Thesis Title Pseudomonas pseudomallei : Immunological Studies with Special Application for the Diagnosis of Melioidosis

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ABSTRACT

Melioidosis, an infection caused by Pseudomonas (Ps.) pseudomallei , is considered to be one of the important health problems in Thailand. Most of the patients reside in the north-eastern part of the country. The clinical features may vary from asymptomatic to acute and fatal septicemia, with a mortality rate of 80-90 % in untreated cases. The disease cannot be readily diagnosed by clinical pictures because its signs and symptoms mimic a number of other infectious diseases. Consequently, a definitive diagnosis of this bacterial infection is based on laboratory findings including bacteriology and serology. Currently, the identification of a causative agent from bacteriological culture is the only available definitive confirmatory diagnosis but it is time consuming. Attempts have been made to diagnose this infection by conventional serological tests but the results are difficult to interprete. Most of these tests are concerned with the detection

of serum antibody including indirect hemagglutination (IHA), complement fixation(CF), indirect fluorescent (IFA), indirect ELISA and most recently gold blotting. These techniques are of limited value particularly because of the high background antibody titer among healthy individuals in the endemic area of infection. More recently, a detection of Ps.pseudomallei antigens has also been attempted, particularly for the diagnosis of acute septicemic melioidosis. There have been two reports describing techniques for the detection of a small quantity of Ps.pseudomallei antigens in the patient sera. However, neither of these has been tested on clinical specimens. In order to develop simple and rapid serological tests that can overcome the problems previously encountered in the early diagnosis of acute septicemic melioidosis, in the present study , a number of previously described serological tests were refined and new test was developed. The polyclonal antibodies to Ps.pseudomallei antigen (prepared by extracting the bacteria with veronal buffer and followed by precipitation with trichloroacetic acid) was used for detecting the Ps.pseudomallei antigen in patient sera by biotin-streptavidin ELISA (B-SA ELISA). Its minimal sensitivity limit was as low as 2.5 ng/ml. However, this system failed to give a positive reaction with 31 sera from septicemic patients. Therefore, if the antigen was present in any of the specimens, it must be present at a concentration below 2.5 ng/ml. The failure to detect free antigen in the serum may also be due to interference by circulating immune complexes. Determination of the specific immunoreactive component(s) in the whole cell lysate of Ps.pseudomallei was evaluated by Western blotting and IgG immunoenzymatic reaction using patient sera. The components with

immunodiagnostic potential have molecular weight of 18.5 and 20.5 KD. The BP.PP$_k$ and other Ps.pseudomallei antigens (culture filtrates) were also used for the detection of antibodies to Ps. pseudomallei antigens by various serological tests including indirect hemagglutination, IgM ELISA, IgG ELISA and latex agglutination (LT). The sensitivity of IHA, IgM ELISA, IgG ELISA and LT were 86.7, 64.5, 83.9 and 86.7 % respectively. The specificity of these techniques were 91.2, 92, 94.4 and 89.6% respectively. IHA agreed well with every method with correlation coefficients of 0.6293,0.6266 and 0.7136 respectively. Comparing with the IHA, the LT was more simple to perform and the results could be more readily obtained. Consequently, the LT can be used instead of the more widely used IHA , particularly for epidemiological surveys. In contrast to the results from previous study , the sensitivity of IHA determined in the present study is similar to the IgG ELISA but better than the IgM ELISA for the diagnosis of acute septicemic melioidosis. It is possible that this is related to the fact that at the time of blood collection for serological study, the immune response has already switched from IgM to IgG antibody production or that the current illness may represent a secondary immune response in these patients. It should be kept in mine also the difference in the nature of the antigens used by different groups may contribute to such a discrepancy results.