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MAJ. SOMPONG TREWATCHAREGON: PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST *PENICILLIUM MARNEFFEI* ANTIGENS AND MOLECULAR EPIDEMIOLOGY OF *PENICILLIUM MARNEFFEI* INFECTION IN THAILAND. THESIS ADVISORS: STITAYA SIRISINHA, Ph.D.; SANSANEE C. CHAIYAROJ, Ph.D.; CHUENCHIT BOONCHIRD, Ph.D.; ANGKANA CHAIPRASERT, Dr.rer.nat. 167 p. ISBN 974-664-591-9

Penicilliosis marneffei, a disease caused by dimorphic fungus *Penicillium marneffei*, is currently the third most prevalent opportunistic infection in AIDS patients in northern Thailand. Incorrect diagnosis and delayed treatment contribute to its relatively high mortality rate. Clinical manifestations of penicilliosis marneffei closely resemble tuberculosis, histoplasmosis, cryptococcosis and other systemic fungal infections, therefore, specific and reliable diagnosis methods are needed.

Four MAbs specific for *P. marneffei*, 3C2, 8C3, 8B11 and 3B9, were produced from hybridomas raised from BALB/c mice immunized with crude culture filtrate (CCF) prepared from mycelial phase of growth. The hybridomas were screened and characterized using different fungal antigens by enzyme-linked immunosorbent assay (ELISA), immunoblotting and immunofluorescent staining. In the immunoblots, MAb 3C2 (IgG1 subclass) reacted specifically with a denatured form of 38-kDa antigen whereas MAbs 8B11 and 3B9 (IgM subclass) reacted most strongly with high molecular weight components (>200 kDa) produced during either mycelial or yeast phase of growth. The immunoreactive epitopes for these MAbs were most likely associated with carbohydrate moieties, judging from their susceptibility to periodate treatment and concanavalin A binding. This is in contrast to the immunoreactive epitopes for MAbs 8C3 (IgM subclass) and 3C2 which were resistant to periodate treatment. In immunofluorescent staining, the three IgM MAbs could react strongly with both mycelial and yeast phase of *P. marneffei*, but not with the yeast phase of *Histoplasma capsulatum* and *Cryptococcus neoformans* whose morphology are closely similar to *P. marneffei*. Thus, these MAbs showed diagnostic potential. They could be used to identify *P. marneffei* in culture and biopsy specimens by immunofluorescent staining.

For genomic epidemiology study of 67 *P. marneffei* isolates using PFGE, 2 macrorestriction patterns (MPs) and 9 MP subgroups were generated by *Not* I digestion of 67 *P. marneffei* isolates. Of the 64 human isolates, 42 isolates (65.6%) were of MPI and belonged to subgroups MPIa (8 isolates), MPIb (11 isolates), MPIc (10 isolates), MPId (3 isolates), MPIe (5 isolates) and MPIf (3 isolates). Whereas 22 isolates (34.4%) were of MPII and belonged to subgroups MPIIa (6 isolates), MPIIb (4 isolates) and MPIIc (7 isolates). Two bamboo rat isolates belonged to subgroup MPIa and one isolate was of subgroup MPIc. No significant correlation between the MP of *P. marneffei* isolates and geographical region nor specimen sources was observed. Notably, isolates obtained before 1995 were of MPI and we have seen increased incidence of infection with MPII isolates since then. However, further studies are necessary to confirm this finding.