Thesis Title Characterization of Host-Bacterial Interaction in *Burkholderia pseudomallei* Infection

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ABSTRACT

Melioidosis is an important endemic disease in Southeast Asia and Australia. Latency and relapse in melioidosis are two of the major problems of this disease because the causative agent, *Burkholderia pseudomallei,* can reside in the human host for years. In this study, the ability of *B. pseudomallei* to enter, survive and replicate in phagocytic and non-phagocytic cell lines were demonstrated. Additionally, PCR amplification method was developed for the detection of *B. pseudomallei* DNA in clinical specimens.

Two strains of *B. pseudomallei* isolated from blood and two strains isolated from liver pus were used to infect cultured cell lines. All 4 strains of *B. pseudomallei* were able to enter phagocytic cells, J774 and freshly isolated neutrophils. Survival and intracellular replication of *B. pseudomallei* were observed in J774 but not in

neutrophils The bacteria were able to enter and survive in non-phagocytic fibroblast cells including MRC5 and HFF, but not in epithelial and lymphoblastoid cell lines. The intracellular bacteria were seen in the vacuole-like membrane at all times during infection and the heavily infected cells had long projections extending out with the bacteria at the tip of each projection.

DNA amplification method was developed for detection of *B. pseudomallei* 16S rRNA gene in clinical specimens. Leukocytes from the buffy coat were used primarily as the main source of specimen because of the superiority of buffy coat to plasma or serum for the detection of *B. pseudomallei* DNA. The nested PCR system developed in this study was highly sensitive and was able to detect as few as two bacteria in the amplification reaction. The system was highly specific to *B. pseudomallei.* Bacterial DNA from 54 clinical isolates of *B. pseudomallei* from various parts of Thailand but not from the other bacteria tested can be amplified. This technique has proven valuable in identifying bacterial pathogens that have resisted detection and identification by traditional microbiological methods. The PCR results were obtained before the bacterial cultures became positive. The PCR method described here allows identification of *B. pseudomallei* directly from buffy coat, sputum, and pus from internal organs and probably from other types of clinical specimens. This technique will allow further investigation on the latency of *B. pseudomallei* in various organs.