form is oval, elliptical or sausage shaped and frequently septated but without budding forms. It stains well routine Wright, Wright-Giemsa, Hematoxylin and Eosin (H&E), Periodic-acid-Schiff (PAS), Grocott methenamine silver (GMS). The organism is characterized by intracellular and extracellular yeast cells that vary in size from 3 to 8 μm

in diameter. Typical unstained bar can also be easily seen in yeast form (Vithayasai, 1994). Extracellular, elongated, and curved (sausage shaped) yeast was up to 13 μm long. Sometimes short hyphae form not longer than 20 μm. is also observed. Usually yeast cell has only one central septum, but occasionally there are two septa dividing the cell into three small chambers (Drouhet, 1993).

D. Clinical manifestation

Penicilliosis marneffei is usually disseminated and progressive mycosis in both normal and immunocompromised hosts.

In immunocompetent patients, the clinical manifestations with varying degree of severity are fever (sometimes accompanied by chills), coughing dyspnea, thoracic pain, pleural effusion, pulmonary infiltration, cutaneous and subcutaneous lesions, lymphadenopathy, hepatomegaly, splenomegaly or hepatosplenomegaly, osteoarticular lesions, oropharyngeal and digestive lesions, weight loss, anorexia and asthenia. The chest x-ray varies from normal to abnormal infiltration both diffuse and localised form. The skin lesions are papules with or without necrosis, acne-like pustules and nodules. The skin lesions are usually seen at face, trunk and extremities (Drouhet, 1993).

The general clinical signs of penicilliosis marneffei in immunocompromised patients, especially in AIDS patients, are similar to those manifestations in immunocompetent patients as described above. The acute onset and intensity of certain symptoms are, however, remarkable in AIDS patients (Drouhet, 1993). Fever, anemia, marked

weight loss, coughing, generalized lymphadenopathy, hepatosplenomegaly, skin lesions, diarrhea, and septicemia are more frequent in AIDS patients (Vithayasai, 1994). Osteoarticular lesion in AIDS patients are not seen (Drouhet, 1993). Hepatosplenomegaly is striking in children (Sirisanthana, 1993).

E. Laboratory diagnosis

The microscopic characteristic of the smears from penicilliosis lesions are the most rapid and reliable mean of diagnosis. Yeast form of *P. marneffei* has been identified in skin smear, oral mucosa, nasal smear, sputum, pleural fluid, blood, bone marrow and cerebrospinal fluid. The yeast form may be oval, elliptical or sausage shaped and frequently septated but without budding. It stains well with Wright, Wright-Giemsa, H&E, PAS and GMS stain (Vithayasai, 1994). Histopathologic examination of the samples are stained with H&E, GMS, PAS, and Ziehl-Neelsen stain.

The isolation of *P. marneffei* from clinical specimens are performed by incubating the specimens on SDA at 25 °C. The mycelial form should be visible within 2 or 3 days. In addition, it is notified for the mold-to-yeast conversion property by subculturing on BHA and incubating at 37 °C (Druohet, 1993).

F. Antifungal therapy of penicilliosis marneffei

P. marneffei is responsive to the principal antifungal agents used for the therapy of deep, systemic mycoses, but delaying treatment may be fatal. The earliest drug trial was nystatin, a polyene macrolide antibiotic reported to be effective in an experimental. *P. marneffei* was *in vitro*. It was sensitive to nystatin at 0.65 μg/ml in a semi-synthetic medium at 24 h and 3.2 μg/ml on the 4th day. *In vivo*, the hamsters were infected with *P. marneffei* and followed with nystatin treatment. The treated hamsters servived during the twenty-day observation period, whereas 50% of untreated died (Segretain, 1959). Segretain is also the first incidentally infected patient with *P. marneffei*, and he was cured by nystatin (Segretain, 1959).

Amphotericin B was first shown to have benefit in patient with spontaneous *P. marneffei* infection in 1973 (Di Savo et al., 1973),

Van Cutsem and Van Gavin showed that oral itraconazole was an effective antifungal agent in systemic *P. marneffei* infection in guinea-pig. Immunosuppressed guinea pigs were cured even by low dosage of itraconazole (Van Cutsem, 1991).

Sekhon and his colleages have also studied the *in vitro* susceptibility of mycelial and yeast forms of *P. marneffei* to amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole (Sekhon et al., 1992).

Drouhet studied the sensitivity of 10 strains of *P. marneffei*, 6 from AIDS patients and 4 from non AIDS patients to amphotericin B, 5 fluorocytosine, ketoconazole, itraconazole, and fluconazole. He was

found that all strains were highly sensitive to all antifungal drugs tested except fluconazole (Drouhet, 1993).

Supparatpinyo and his collaborators reported the *in vitro* susceptibility of *P. marneffei* to miconazole, itraconazole, ketoconazole, and 5-fluorocytocine but intermediate susceptibility to amphotericin B (Supparatpinyo et al., 1993). In clinical, itraconazole or ketoconazole should be considered to be the drug of choice in the treatment of mild to moderately severe *P. marneffei* infection. In clinical practice, patient which serious illness, treatment is routinely started with intravenous amphotericin B and followed by oral therapy with itraconazole or ketoconazole until culture results were negative and clinical findings had resolved. However, several patients relapsed after the cessation of the initial therapy, in this case, maintenance with itraconazole or ketoconazole is necessary (Supparatpinyo et al., 1993).

G. Antigen and antibody detection of *P. marneffei*

Van Cutsem and his collaborators detected a circulating *P. marneffei* antigen which contain galactomannam and/or mannan by latex agglutination test, a test kit for detection of a circulating *A. fumigatus* galactomannan in aspergillosis patients. However the titer of *P. marneffei* antigen was lower than *A. fumigatus* antigen (Van Cutsem et al., 1990). The monoclonal antibody, EB-A1 specific for *A. fumigatus* galactomanan cross-reacted with an antigen from *P. marneffei* (Pierard et al., 1991). This antibody was used to detect yeast-hyphae or remnants of filaments of *P. marneffei* in cytoplasm of some phagocytic cells by

immunohistochemical method (Estrada et al.,1992). The monoclonal antibody, 7D7, raised against *Pneumocytis carinii,* has cross reactivity with antigen of *Aspergillus spp., Candida albicans, Histoplasma capsulatum,* and *Penicillum spp.* except *P. marneffei* by immunofluorescent technique (Lundgren et al., 1992). Kaufman and his colleagues reported that rabbit antiglobulins against both culture filtrate antigens at 37 ^{0}C and whole yeast-like cellular antigens of *P. marneffei,* by indirect fluorescent antibody reacted with the yeast-like cells of *P. marneffei* and *H. capsulatum,* but not with the mycelial forms of *P. marneffei, H. capsulatum* and other fungi (Kaufman et al., 1995).

Sekhon prepared that crude mold form of *P. marneffei* (Di Savol strain, and Indochina strain of human and bamboo rat origin) from six-week-old culture filtrate, as an antigen for serological test of *P. marneffei* by microimmunodiffusion (ID) technique. The antigen did not either react with rabbit antisera specific to five species of *Aspergillus* or with four dimorphic systemic fungi *(B. dermatitidis, C. immitis, S. shenckii,* and *Micropolysporafaeni*) . In addition, rabbit anti *P. marneffei* antiserum did not react with those fungi but react with histoplasmin, blastomycin and cocidiodin in the complement fixation test (titer 1:32 to 1:64) (Sekhon et al., 1982). Sekhon also found that crude mold form exoantigen of eight isolates of *P. marneffei* from Segretain strain, the DiSalvo strain (ATCC 24100), and other Chinese and Thai strain of human and bamboo rat origin produced 2 to 4 specific antigens which were distinct from those of *P. primulinum* (Sekhon et al., 1989). Mycelial culture filtrate antigen of *P. marneffei* was applied to detect the anti *P. marneffei* antibody in

sera from the patient infected with this fungus, by ID test. It was found that, sera taken early in the course of disease gave positive antibody reactions. Whereas sera taken 3-5 months flollowing therapy were negative (Vivieni et al., 1993). Yeun and his colleagues were established an indirect immunofluorescent antibody test (IFAT) for detection of antibody against *P. marneffei* in serum, by using germinating conidia and yeast hyphae form as antigens. Eight out of 103 patients diagnosed as penicilliosis marneffei with persistent fever had an IgG titer of 160 or more while the other patients without penicilliosis marneffei (tuberculosis, typhoid fever, melioidosis, disseminated cryptococcosis, candidaemia, intra-abdominal sepsis, autoimmune disease, and lymphoreticular cancer) and 15% of 78 healthy controls had an IgG titer of 10-40 (Yuen et al., 1994). Mekaprateep studied the crude extracellular proteins of *P. marneffei* secreted during growth of yeast form by immunoblot assay. It was fuond that, the IgG antibody in 31 out of 33 sera from AIDS patients with penicilliosis marneffei recognized one or more of the four major proteins (200, 88, 54, and 50 kD). About half of them had strong reactivities to the 88, 54, and 50 kD proteins, whereas, 4 out of 38 sera from non- *P. marneffei* infected patients and any healthy sera had strong reaction to these proteins (Mekaprateep, 1995).

H. HIV

1. The structural of HIV

The human immunodificiency virus (HIV) is a member of retrovirus family. Retroviruses are small envelope viruses that contain a diploid, single-stranded RNA genome. The virus particle contains an inner core that contains the viral nucleic acids, as well as enzymes required for early replication events. This inner core is surrounded by a capsid proteins. The capsid itself is surrounded by a lipid membrane. A virus matrix protein inserts into the inner surface of the lipid membrane. An integral membrane protein (part of the envelope glycoprotein) protrudes through the lipid membrane and forms the outer surface of the virus particle (Haseltine, 1991).

2. The life cycle of HIV

HIV uses the CD4 receptor to gain entry into the cell through the high-affinity binding of the HIV envelope glycoprotein (gp 120) to a specific region of the CD4 molecule (Mc Dougal et al., 1986). One bound to CD4, HIV internalizes into the cytoplasm of the cell. This process is not well understood but is thought to involve the fusion of cell membrane with an envelope glycoprotein (gp 41) which noncovalently associates with gp 120. After the virus enters the cell, the virion-associated reverse transcriptase (RT), in conjunction with ribonuclease H, transcribes the viral RNA into double-stranded DNA (Varmus, 1988). The double-stranded HIV DNA then enters the nucleus where it inserts into the host cell genome via the action of viral integrase. At this phase of HIV life cycle,

the HIV genome designs a provirus. Once the HIV provirus has incorporated into the cellular DNA, both cellular and viral factors are required to initiate expression of viral genes (Varmus, 1988). The cellular factors may be constitutively produced by the cell or may be induced by a variety of activating signals including antigens, mitogens, heterologous gene products, cytokines, ultraviolet light, and heat. After activation of the HIV provirus by cellular factors, the first viral genes to be expressed are those that encode nonstructural proteins with regulatory function (Rosenberg, 1989; Greene, 1991). The most important of these proteins is Tat, which is a powerful transactivator of HIV gene expression and is essential for HIV replication. Tat is thought to exert its effect in two ways: by initiating RNA transcription of the HIV provirus or by stimulating production of full-length RNA transcripts. The full-length RNA transcripts multiply, splice and transport to the cytoplasm where the regulatory proteins of HIV are expressed. At this stage, another essential regulatory protein, Rev, becomes dominant. The main function of Rev is to effect the transport of unspliced and singly spliced mRNAs from the nucleus to the cytoplasm. These unspliced and singly spliced mRNAs encode the structural and enzymatic proteins of HIV that are essential for assembly of the infectious virion. The virion RNA and core proteins associated with viral envelope protein locating at the cell membrane, and the mature virion form by budding from the cell surface (Rosenberg, 1989; Greene, 1991).

Ho and his colleagues indicated that replication of HIV *in vivo* was continuous and highly productive, and it drove the rapid turnover of CD4

lymphocytes (Ho et al., 1995). The mean half-life of CD4 cells was calculated to be approximately two days, with a mean of approximately 2.0 x 10^9 cells turned over every day. Similarly, virus half-life was calculated to be approximately two days, representing a mean of 1.1 to 6.8 x 10^8 virions (30% of the total virus population) turned over every days. However, slightly greater numbers of virus than CD4 cells survive, building to a critical mass that ultimately makes its presence known through the clinical presentation of AIDS-related symptoms (Ho et al., 1995; Wei et al., 1995).

3. Immune response to HIV

3.1 Human response to HIV

3.1.1 General characteristics

The existence of antibodies to HIV was noted early in the AIDS epidemic, and testing for such antibodies constitutes the basis for the HIV enzyme-linked immunosorbent assay. It was subsequently shown that the viral gp120 and gp160 env glycoproteins were the major protein recognized by HIV-infected individuals at all stages of infection (Allan et al., 1985). Further studies showed that approximately 50% of HIV-infected individuals produced non-neutralizing antibodies to a highly conserved region, 15 amino acid (504 - 518) of the carboxy terminus of gp120 (Rosenberg, 1989). Gnann and his colleagues showed that 100% of HIV-infected individuals produced antibodies to 12 amino acid immunodominant sequence (598-609) in gp 41 transmembrane protein of the envelope (Gnann et al., 1987). The most studies indicated that the

titer of antibodies to p24 core structural protein was very high in early HIV infection, decreased to the lowest at late stages of disease (de Wolf et al., 1988). The antibodies to HIV reverse transcriptase are also detected in infected individuals (Pan et al., 1987).

The antibody isotypes vary according to the specific protein and the route of infection. IgG1 appears to be the major isotype that is produced in response to HIV (Sundgvist et al., 1986). The antibody response to env is restricted to the IgG1 subclass, whereas the response to gag and nef proteins are IgG1, IgM, IgA. In case of gag, IgG3, IgG4 and IgE present only in infected hemophiliacs (Rosenberg, 1989).

3.1.2. Neutralizing antibodies

Among the spectrum of antibodies produced against HIV, low titers of neutralizing antibodies were demonstrated in HIV-infected individuals (Weiss et al., 1985). In contrast several studies showed no significant correlation between neutralizing antibody titers and clinical status. The other investigators reported the high titer of neutralizing antibody in HIV-infected individuals with asymptomatic compared to those diagnosed with AIDS (Ho et al., 1987). Several studies indicated that an increase in neutralization titers over time correlated to stable clinical course and a decrease in neutralization titers was the indicator of disease progression (Sei et al., 1985).

Stimulation of neutralizing antibodies to HIV was first achieved by inoculation of animals with either genetically engineered or purified natural gp120 (Lasky et al., 1986). Deglycosylated recombinant gp120

was also able to elicit neutralizing antibodies (Putney et al., 1986). The HIV-specific neutralizing antibodies reacted predominantly with the env glycoprotein, similar to that observed in other retroviral systems. And the gp120-specific neutralizing antibodies appeared to be type specific in goats immunized with ether recombinant or purified viral gp120, whereas group-specific neutralizing antibodies have been detected in human sera from HIV-infected individuals (Rosenberg, 1989). Matsushita and his colleagues showed that both human and animals generated type specific neutralizing antibodies to a region of gp 120 that encompasses amino acids 303-331, 307-330 and 296-331 (Matsushita et al., 1988). Neutralizing antibodies to this type-specific region did not inhibit gp120-CD4 binding but presumably interfered with HIV replication at a postreceptor binding stage (Skinner et al., 1988).

Since HIV can be transmitted by cell-to-cell fusion, the presence of antibodies able to block cell fusion is necessary for the control of the spreading of HIV *in vivo*. Several studies have shown that type-specific antibodies that inhibit cell fusion could be produced in animals immunized with glycosylated gp120 (Putney et al., 1986).

The rapid mutation occurred in the HIV genome during replication may result in the creation of new viral strains that can escape the neutralization. It was shown that the neutralizing antibodies in chimpanzees immunized with recombinant gp120 did not afford the protection from subsequent challenge with HIV. And similarly, chimpanzees passively immunized with HIV immunoglobulin became infected after inoculation with HIV (Rosenberg et al., 1989).

However, a recent study has shown that passive immunization of AIDS or ARC patients with pooled, high-titered plasma from healthy HIV-infected individuals resulted in the sustained elimination of p24 antigen in these patients (Karpas et al., 1988).

The further research in the role of neutralizing antibodies in the prevention of HIV infection and disease progression is necessary, particularly for the development of an effective vaccine against HIV.

3.2. Cell mediated cytotoxicity to HIV

Cytotoxic T lymphocytes may be necessary to eliminate cells already infected that serve as a reservoir for HIV. Walker and his co-workers as well as Tsubota and co-workers demonstrated that $CD8^+$ CTL cells could prevent outgrowth of HIV from infected cells in tissue culture (Walker et al., 1986; Tsubota et al., 1989). Like helper T cells, $CD8^+$ CTLs recognized processed protein antigens only in association with MHC molecules, certainly class I rather than class II (Shearer, 1977). A critical difference from $CD4^+$ helper T cells is that $CD8^+$ CTLs restrictedly recognized the endogenous peptide processed intracellular not exogenous peptide (Germain, 1986). Meanwhile, Townsend and co-workers demonstrated that short peptides containing the antigenic site could directly bind to class I MHC molecules and render target cells susceptible to lysis by CTLs without entering the endogenous processing pathway (Townsend et al., 1986).

MHC-resticted HIV-specific CTLs have been detected in the peripheral blood, lungs and cerebrospinal fluid of HIV infected

individuals (Sethi et al., 1988). Circulating CTLs were shown to be capable to kill env-, gag-, and pol-expressing target cells (Nixon et al., 1986). The study by Koenig and his co-workers found that cloned CTLs were able to lyse cells expressing HIV reverse transcriptase as well as Env proteins from highly divergent HIVs, suggested that the epitope on gp120 recognized by CTLs is conserved (Koenig et al., 1988).

Suppression of HIV replication by $CD8^+$ lymphocytes has been reported by Walker and his co-workers. They found that HIV reverse transcriptase activity substantially increased when $CD8^+$ cells were removed from cultured PBMCs of HIV-infected individuals. The addition of autologous $CD8^+$ cells suppressed both initial and ongoing virus replication in a dose-dependent manner (Walker et al., 1986).

4. Mechanisms of $CD4^+$ T cell killing

Although a direct relationship between HIV burden and $CD4^+$ T cells loss has been established (Schnittman et al., 1990; Simmonds et al., 1990; Genesca et al., 1990), the precise mechanisms by which HIV causes a decreasing number of $CD4^+$ T cells is not well understood. Several different mechanisms have been considered in an attempt to shed light on this important area of HIV pathogenesis (Rosenberg, 1989). Clearly, as HIV causes extensive cell death during *in vitro* infection of $CD4^+$ T cells HIV may destroy $CD4^+$ T cells *in vivo* as a direct result of infection and replication. It has been suggested that massive budding of HIV particles damages the integrity of outer cell membrane, leading to ionic imbalance and death. Another potential pathway of HIV-induced cytopathicity may

be similar to that observed in other retroviral systems, namely a positive relationship between the accumulation of cytoplasmic retroviral DNA and cell killing. High level of unintegrated viral DNA have been observed in the cytoplasm of HIV-infected cells and though to increase as a result of reinfection of the cells (Pauza et al., 1990). It has been shown that unintegrated DNA can serve as a template for the production of HIV antigens which may in turn, impair cell viability or functions. The high-affinity binding of gp 120 to CD4 has also been implicated in HIV-induced cell killing (Hoxie et al., 1986). In addition to gp120-CD4 binding that occurs on the cell surface during infection on the $CD4^+$ cell, the binding of these two proteins can also intracellularly occur and interfere with normal cell metabolism (Koga et al., 1990). The binding of gp120 to CD4 can also induce the formation of multinucleated giant cell or syncytia resulted from the fusion of HIV-infected cells with uninfected cells. A single HIV-infected $CD4^+$ T cell expressed enough gp120 on its cell surface ready to bind CD4 molecules on the surface of tens or perhaps hundreds of uninfected cells. It is conceivable that uninfected $CD4^+$ T cells can be eliminated at the same time of infected cell killing process. Several other mechanisms of indirect killing of uninfected $CD4^+$ T cells have been proposed. The destruction of bystander $CD4^+$ T cells by autoimmune phenomena is one such prospect. As the viral envelope proteins interact with the CD4 or MHC II on the surface of $CD4^+$ T cell, it is possible that antibodies directed against certain epitope of these viral protein may crossreact with the usual ligand for CD4 or MHC class II. In addition, uninfected $CD4^+$ cells may bind free gp120 molecules and become

targets for lysis by antibody-dependent-cellular cytotoxicity involving anti-gp 120 antibodies (Weinhold et al., 1989). Furthermore, $CD4^+$ T cells can effectively process soluble gp120, present the processed antigen to $CD4^+$ gp120 specific cytolytic clones, and be lysed by a CD4-dependent autocytolytic mechanism (Rosenberg, 1989). Moreover, $CD4^+$ T cells in patients with HIV infection may be induced cell death or apoptosis (Ameisen, 1991; Laurent et al., 1991). Apoptosis can be induced in $CD4^+$ T cell of HIV-infected patients by cross-linking CD4 molecules to one another and triggering the T-cell antigen receptor with a specific antigen or superantigen (staphylococcal enterotoxin B) (Groux et al., 1992).

5. Functional abnormalities of $CD4^+$ T cells and opportunistic infection

While long-term infection with HIV is characterized by a reduction in the absolute number of $CD4^+$ T cells, function abnormalities of viable and normal-appearing cells have been noted at all stages of HIV infection. Prior to the discovery of HIV, investigators observed that $CD4^+$ T cells from AIDS patients were defective in their ability to induce B cells to secrete immunoglobulin and to respond to alloantigens (Lane et al., 1983; Gupta et al., 1984). It was subsequently shown that lymphocytes of AIDS patients, while normally responding to mitogens, were defective in their ability to recognize and proliferate in response to soluble antigens (Lane et al., 1985; Shearer et al., 1986; Hofmann et al., 1989). Abnormal T cell response to soluble antigen were observed early in the course of HIV infection prior to a significant drop in $CD4^+$ T cell number (Lane et al.,

1985; Hoy, 1988). Margolick and his colleagues was demonstrated that viable $CD4^+$ T lymphocytes from AIDS patients had markedly reduced proportions of clonable cells compared to the HIV-negative donors (Margolick et al., 1985). Winkelstein and his collaborators showed that defective T cell colony formation and impaired expression of IL-2 receptors occurs throughout HIV disease with a more profound defect appearence at later stages of illness (Winkelstein et al., 1988). Other investigators described a range of immunological defects in HIV-infected individuals, including defective antigen-induced IL-2 production (Antonen, 1986), defective mitogen-induced IL-2 production (Prince,1987) and depressive HLA class I-restricted cytotoxic T lymphocyte (CTL) response to cytomegalovirus (CMV) and influenza (Shearer et al., 1986). This latter defect is thought to be primarily due to $CD4^+$ T cell/inducer dysfunction, since *in vitro* CTL activity against CMV can be restored by the addition of IL-2 (Rook et al., 1983).

Suppression of *in vitro* lymphocyte activation of normal T cells has been shown to occur upon exposure to sera from AIDS patients (Siegel et al., 1985) or supernatants of peripheral blood mononuclear cells (PBMCs) from AIDS patients (Laurence et al., 1983). The inhibitory activity of AIDS sera occurred even when adding several hours before or after phytohemagglutinin stimulation of normal T cells. The inhibitory factor(s) inhibited the production of IL-2 and caused a decline in IL-2 receptor expression (Siegel et al., 1985; Donnelly et al., 1987). The inhibitory factor (s) was not known. No study demonstrated which one's effect but Siegel showed that the inhibitory factors were not either interferon,

cortisol, immunoglobulin G or M, or immune complexes. (Siegel et al., 1985). And it has been shown that serum-inhibitory factor are also present in HIV-infected individuals at early stages of infection, and are found to increase in patients with low CD4 cell counts (Israel-Biet et al.,1988). However, the inhibitory factor in serum of HIV-infected patients may be p29, the inhibitory factor that derived from HIV-infected adherent cell or gp120 of HIV or Nef proteins. The preliminary report indicated that all of p29, gp120 and Nef protein could inhibit IL-2 production and decrease expression of IL-2 receptor, therefore the p29 could inhibit PHA-P induced normal T cell proliferation (Siegel et al.,1985; Ammar et al.,1992). Moreover, the immunosuppresive might be effect of polysaccharide (glucuronoxylomannan or mannan), the circulating antigens of *C. neoformans* or *C. albicans*. Since the study of Durandy demonstrated that T lymphocytes from normal subjects preincubated with excess mannan or glucuronoxylomannan, resulted in a strong suppressive effect of both T and B cell but activated the effect of T suppressor (Ts) cells (Durandy et al., 1987). These circulating antigens were frequently observed in AIDS patients infected with *C. neoforman* or *C. albicans* and disappeared following the appropriates effective therapy of ketoconazole (Drouhet, 1983). Furthermore, Gammon indicated that 411F, the metabolite of *P. glabrum* could inhibit antigen-induced CD4-dependent T cell proliferative response of PBMC (Gammon et al.,1994) Therefore, *P. marneffei* antigen (mannan) and its metabolite may be an immunosuppressor of cellular immunity as has been shown for the antigens *of C. neoformans, C. albicans* and the metabolite of *P. glabrum.*

The finding that T-cell proliferation and IL-2 production decline in HIV-infected individuals, while B-cell activity increases, lead to the speculation that the switching of Th1 to Th2 cytokine phenotype might be important in the pathogenesis of the disease progression in HIV infection (Clerici, 1993). This hypothesis was based on two distinct observation. Firstly, Clerici and his colleagues found that individuals exposed to HIV who were tested negative for the virus and did not seroconvert, showed evidence of HIV-specific cell-mediated immunity as measured by IL-2 production of PBMC in response to HIV envelope peptides (Clerici et al., 1992). Secondly, in other series of experiments, Clerici and his collaborators demonstrated that 50% of asymtomatic, HIV-infected patients showed a gradual shift from a predominance of Th1 to Th1 type responses in the course of HIV infection. Loss of initially good IL-2 responses to soluble antigens or HIV peptides was often accompanied by increased mitogen-induced IL-4 production (Clerici et al., 1993). However, these findings were not fully convincing. The different experimental approaches have been pursued. Graziosi and his collaborators found that a similar pattern of cytokine expression wasobserved after PHA stimulation *in vitro* of purified $CD4^+$ T cell populations obtained from HIV-infected individuals at different stages of dissease. It was indicated that the switching of Th1 to Th2 cytokine phenotype did not occur during the progression of HIV disease (Graziosi et al., 1994). This finding was similar to what obtained by another group (Maggi et al., 1994; Romagnani, 1994). Estaquir and his colleagues reported that the loss of $CD4^+$ Th1 cell function in HIV-infected persons

were not related to Th1 to the Th2 cytokine switching but to the process of activation induced death of $CD4^+$ Th1 cells (Estaquier et al., 1995). However, it is not easy to demonstrate the switching from a Th1 to Th2 state, since Th1 and Th2 markers are currently not available. Thus, the mechanisms of Th1 to Th2 cytokine switching are still unclear. One explanation is, it reflects the altered balance of cytokines produced by HIV-infected macrophages. Cytokines released by macrophages play a critical role in determining the differentiation of T cells into distinct functional phenotypes at the time of antigen presentation (Hsieh et al., 1993). It is worthnothing that the production of IL-12 and IFN-α (both Th1-inducing agents) is defective in HIV-infected patients (Maggi et al., 1994) whereas the production of IL-1, IL-6, IL-10,TNF-α and GM-CSF is normal or increased (Poli, 1992). Therefore, the combined IL-12 and IFN-α defective production by HIV-infected macrophages may favor the enhanced expression of Th2-type cytokines even in response to antigens usually evoking Th1 responses.

From the explanation described above of the depletion of both number and function of $CD4^+$ T cells. This was belive to increase the risk of an opportunistic infection and malignancies. The most common opportunistic pathogens causing disease include *Pneumocystis carinii*, *M. avium*, *M. tuberculosis*, Cytomegalovirus, Herpes simplex virus, Papova virus, *T. gondii*, *Candida spp.*, *Cryptosporidium spp.*, *C. neoformans*, *H. capsulatum* and *P. marneffei*. Vithyasai indicated that 174 AIDS patients were infected with *M. tuberculosis* (35.06%), *S. cholerasuis* (6.32%), *C. meningitis* (22.99%), *C. albicans* (71.26%), *H. capsultum* (1.72%), Herpes

simplex virus (2.30%), *P. carinii* (8.05%), and *P. marneffei* (20.11%). The most common cancer was found in 174 AIDS patients include Kaposi's sarcoma (2.87%), non Hodgkin' s lymphoma (1.13%), and, ALL (0.57%) (Vithayasai, 1992). The opportunistic infection in AIDS patients are caused by the low virulence organisms and commonly found in environment. For example, *P. marneffei*, it is an important emerging mycosis, especially in AIDS patients, and is considered as an AIDS defining illness in the endemic areas for this pathogenic fungus in Southeast Asia and China. The reason that Thailand is an endemic area for penicilliosis marneffei, thus it is the second common systemic fungal infection manifested in AIDS patients after cryptococcosis (Imwidthaya, 1994). Humans as well as bamboo rats are probably infected this fungus from a common environmental source (Deng et al., 1986).