CHAPTER 6

DISCUSSION

Candida albicans is found as a normal commensal of the oropharyngeal and as a pathogen in this site and in the vagina, urinary tract, and skin. In addition, organ and deep tissue infections, although less common, are serious and may lead to septicaemia, which is frequently in fatal. The difficulty of diagnosis is that isolation of the organism only rarely indicates infection while biopsy to demonstrate tissue invasion can be justified only the certain circumstances. For the serological tests, several laboratories are currently investigating methods to detect circulating *C. albicans* antigens or metabolites such as cell wall mannan (148, 149), mannose (150), arabinitol (151, 152), extracellular proteinases (153), and cytoplasmic proteins (154). It is not yet clear whether the detection of these particular cellular components will significantly improve serodiagnosis. Agglutination (155), fluorescent antibody (156), crossed immunoelectrophoresis (157, 158), and radioimmuno asaay (159) tests have been used for this purpose, although the most widely used procedures are probably the double gel diffusion precipitin (Ouchterlony) and immunoelectrophoresis test. The numerous approaches for the serological diagnosis of candidiasis have concentrated on the detection of *C. albicans* derived molecules. These molecules were detected either on the basis of their antigenicity or through biochemical – enzymatic procedures. Many serologic studies used whole phosphomannan complexes as antigenic preparations and did not identify individual epitopes recognized by different antibodies. (24)

All HOC and the systemic candidiasis patients were confirmed for the criteria (in materials and methods) since *Candida* was isolated from all the oral swabs and hemocultures. In systemic candidiasis patients, three patients were positive for *Candida tropicalis,* and two patients were positive for *Candida albicans* whereas all member of HOC were positive for *Candida albicans.* In this study, the semiquantitative of the antibody or the titer of immunoglobulin G

Compare to the molecular standard sizes, the twelve components were 52, 50.5, 46.5, 45.5, 43.5, 42, 38, 36.5, 32.5, 29, 25, and 22.5 kilodaltons. All the components were transferred to immobilon membrane and the size of components was confirmed as explained in the Chapter 3 and Chapter 4 (Figure 16 and 17). After all the components were reacted with each serum, eight in thirty healthy individuals' sera concealed any bands. The ELISA titers of these eight cases were less than 1:100 whereas the other cases whose the titers were < 1:100 both in healthy individuals, HOC and other fungal infection patients demonstrated the reactive bands on the membranes. In additional, protein component with molecular weight 22.5 kDa was not reacted with all sera. It may be low immunogenicity. This experiment was repeated twice and confirmed again by using two-fold dilution from original sera to react with the twelve protein components. The result was the same that was no bands were detected. The possibility is that the detectable antibody in ELISA were stimulated by the different serotypes of *Candida* or stimulated by the other similar antigen in the hosts.

Regarding the dilution of the antibody using in the immunoblot analysis, ten-fold dilution less than of the titer from ELISA method (first dilution) and two – fold dilution of the above titer (second dilution) were used to detect the antibody pattern on the membrane. More reactive bands were demonstrated in the second dilution than in the first dilution. Moreover, the cases in which the titers were less than 1:100, two-fold dilution of the original serum (the last dilution) was prepared to react also. The results demonstrated that the second dilution and the last dilution after reacted with the antigen on the membrane revealed the same pattern but different intensity. This indicated that the dilution of the serum using in the Western blot is one of the important factor which influence the interpretation. From this study, we recommended to use ≤ ten-fold dilution of the titer from ELISA and two – fold less than above dilution in some cases which had IgG antibody level less than 1:100 for the Western blot experiment.

Western blot of the healthy individuals revealed total fifteen patterns (Figure 20A). Among these patterns, eleven of them (no. 1-11 in Figure 20A) were

However, the more samples with the data of sample collection was recommended for this study.

Nine cases in other fungal infection patients demonstrated nine different profiles. Pattern no. 1 – 2 was from penicillosis marneffei patients, no. 3 – 5 was from aspergiollsis patients, no. 6 – 7 was from cryptococcosis patients and no. 8 and 9 was from pythiosis patient and chromoblastomycosis patient, respectively. The common component in sera from aspergillosis cases was 36.5 kDa, f rom cryptococcosis was 45.5 kDa and from penicillosis marneffei were 46.5 and 42.5 kDa. The 36.5 kDa component was found in pythiosis and chromoblastomycosis, also. The sera from almost all the cases but cryptococcosis did not react with the 46.5 kDa protein component. I think that some special mechanisms in this cryptococcosis patients effect the stimulation of the specific antibody against 46.5 kDa component.

In conclusion, the 46.5 kDa antigenic component was eminent among all sera including the other fungal infection (84%). This predominate component must have the high immunogenicity thus it can stimulate specific IgG antibody against *Candida albicans*, both normal flora and pathogens. The similar size to this component was reported (161, 162, 163, 164, 165). The 47 kDa, immunodominant antigen in whole-cell extracts of *Candida* was found in sera with systemic candidiasis and identified as a heat-stable breakdown product of hsp90 (166). Antibodies against this 47 kDa appear to provide some protection against *candida.* (167). Furthur study should be performed on proving whether 46.5 kDa protein and 47 kDa is the same component. Another 36.5 kDa protein was demonstrated in candidiasis patients (80%), both oral infection and systemic manifestation, and other fungal infection (56%) with higher frequency than the healthy individuals (31.8%). This indicated that this component might play an important role in the pathogenicity of candidiasis. As far as the literatures were reviewed, no report on 36.5 kDa protein was mentioned. One more interesting component which reacted with antibody from HOC only was 29 kDa. This result was contrast to the study by Zoller *et al.*, who reported that 29 kDa reacted with sera from systemic candidiasis (171). This point is hard to discuss since the sample was too small. The smallest component in this study, 22.5 kDa was not

reacted with all sera. It might due to its poor immunogenicity or inability to stimulate the specific IgG.

Further study on the characterization of the 52, 50.5, and 29 kDa should be performed to confirm their antigenic activities or whether it can be the remarkable antigenic markers for differential diagnosis of systemic candidiasis and HOC.