II. LITERATURE REVIEWS

A. History

Penicillium marneffei is the only thermal dimorphic pathogenic *Penicillium* among several hundred species of *Penicillium*. The initial isolate was obtained in 1956 by Capponi and his colleagues from the captive bamboo rat (*Rhizomys sinensis*) from the highlands of central Vietnam (Capponi et al, 1956). These rats has been maintained for experimentally inoculation with *Rickettsia orientalis* at the Pasteur Institute of Indochina at Dalat, South Vietnam. They first observed the spontaneous death of three bamboo rats due to a reticuloendothelial mycosis. At autopsy, a slightly hypertrophic spleen, ascites and epiploic nodules showed fungus cells in their macrophages. The fungus isolated from a rat was used to infect a laboratory mouse, and then both fungus and animal were sent by airplane to the Pasteur Institute in Paris to be studied. The fungus was identified by Segretain as a new species, *Penicillium marneffei* 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. The typical feature of this pathogenic fungus is the thermal dimorphism; unicellular oval or elongated cells which multiply by fission in vivo in histiocytes and macrophages or in vitro at 37°C. At 25°C, they are present as mycelia with a characteristic diffusing red pigment.

Segretain pricked his finger accidentally during experimental studies, with a needle used to inoculate hamster. He developed a small nodule at the site of inoculation 9 days later, followed lymphangitis and axillary lymphnode hyperthrophy (Segretain, 1959). Antifungal sensitivity studied at the time demonstrated a high in vitro sensitivity of this fungus to nystatin. An intensive treatment with oral nystatin (20 million units, 13 times the usual daily dose) for 30 days cleared the infection. This accidental infection emphasized the possibility of human pathogenicity.

There have been no additional reports 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 traveled in Southeast Asia (DiSalvo et al, 1973). In 1984, Pautler and his colleagues also reported the *P. marneffei* infection in a 59-year- old man who had traveled extensively in Far East and currently 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 infections of *P. marneffei* were reported as the imported disease in the USA because of the history of their history records of previous travelling in Southeast Asia where *P. marneffei* is endemic. Ever since, numerous cases of penicilliosis marneffei have been reported in many countries among healthy and immunocompromised patients, especially AIDS patients.

B. Epidemiology

The first isolate of *P. marneffei* was recovered in 1956 from a captive Chinese bamboo rat (*Rhizomys sinensis*) in Vietnam (Capponi et al, 1956) that had been experimentally inoculated with scrub typhus bacterium- *Rickettsia orientalis* now designated as synonym of *R. tsutsugamushi.* The organism was isolated from liver, spleen and ascites fluid of the bamboo rat. Thirty years later in 1986 Deng et al investigated bamboo rats in Southern China, Guangxi Zhuang, to determine their relationship to *P. marneffei.* The result was shown that bamboo rats, *R. sinensis*, is less frequent than *R. pruinosus* (Deng et al,1986). The organism was isolated from one or more of their internal organs; liver, lung, mesenteric lymph nodes, pancreas and spleen. Thereafter, *P. marneffei* was also isolated from soil from three burrows of bamboo rat, *R. pruinosus*, in 1988 by Deng et al (1988). No such study was performed in Thailand, until recently two separate studies found that another member of the family *Rhizomydae*, *Cannomys badius*, could be identified in the central part and the northern provinces of Thailand. In addition, a new animal reservoir of *P. marneffei*, *Rhizomys sumatrensis*, was reported in Chiang Mai province in the northern Thailand. The fungi were isolated from rat's internal organs about 75% of *R. pruinosus* and 19.4% of *C. badius* , captured in the central part of Thailand (Ajello et al,1995), and 92.8% of *R.sumatrensis*, and 30% of *C. badius* (reddish brown in color), captured in the northern province of Thailand (Chariyalertsak et al,1996). In addition,

one soil sample from the burrow of a *R. sumatrensis* bamboo rat was positive for *P. marneffei* (Chariyalertsak et al,1996). Therefore, they suggested that these animals may be important animal hosts of *P. marneffei* in northern Thailand and contaminated soil may be a common environmental source of infection for bamboo rats and people via respiratory route.

The first report of *P. marneffei* infection was an accidentally laboratory infection (Segretain, 1959). Subsequent publications have reported naturally acquired infection in both healthy and immunocompromised persons who have resides in or have visited the endemic area in Southeast Asia, China and Indonesia. 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 described in Bangkok, Thailand, by Jayanetra et al (1984). The patients came from various parts of the country. In the same year the other 2 cases were reported (Tanphaichitra et al, 1984). Additional 5 cases were reported from Maharaj Nakorn Chiang Mai Hospital, in the northern Thailand, during the year 1987-1989 (Supparatpinyo et al,1992). The first eight Chinese cases of *P. marneffei* from Guangxi Zhuang Autonomous Region, the southern China, were described in 1985 by Deng and Connor (1985). These patients had no predisposing illness or evidence of altered immunity and most of them originally misdiagnosed as histoplasmosis. Moreover, 3 cases with disseminated penicilliosis marneffei that are all natives of Guangxi Zhuang Autonomous Region were reported by Li et al (1991). In the same year, a few cases were also reported from Hong Kong, which belongs geographically to southern continental China situated at the latitude as Guangxi region (Yuen et al, 1986; Tsang et al, 1988; Chan et al, 1989; Chan and Woo,1990;). In recent report, there were three patients in Taiwan who did not have a history of travel to areas of endemically have been identified with disseminated penicilliosis marneffei at National Taiwan University Hospital between January 1987 and December 1996 (Hung et al,1998).

Since 1988, the occurrence of penicilliosis marneffei increased substantially and numerous cases of systemic penicilliosis due to *P. marneffei* were reported among AIDS patients who travelling in Southeast Asia, Hong Kong and/or southern China, including patients from France (Hilmarsdottir et al, 1991, 1993), Italy (Viviani et al,1993), Netherlands (Hulshof et al,1990), USA (DiSalvo et al,1973; Pautler et al,1984), UK (Piehl et al, 1988), Sweden (Julander and Petrini, 1997), Canada (Sekhon et al,1994)

and Australia (Jones et al,1992; Heath et al,1995). The first native case of *P. marneffei*–infected AIDS patient was reported in 1989 from Bangkok, Thailand (Sathapatayavongs et al, 1989). During the years 1987-1992, 86 adults (Supparatpinyo et al, 1994) and 5 children (Sirisanthana et al, 1993) of AIDS patients were diagnosed from Chiang Mai, Thailand. Therefore, it can be concluded that the northern region of Thailand appears to be the most important endemic area of *P. marneffei* and the disease is generally associated with HIV infection. From 1991 to 1994, approximately 550 AIDS patients infected with *P. marneffei* were diagnosed at Chiang Mai University Hospital (Chariyalertsak et al, 1996 a). By 1995, the number of *P. marneffei* infection in Chiang Mai had more than doubled, to over1,300 cases (Phillips, 1996). Penicilliosis has become a very common infection in the endemic area.

In the northern Thailand *P. marneffei* infection is the third most common opportunistic infection, after tuberculosis and cryptococcosis, and occurs in up to one third of HIV-infected patients (Supparatpinyo et al, 1994). The exact route of *P. marneffei* infection in humans is not known, although it is assumed that like other endemic mycoses it is acquired by inhalation of the conidia (Sirisanthana, 1996 a). 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). One interesting study, performing between 1991 and 1994 at Chiang Mai University Hospital, found that there was a seasonal association of human infection with *P. marneffei*. Penicilliosis was more frequent in the rainy season than in the dry season; this was in contrast to cryptococcosis, which occurred at the same frequency year round (Chariya-lertsak et al, 1996 a). In addition, patients with a history of occupational or other exposure to soil, especially during the rainy season, were more likely to present with *P. marneffei* infection (Chariyalertsak et al, 1997). However, the significance of this observation is unclear. It has been noted that bamboo rats breed during the rainy season, this may be coincidental and the season association may reflect disturbances in the natural niche of the fungus.

C. Mycology and cultural aspects

1. Mycelial phase

P. marneffei grows as mold on Sabouraud's dextrose agar (SDA) at 25°C -30°C and young colonies became visible within 3 days. They are generally thin, flat,

powdery to velvety and yellowish green to bluish gray in color in the center. As the colony matures, it becomes red to reddish brown and produces a pink or rose-red pigment that diffuses into the medium. The eight-day-old colony is about 37 mm in diameter. The colonial pleomorphism can occur rapidly by subculturing on SDA. It's characteristic feature can, however, be induced by subculturing on malt extract (ME) agar or modified ME (Vanittanakom et al, 1993). This fungus can grow well on wort agar and potato dextrose agar (PDA), but slowly on Littmann Oxgall agar and Czapek dox agar.

Microscopically, *P. marneffei* shows septate branched hyphae with lateral and terminal conidiophores which are smooth walled, sometimes multiple septate. Penicilli are monoverticillate or/and biverticillate, either symmetrical or asymmetrical (DiSalvo et al, 1973; Pracharktam et al,1992; Vanittanakom et al,1993). The conidiophores consist of basal stipe bearing terminal verticils of three to five metulae. Some metulae bear 3-16 phialides that produce long basipetal unbranched chains of ellipsoidal, smooth-walled conidia, however, single phialide can be found (Vanittanakom et al, 1993). The conidia are approximately 2x3 µm in size and join in chains by prominent disjunctors. In biochemical characteristics, *P. marneffei* can assimilate glucose, lactose, xylose, maltose, levulose and mannitol (Segretain, 1959).

2. Dimorphism and yeast-like phase

Within 2 or 3 days of incubation at 37°C on brain heart infusion agar, the fungus grows as a yeast, forming white-to-tan, soft colonies. Conidia of *P. marneffei* undergo a phase transition whereby the predominant morphology is a single celled, yeastlike entity that divides by fission. Within 6 to 12 h of inocubation at 37°C, individual conidia swell and begin a short period of isotropic growth. Subsequently, one or two loci at the surface of the conidium begin to bulge. These continue to develop apical, resulting in a septate hyphal element. These septa possess intracellular pores and Woronin bodies characteristic of fungi belonging to the Ascomycota (Popov V and Cooper CR, unpublished data, 1997). However, hyphal growth is determinant, and segments of varying length are formed. As the hyphae age, they appear to fragment along septal planes. This generates single cell

that tend to continue to reproduce by fission. As the culture ages, more and more of these cells are formed. Notably, the production of the red pigment characteristic of the *P.marneffei* mold form is absent or greatly reduced during yeastlike growth (Cooper and McGinnis, 1997). 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

The initial host response to *P. marneffei* is generally histiocytic in nature. Histiocytes contain a few or many globose-to-oval yeastlike cells of *P. marneffei* that measure 2-3 x 2-7 μm. As the lesions progress, the intracellular fungal cells are released following cellular necrosis and subsequent abscess formation. In histiologic material, neither the cell wall nor the cytoplasm of the *P. marneffei* cells stained well with hematoxylin-eosin stain. This can result in the false impression that a capsule is present. These cells, especially when found in histiocytes, resemble those of *H. capsulatum* var *capsulatum* and *H. capsulatum* var *farciminosum.* When found extracellularly, however, *P. marnefffei* cells are generally much larger than those of *H .capsulatum*. Many of the extracellular *P. marneffei* cells are elongate, occasionally curved, and measure 8 to 13 μm in length. Rarely, short hyphae no longer than 20 μm are observed. Because *P. marneffei* reproduces by schizogony (fission), the dividing cells of *P. marneffei* characteristically contain a single, centrally located, transverse septum, although cells with two septa can be observed. In contrast, the yeast cells of *H. capsulatum* var *capsulatum* and *H. capsulatum* var *farciminosum* are smaller in size (2-4x 3-4 μm) and divide by budding. *H. capsulatum* var *duboisii* can be readily distinguished from these fungi by its larger size (8-15 μm) (Chandler and Watts, 1987).

D. Clinical manifestations

Penicilliosis marneffei is usually disseminated and progressive mycosis in both normal and immunocompromised hosts. The exact route of infection is not known, but assumed that it is acquired by inhalation of the spores. The organism proliferates in macrophages and is disseminated throughout the body, especially to the

reticuloendothelial system. Consequently, liver, lymph nodes, bone marrow, and spleen are commonly involved (Kudeken et al, 1996; Cui et al, 1997). The clinical manifestations of symptoms penicilliosis marneffei varying with degree of severity are chronic productive cough, pulmonary infiltrates, generalized lymphadenopathy, septicemia, hepatosplenomegaly, anemia, leukocytosis, intermittent fever (38° C to 39°C) with or without chill, weight loss, osteoarticular lesions, pericarditis, and multiple subcutaneous abcesses and papulelike ulcers (Drouhet, 1993; Drouhet and Dupont, 1995; Duong, 1996; Sirisanthana, 1996b; Wortman, 1996; Li et al, 1992). Although most of these symptoms are common to both HIV-positive and HIV-negative patients, their acute onset and intensity are notable in AIDS patients. Moreover, these signs and symptoms can mimic those of other diseases. In particular, the skin lesions can be confused with tuberculosis, molluscum contagiosum, cryptococcosis, and histoplasmosis. A number of *P. marneffei* cases reported between 1965 and 1983 were misdiagnosed as acute histoplasmosis (Deng et al, 1986; Deng and Connor, 1985; Jayanetra et al, 1984; Li et al, 1991).

Skin lesions occur in approximately 68% of patients. They are often the first indication of penicilliosis marneffei disseminated infection, as well as an HIV infection. The cutaneous lesions frequently occur on the face, upper trunk, and extremities. The lesions may consist of papules, a generalized papular rash, necrotic papules, or nodules. Some patients have papules with central necrotic umbilication. The molluscum contagiosum-like papulonecrotic lesions caused by *P.marneffei* and *H.capsulatum* cannot be clinically differentiated from each other.

P. marneffei is a primary pulmonary pathogen that disseminates by hematogenous means. This accounts for the 55% of HIV patients whose septicemia has been documented by blood culture (Drouhet, 1993). At autopsy, the most frequent sites involved included lymph nodes, liver, lung, kidney, bone, and bone marrow. Culture of bone marrow, blood, skin, and respiratory specimens are the most useful in making a laboratory diagnosis.

E. Laboratory diagnosis

The most reliable means of diagnosis in HIV patients infected with *P. marneffei* is the microscopic observation of fungus in biopsy specimens, such as skin or bone marrow, or by culturing *P. marneffei* from skin, blood, or respiratory

specimens , such as sputum and bronchoalveolar lavage. In non HIV-infected patients, the most valuable clinical materials for either examination or culture include skin, lung, liver, lymph nodes, and bone-joint specimens. Microscopically, the cells of *P. marneffei* are frequently seen in bone marrow aspirates, bronchoalveolar lavage, and skin scrapings. In tissue, *P. marneffei* are seen as intracellular yeast cells of 3 μm in diameter that divide by fission. Occasionally, elongated sausage shaped cells, up to 8 μm long, with septa may be present (Supparatpinyo, 1994). The cell is well stained with Wright's stain, Giemsa's stain, H&E, PAS and GMS (Vithayasai,1994). Histopathologic examination of the samples are usually stained with H&E, GMS, PAS, and Ziehl-Neelsen stain.

Routine mycologic media, Sabouraud's dextrose agar (SDA) is adequate for the isolation of this fungus. Bone marrow, blood, and skin scrapings provide the highest recovery of *P. marneffei.* 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 (Drouhet, 1993). Differentiation of *P. marneffei* from other species of *Penicillium*, including those that also produce a diffusible reddish-brown pigment when grown on a medium like Sabouraud dextrose agar, must be made carefully. Moreover, conversion of the mold form to the yeastlike form may not be appreciated well enough to reach a conclusive identification. For example, strains of *Paecilomyces lilacinus* and *Paecilomyces viridis*, which morphologically resemble *Penicillium*, can cause infections in animals and compromised hosts (Drouhet,1993; Gordon,1984; Kwon-Chung and Bennett,1992). Like *P. marneffei*, the tissue phase of these strains can occur as yeastlike cells; but in contrast, they produce blastoconidia. The mold-to-yeast conversion of *Paecilomyces lilacinus* can be produced by culturing the fungus at 35°C to 37°C on enriched medium.

F. Antifungal susceptibility and therapy

The in vitro susceptibility of clinical *P. marneffei* isolates to various antifungal agents has often been included in various reports. In general, the results indicate that isolates of *P. marneffei* are generally susceptible to the principal agents used to treat deep-seated mycoses (Drouhet, 1995; Wortman, 1996). However, in separate

investigations by Sekhon et al (1992, 1993), some isolates were found to be resistant to one or more antifungal agents. These investigators showed that the yeast form of *P. marneffei* is more sensitive to fluconazole and itraconazole than the mold phase. Conversely, the mold phase was found to be more sensitive to amphotericin B and 5-fluorocytosine than the yeast form. More recently, 25 isolates of *P. marneffei* were evaluated against the new triazole antifungal agent voriconazole. The minimal inhibitory concentration ranged from $\leq$0.03 to 0.06 μg/ml (geometric mean $\leq$0.03 μg/ml), suggesting that this antifungal agent may also be important in managing penicilliosis marneffei. Only limited studies have been performed to correlate in vitro susceptibility and outcome of infection. In one investigation, guinea pigs infected with *P. marneffei* were successfully treated with low dosages of itraconazole (Cutsem et al,1991; Cutsem, 1991). In another study, the correlation of in vitro susceptibility and outcome of infection in adult HIV-infected patients was examined (Supparatpinyo et al, 1994; Supparatpinyo et al, 1993). The mean minimal inhibitory concentration of at least 28 isolates from 86 patients indicated that *P. marneffei* was highly susceptible to itracona-zole, ketoconazole, miconazole, and 5-fluorocytosine; moderately susceptible to amphotericin B, and generally resistant to fluconazole. These in vitro data paralleled the clinical outcome of patients. The frequency of treatment failure or relapse was greater with fluconazole treatment, but significantly less than that of patients receiving either amphotericin B or itraconazole. In contrast, pediatric HIV-patients appeared to respond better to treatment than adult ones (Sirisanthana, 1995). For the clinical and microbiological response, itraconazole or ketoconazole should be considered to be the drug of choice in the treatment of mild to moderately severe *P. marneffei*-infection. For patients who seriously ill, therapy could be started with itraconazole or ketoconazole until cultures were negative and clinical findings had resolved. However, several patients relapsed by *P. marneffei* after the cessation of the initial therapy, maintenance oral therapy with itraconazole or ketoconazole may be necessary (Supparatpinyo et al, 1993). Nevertheless, it is clear from all studies and case reports that the most effective treatment of penicilliosis marneffei includes initial therapy with amphotericin B followed by a prophylactic azole maintenance regimen. The latter usually involves the

use of itraconazole, although reports have indicated that fluoconazole and ketoconazole are efficacious (Kok et al, 1994; Peto et al, 1988).

In vitro susceptibility testing is appropriate when valuating the cause of treatment failure in which the fungus may have developed resistance or cross resistance to azole during therapy. Because *P. marneffei* is extremely sensitive to several triazole antifungal agents, routine in vitro testing is not necessary.

G. Antigen and immunity

Van Cutsem and collaborators (1990) detected a circulating proteins antigen of *P .marneffei* which contain galactomannan 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. The monoclonal antibody, EB-A1 specific for *A. fumigatus* galactomannan was used to detect yeast-hyphae or remnants of filaments of *P .marneffei* and hyphae of *Aspergillus* species in tissue biopsy of infected-patients by immunohistochemical method (Pierard et al,1991; Estrada et al,1992). The monoclonal antibody, 7D7, raised against *Pneumocystis carinii*, has cross reactivity with antigen of *Apergillus* species, *Candida albicans*, *Histoplasma capsulatum*, and *Penicillium* species except *P. marneffei* by immunofluorescent technique (Lundgren et al, 1992).

Sekhon and collaborators (1982) prepared the crude mold form antigens of *P. marneffei* (DiSalvo strain, and Indochina strain of human and bamboo rat origin) from six week-old culture filtrate defined as an exoantigens. The antigens were used in serological test of *P. marneffei* infection by microimmunodiffusion (ID) technique. The antigen did not react neither with rabbit antisera raised against five species of *Aspergillus* or with four dimorphic systemic fungi (*B. dermatitidis*, *C. immitis*, *S. schenckii* and *Micropolyspora faeni*). In addition, rabbit anti *P. marneffei* antiserum did not react with those fungal antigens but react with histoplasmin, blastomycin and coccidioidin in the complement fixation test (titer 1:32 to 1:64) (Sekhon et al, 1982). An exoantigen test was applied to detect the anti-*P. marneffei* antibody in sera from the patients 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 following therapy were negative (Viviani et al,1993). Kaufman et al (1996) used immunodiffusion and latex agglutination test to diagnose antigenemia during *P. marneffei* infection in

10 and 13 of 17 patients, respectively. These highly specific tests did not cross-react with antigen from 6 serum samples containing cryptococcal antigen or 6 urine specimens positive for *Histoplasma* polysaccharide antigens. Recently, Imwidthaya et al (1997) also detected both antibodies and antigens in serum specimens of their AIDS patients with penicilliosis by microimmunodiffusion (MID) test. It was found that 2 of 8 sera positive for antibodies and 7 of 8 sera positive for antigens against *P. marneffei.*

Yuen and his colleagues 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 IgG antibody titer 1:160 or more while the other patients without penicilliosis marneffei (tuberculosis, typhoid fever, melioidosis, disseminated cryptococcosis, candidiasis, intra - abdominal sepsis, autoimmune disease, and lymphoreticular cancer) and 15% of 78 healthy controls had an IgG antibody titer 1:10–1:40 (Yuen et al, 1994). Thereafter, IFAT for detection of *P. marneffei* infection was also studied by Kaufman et al (Kaufman et al, 1995). The result of study was similar to that both types of antiglobulins, raised to whole yeast cells and yeast culture filtrate antigens, reacted with yeast cells of *P. marneffei* and *H. capsulatum*, but not with their respective mycelial forms including other fungi. This result correlated with the other experiment conducting by Wheat et al (1997). They identified fungal antigens causing endemic mycoses in patient urine samples with disseminated fungal infection by using antibodies raised to *H. capsulatum* var. *capsulatum* antigen. This experiment was carried out with solid-phase enzyme immunoassay that biotinylated antibody to *H. capsulatum* var. *capsulatum* was used in place of radiolabeled antibody. The result revealed cross-reaction in 17 of 18 patients with *P. marneffei* infection, 12 of 19 with blastomycosis, 8 of 9 with paracoccidioidomycosis, and in one with disseminated *H. capsulatum* var. *duboisii* infection. Cross- reactions were not observed in the assay for six patients with disseminated coccidioidomycosis.

Vanittanakom et al (1997), studied the crude extracellular proteins of *P. marneffei* secreted during growth of yeast form by immunoblot assay. It was found that the IgG antibody in 31 out of 33 sera from penicilliosis marneffei patients with AIDS recognized one or more of the four major proteins in sizes of 200, 88, 54, 50 kDa . About half of them had strong reactivities to the 88, 54, and 50 kDa protein

antigens, whereas, 4 out of 38 sera from non *P. marneffei* infected patients and none from 80 healthy individual had strong reaction to these proteins. In contrast with the other study appeared that specific antigen of *P. marneffei* which was produced and secreted in culture medium for 6 weeks was a 38 kDa antigen. This component could not be detected in antigenic extracts of *H. capsulatum*, *C. neoformans*, *A. niger*, *A. fumigatus*, *A. flavus*, *A. terreus*, *C. albicans*, and two species of *Penicillium* by immunoblot analysis against the sera from patients with culture–confirmed penicilliosis marneffei, however, it also could be seen in other patient's sera; cryptococcosis, and candidiasis (Chongtrakool et al,1997). A study of immunogenic proteins in cytoplasmic yeast antigens of *P. marneffei* was conducted to identify and purify specific antigens, and then analysed their recognition by human immune sera (Jeavons et al,1998). The procedure was carried out with liquid isoelectric focusing, and the fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. There were three antigens; 61 kDa, 54 kDa, and 50 kDa, which were specifically recognized by IgG antibodies in pooled sera from *P. marneffei*-infected patients (n=21), whereas negative reaction was observed with pooled sera from patients with aspergillosis (n=20), candidiasis (n=10), cryptococcosis (n=9), and histoplasmosis (n=11). When these antigens were tested with individual penicilliosis sera (n=21), it was found that protein antigens of 61 kDa, 54 kDa, and 50 kDa were recognized from sera about 86%, 71%, and 48%, respectively. In total, 18 of 21 (86%) of all serum samples recognized at least one of these antigens. N-terminal amino acid sequence analysis of 61 kDa antigen revealed that it had a strong homology (87% identity) with the antioxidant enzyme catalase.

In current study, *MP1* gene, which encodes an abundant antigenic cell wall mannoprotein from *P. marneffei* was cloned by Cao and his colleagues (1998a). The MP1 was a unique gene without homologue in sequence databases, encoding for a protein, Mp1p, of 462 amino acid residues and contained two putative N-glycosylation sites, a serine- and threonine - rich region for O-glycosylation, a signal peptide, and a putative glycosylphosphatidylinositol attachment signal sequence. Western blotting analysis of the recombinant Mp1p protein and anti-Mp1p antibody revealed that Mp1p has predominant bands with molecular mass of 58 and 90 kDa. This protein belonged to a group of cell wall proteins that could be readily removed from yeast cell surface by glucanases digestion. In addition, Mp1p was an abundant glycoprotein and had

highly affinity for concanavalin A, a characteristic indicative of mannoprotein. Furthermore, ultrastructural analysis with immunogold staining indicated that Mp1p is present in the cell walls of the yeast, hyphae, and conidia of *P. marneffei* (Cao et al, 1998 a). Finally, it was used to detect antigen and antibody in individual serum from penicilliosis patients by ELISA method. The results revealed that 17 (65%) of 26 penicilliosis patients were positive for Mp1p antigen detection, whereas 6 out of the 9 patients that antigen test negative were Mp1p antibody test positive and non of 85 control sera was positive in either test. Furthermore, the protein, Mp1p, did not react with antibodies raised against other pathogenic fungi (*Histoplasma, Blastomyces, Candida,* and *Aspergillus*) and with lysate as well as culture supernatant antigens of *C. albicans, H. capsulatum*, and *C. neoformans* on Western blotting and ELISA. It can be indicated that this protein was highly specific for host humoral immunity representing a good cell surface target of *P. marneffei* (Cao et al,1998 b; Cao et al, in press).

Chan and Chow studied host response to *P. marneffei* infection by electron microscope in two patients, one was immunocompetent and the other an immunosuppressed renal graft recipient. All yeast cells were phagosytosed by macrophages of the first patient, whereas, in the second one, the effect of corticosteroids might account for the large number of nonphagocytosed fungi in tissue space (Chan and Chow,1990). Disseminated penicilliosis marneffei may be associated with alteration of T cell-mediated immunity as has been shown in disseminated histoplasmosis (Lehmann et al,1983). Kudeken and his colleagues (1996) demonstrated that the cell-mediated immunity played a central role in a host defence mechanism against *P. marneffei* infection in euthymic and athymic mice model. It was found that microorganisms inoculated intratracheally multiplied progressively in the lungs and disseminated to the liver and spleen. In euthymic mice, the organisms decreased gradually in these organs, but congenitally athymic mice developed severe pulmonary and disseminated systemic mycosis. However, when activated nylon wool non-adherent spleen cells were transferred into athymic mice, they can be significantly reduced the number of yeast in the internal organs of tested mice.