CHAPTER I
INTRODUCTION

1. Rationale and background

Burkholderia pseudomallei is a facultative intracellular Gram-negative bacteria. It causes melioidosis which is found in man and animals. The disease is most common in tropical regions especially in Southeast Asia and Northern Australia (Ashdown & Guard, 1984; Leelarasamee & Bovornkitti, 1989) where it is a major cause of community acquired septicemia and acute pneumonia. It has a case fatality of up to 70% (Chaowagul et al., 1989; Currie et al., 2000). In Thailand, especially in the northeast, *B. pseudomallei* is a major cause of morbidity and mortality causing one-fifth of all community-acquired septicemia (Chaowagul et al., 1989). Infection is probably caused when skin abrasions come to contact with contaminated soil or water or inhalation of *B. pseudomallei* from environmental sources (Leelarasamee & Bovornkitti, 1989; Pruekprasert & Jitsurong, 1991). As a result, a relatively high percentage of the case in Thailand is the farmer who works in rice fields without a proper protection (Chaowagul et al., 1989). The clinical manifestation of melioidosis present as a long spectrum of severity ranging from acute fulminating septicemia, which carries high mortality rates to chronic and subclinical infections. The range of clinical presentations and the potential for asymptomatic infection depended on the differences in the route of inoculation, inoculum size, virulence of the infecting strains, immune competence and genetic predisposition of their host. Antibiotics therapy is complicated by antibiotic resistance in clinical isolates, resulting in frequent relapse or reactivate decades later (Jenney et al., 2001; White, 2003). Furthermore, recurrent infection can occurred despite adequate and prolong therapy (White, 2003). Severe septic melioidosis is usually associated with underlying diseases such as diabetes and chronic renal failure, although it sometimes occurs in previously healthy individuals (Brett & Woods, 2000). Asymptomatic seroconversion has been observed in areas of endemic such as Northeast Thailand, where antibodies were found in about 80% of children by the time they were 4 years old (Kanaphun et al., 1993).

In order to understand the pathogenesis of the disease and the mechanism of host resistance, a murine model that mimics the acute and chronic forms of human melioidosis has been established (Leakey et al., 1998). It was demonstrated that BALB/c mouse is highly susceptible to infection with virulent *B. pseudomallei*, while C57BL/6 mouse is relatively resistance. Although much is known about the epidemiology, clinical manifestations, and its response to antimicrobial agents, the host immune response towards the infection is not yet completely defined (Anuntagool et al., 1993; Suputtamongkol et al., 1994; Wilson et al., 1987). Because *B. pseudomallei* is an intracellular organism (Pruksachartvuthi et al., 1990), the development of a cellular adaptive immune response would be important in the eventual clearance or control of the infection, which essential for the survival of host (Barnes et al., 2004; Ketheesan et al., 2002). Antibodies which are bactericidal or promote phagocytosis may have an important role in the initial clearance of the bacteria. An induction of both humoral and cell mediated immune responses was performed by immunized BALB/c mice with dendritic cells pulsed with heat-killed bacteria followed by killed bacteria in adjuvant (Healey et al., 2005). The survival mice were found to be increased with the use of this combine vaccines. This indicated that both humoral and cell mediated immunity play an important role in the control of the infection; as pulsed dendritic cells activate a good cell-mediated response while bacteria in adjuvant enhance antibody response. Humoral immunity probably helps to control infection through opsonisation of the bacteria and triggering the classical complement pathway (Casadevall, 1998). Cell-mediated immunity is useful in clearing the infection by activating infected macrophages to kill intracellular bacteria. Therefore, both arms of the adaptive immune response are likely to be complementary in controlling the infection. This understanding will be important in the development of vaccines as well as immunotherapy.

One of the major limitations of antibiotic therapy for *B. pseudomallei* infection is the lack of the pattern of *B. pseudomallei* and antibody responses in blood which can be used to monitor the effectiveness of treatment in eradication of the organism. Relapse after 3–6 months of maintenance therapy with antibiotics is common in melioidosis (Jenney et al., 2001). The knowledge of the pattern of *B. pseudomallei* and antibody response to the organism during infection would be of great importance

in evaluating the progression of melioidosis. Furthermore, current laboratory diagnosis of melioidosis is still depended on the culture as the gold standard method. Although the serological and molecular biological methods were developed and the sensitivity and specificity are varied from one laboratory to others (Appassakij et al., 1990; Ashdown et al., 1989; Charoenwong et al., 1992; Dharakul et al., 1997; Petkanjanapong et al., 1992; Rugdech et al., 1995; Wongratanacheewin et al., 1995). All methods developed did not give satisfactory result when compared to the culture. Moreover, they are not rapid enough for diagnosis of sepsis melioidosis with high mortality rate. Nevertheless, one of the reasons that make all tests not satisfy is the unknown window period of *B. pseudomallei* and antibody presence in blood circulation. Several question concerning the immune responses and the antigens in the circulation after exposed to the organisms still controversial these include 1) How long *B. pseudomallei* persistent in blood? 2) When will the antibody response occur? and 3) How long it persist? In order to make use of the antigen and antibody detections for diagnosis of melioidosis, the kinetic of antigens and antibodies should be investigated. Therefore, in this study, the kinetic growth of *B. pseudomallei* and its DNA in blood were determined by the conventional culture and polymerase chain reaction (PCR) methods. The antibody responses were measured by the enzyme linked immunosorbent assay (ELISA) in serum of BALB/c mice after intraperitoneal injection with various dose of *B. pseudomallei* for acute and chronic infections. The knowledge of this study might be used as a guideline to develop sensitive and specific laboratory diagnosis and monitoring the progression of the disease which leading to a better treatment of melioidosis.

2. Objectives of the study

2.1 To determine kinetic growth of *B. pseudomallei* and its DNA in blood of BALB/c mice in acute and chronic infections.

2.2 To determine the kinetics of antibody responses in BALB/c mice infected with *B. pseudomallei* in acute and chronic infections.

3. Limitations of the study

To determine the kinetics of *Burkholderia pseudomallei* and antibody response in infected BALB/c mice. The result may not be the same as in human.

4. Location of research conducting

4.1 Experiments in animals will be performed at the Laboratory Animals Breeding Unit, Faculty of Medicine, Khon Kaen University.

4.2 All laboratory analysis will be performed in microbiology laboratory, Department of Microbiology, Faculty of Medicine, Khon Kaen University.

5. Anticipated outcome

Kinetics of *B. pseudomallei* in blood and antibodies response in infected mice will be useful as a guideline to develop a sensitive and specific laboratory diagnosis and monitoring the progression of the disease which leading to a better treatment of melioidosis patients.