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WIMOL PETKANCHANAPONG : SIGNIFICANCE OF PROTEASES FROM *BURKHOLDERIA PSEUDOMALLEI*, DEVELOPMENT OF MONOCLONAL ANTIBODY AND ENGINEERING OF A CHIMERIC PROTEASE-BINDING PROTEIN. THESIS ADVISORS: VIRAPONG PRACHAYASITTIKUL, Ph.D., LEIF BÜLOW, Ph.D., VINITA BORIRAJ, MD., Ph.D. 189 p. ISBN 974-665-250-8

Burkholderia pseudomallei is one of the major causative agents of bacterial septicemia in Thailand. Mortality occurs ca. 50% of patients after 48 hours of hospital admission. Development of laboratory diagnosis to facilitate rapid management, particularly on acute melioidosis, have been widely proposed. This study aimed to construct a chimeric protein and a monoclonal antibody to provide binding to protease of *B. pseudomallei.*

Three distinguished proteases (P_1, P_2 and P_3) were discovered. They possessed a variety of different pathogenic properties e.g. immunoglobulin destruction, cytotoxicity, hemolytic activity, antigenicity, coagulation and plasma protein detardation. P_1 was a major component released extracellularly. Most of melioidosis patients (90%) possessed anti-P_1 antibody detected by ELISA. This confered its roles on pathogenic and immunologic activity *in vivo*. Therefore, P_1 was selected to be used as the target antigen for further development.

An IgM monoclonal antibody to P_1, MAb $P_1H_2C_4$ was successfully produced. Immunoblot analysis demonstrated that the MAb $P_1H_2C_4$ specifically recognized a 41 kDa P_1. Moreover, sandwich ELISA and IFA confirmed that the MAb $P_1H_2C_4$ reacted directly with the P_1 in both culture supernatant and intact *B. pseudomallei*. By Dot-ELISA, MAb $P_1H_2C_4$ showed the same specific binding. It did not react to trypsin, subtilisin and proteinase K. Using sandwich ELISA, all 25 different strains of *B. pseudomallei* but none of 45 other bacterial species reacted with the MAb $P_1H_2C_4$. Both sensitivity and specificity of this test were 100% and provided detection down to 10^4 cells/ml of *B. pseudomallei.*

The chimeric protease binding-green fluorescent protein (PB-GFP) was successfully constructed. The PB-GFP bound to the P_1 while it exhibited fluorescence emission. The lower limit of detection was 250 ng of P_1. It did not bind to subtilisin, trypsin, proteinase K and RNase.

Hybridoma agglutination test (HAT) was performed by using the 2% glutaraldehyde treated hybridomas producing MAb $P_1H_2C_4$. This newly developed HAT for detection of *B. pseudomallei* was evaluated via field trial. Field evaluation of the HAT among 520 hemocultures showed high sensitivity (96.3%) and specificity (97.95%) as compared to conventional culture detection. In addition, the identification of *B. pseudomallei* by HAT is rapid (2-5 min) and simple to perform in most laboratories.

The purified P_1 was also applied as antigen for serodiagnosis. Using immobilized P_1, specific IgG and IgM could be detected with high sensitivity (90%, 83.3%, respectively) and specificity (91.3% and 93.1%, respectively) for diagnosis of melioidosis.

The success of early detection of *B. pseudomallei* using protease as target will be very helpful for life saving of patients. Both the chimeric PB-GFP and MAb $P_1H_2C_4$ are ongoing to improve their efficacy for clinical detection.