

# ***Burkholderia pseudomallei*: AN UPDATE ON DISEASE, VIRULENCE AND HOST INTERACTION**

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## **ABSTRACT**

*Burkholderia pseudomallei* is the etiological agent of melioidosis, a life-threatening disease of humans and animals that occurs primarily in Southeast Asia and northern Australia. The distribution of *B. pseudomallei* and occurrence of melioidosis globally is very much evident. This soil and water-dwelling saprophyte is resilient to various environments. This organism has gained further notoriety following the Centre for Disease Control's classification of *B. pseudomallei* as a Tier 1 biological agent. Despite several decades of clinical research, the mortality rate for melioidosis remains high. Genomics-based studies have demonstrated the plasticity of the *B. pseudomallei* genome and the coding sequences consists of a myriad of functions that enable the bacteria to adapt to these hostile environments as well as various hosts. Diagnosis is mainly based on bacterial culture or serological assays whilst treatment is limited to third generation cephalosporins. To date, no vaccine is currently available as an immunoprophylaxis for melioidosis. By utilising the available genome sequence, a number of virulence factors have recently been identified and have provided more insight into the pathogenesis of *B. pseudomallei*. These factors include surface associated proteins as well as secreted effector proteins and their corresponding secretion systems. In addition, a number of recent studies on host-pathogen interaction have also demonstrated how the pathogen is able to subvert the host immune system and survive within the cell. This review presents an overview of the current understanding on *B. pseudomallei* pathogenesis and the disease melioidosis.

**Key words:** *Burkholderia pseudomallei*, melioidosis, pathogenesis

## **INTRODUCTION**

*Burkholderia pseudomallei* (previously known as *Pseudomonas pseudomallei*) was first described as *Bacillus pseudomallei* by Alfred Whitmore, a British pathologist and C. S. Krishnaswami, an assistant surgeon, following its isolation from a young boy dying of pneumonia in Yangon, Myanmar in 1911 (Whitmore and Krishnaswami, 1912). Whitmore noted the clinical resemblance of this disease to glanders, an abscess-forming infection caused by *Burkholderia mallei* (Whitmore, 1913). The name of this bacterium continued to evolve over the years until 1992, when this bacterium and a number of closely related species were incorporated into a new genus, *Burkholderia*, based on the 16s ribosomal ribonucleic acid sequences, deoxyribonucleic acid (DNA)-DNA homology values, cellular lipid and fatty acid composition and phenotypic characteristics (Yabuuchi *et al.*, 1992).

## **BACTERIOLOGY AND GENOMICS**

### **General bacteriology**

*B. pseudomallei* is a Gram negative, motile, non-spore-forming bacillus, 0.4-0.6 µm in width and 2-5 µm in length (Yabuuchi and Arakawa, 1993). It is an environmental saprophytic organism which can be isolated from water and soil in rice paddy fields in endemic areas (White, 2003). *B. pseudomallei* is a resilient organism that is capable of surviving hostile environmental conditions, including a wide temperature range (37 - 42°C); some strains appear to survive for long periods (greater than 90 days) at 5°C under laboratory conditions (Yabuuchi *et al.*, 1993), acidic environments (pH 4.0 for more than 70 days) (Tong *et al.*, 1996), prolonged nutrient deficiency (Wuthiekanun *et al.*, 1995) and dehydration (soil water content of < 10% for up to 70 days) (Chen *et al.*, 2003) but not exposure to UV light (Tong *et al.*, 1996).

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When isolated from blood, sputum, pus and other body fluids, *B. pseudomallei* appears like safety-pins (bipolar) under the microscope (White, 2003). In the laboratory, it grows aerobically on most agar media and produces clearly visible colonies within 24 hours at 37°C. Nevertheless, colony morphology often demonstrates considerable variability between rough and smooth both within and between samples (Chantratita *et al.*, 2007a). Ashdown's selective medium which contains crystal-violet, glycerol and gentamicin is commonly used to culture the organism. The colonies develop a characteristic appearance (wrinkled and dry daisy-head) and they take up crystal-violet dye from the medium (Ashdown, 1979) (Fig. 1). More improved *B. pseudomallei* selective media such as *Burkholderia pseudomallei* selective agar (BPSA) (Howard and Inglis, 2003) or Francis medium (Francis *et al.*, 2006) have since been developed to improve the recovery of *B. pseudomallei*. The organism is oxidase positive, uses glucose by an oxidative pathway and can be distinguished from the closely related but less pathogenic *Burkholderia thailandensis* (Ara<sup>+</sup>) by the ability to assimilate arabinose (Smith *et al.*, 1997).

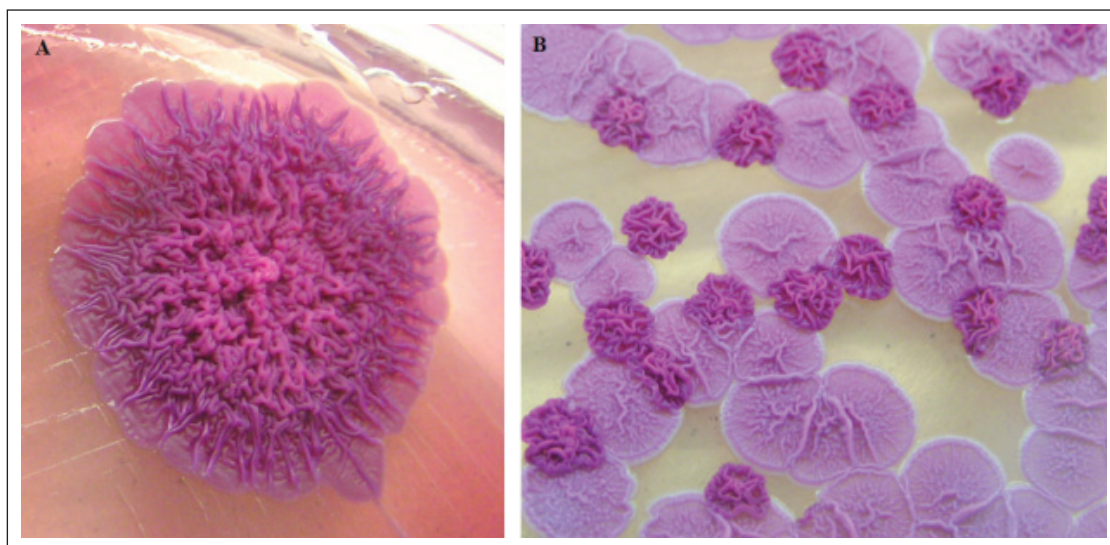
#### Drug susceptibility

*B. pseudomallei* is resistant to a diverse group of antimicrobials including third generation cephalosporins whilst quinolones and aminoglycosides have no reliable effect (Puthuchery and Sam, 2012). Therefore, therapeutic options are limited and the continuous presence of the organism in patients is not fully understood. Some rare, naturally occurring mutants are susceptible to both aminoglycosides and macrolides because they lack

a multidrug efflux pump to export these antibiotics (Moore *et al.*, 1999).

#### The *B. pseudomallei* genome

The first genome sequence of *B. pseudomallei* was released in 2004. The genome is relatively large, 7.24 Mb, divided unequally between two chromosomes (4.07 Mb and 3.17 Mb), with a G + C content of 68% (Holden *et al.*, 2004). At 7.2 Mb, the genome represents one of the most complex bacterial genomes sequenced to date. The large chromosome encodes many of the core functions associated with central metabolism and cell growth, whereas the small chromosome carries more accessory functions associated with adaptation and survival in different niches. The *B. pseudomallei* genome is estimated to carry approximately 5855 coding sequences involved in a myriad of functions, allowing microbial survival in extreme environments and virulence in diverse host species including humans, gorillas, pigs and fish. A large proportion of *B. pseudomallei* genes are still unannotated or poorly characterized, raising the need for systematic approaches to link discrete sets of *B. pseudomallei* genes to their specific biological and cellular functions. A striking feature of the genome is the presence of genomic islands (GIs) that together make up 6.1% of the genome. Genomic islands contain regions of mobile genetic elements, such as insertion sequence (IS) elements, bacteriophages and plasmids directly acquired by horizontal transfer from other microorganisms and are likely to represent the major evolutionary drivers of *B. pseudomallei* virulence. This also led Holden *et al.* (2004) to propose that variable horizontal gene acquisition by *B. pseudomallei* is an important



**Fig. 1.** *Burkholderia pseudomallei* in culture. A. Typical colony morphology of *B. pseudomallei* on Ashdown's selective medium after 72 hours incubation at 37°C. B. Variable colony morphology that can be seen from a single sample on Ashdown's selective medium after 72 hours incubation at 37°C.

feature of recent genetic evolution and that this has resulted in a genetically diverse pathogenic species.

A separate study using whole-genome *B. pseudomallei* microarrays to examine patterns of gene presence and absence across 94 South East Asian strains isolated from a variety of clinical, environmental or animal sources demonstrated that 86% of the *B. pseudomallei* K96243 genome was common to all strains representing the *B. pseudomallei* “core genome”, comprising genes largely involved in essential functions (e.g. amino acid metabolism, protein translation). In contrast, 14% of the *B. pseudomallei* K96243 genome, namely the accessory genome, was variably present across the isolates. The accessory genome comprises of multiple genomic islands, paralogous genes and insertions/deletions, including three distinct lipopolysaccharide (LPS)-related gene clusters. Noticeably, genomic islands were generally absent in strains derived from the environment suggesting that the *B. pseudomallei* accessory genome plays an important role in microbial adaptation and virulence in clinical isolates (Sim *et al.*, 2008).

## EPIDEMIOLOGY

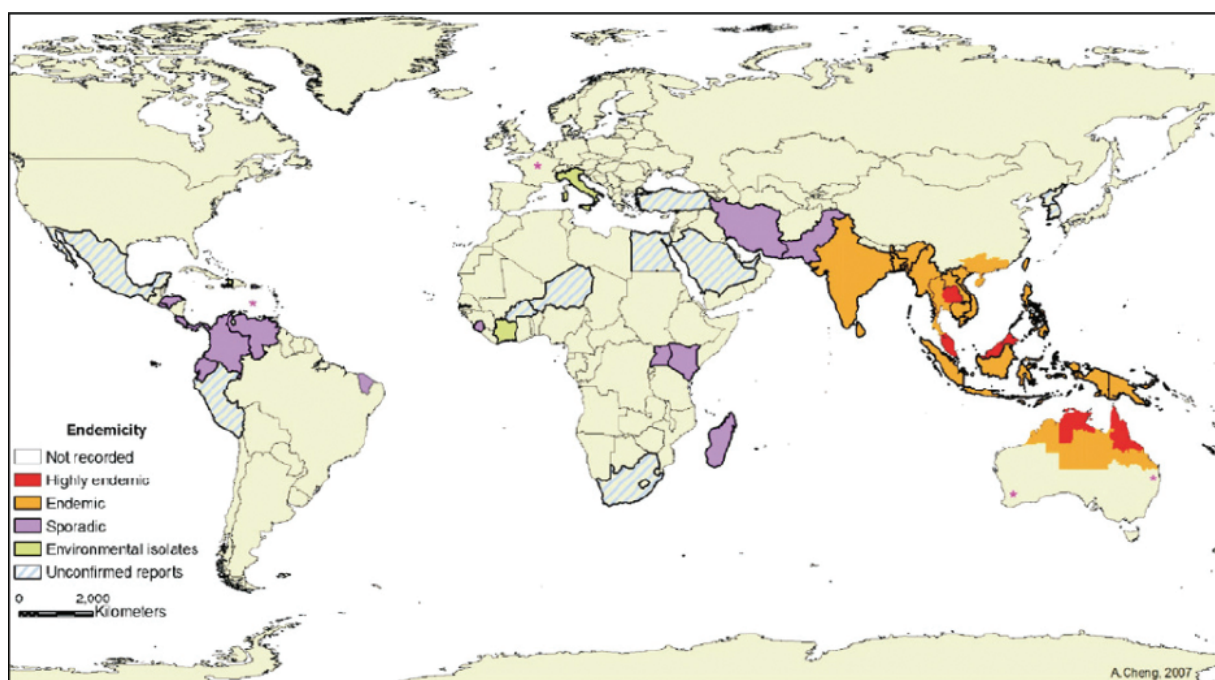
The geographical distribution of *B. pseudomallei* and melioidosis was comprehensively reviewed in 2005 (Cheng and Currie, 2005) and updated in 2008 (Currie *et al.*, 2008). It is well recognized that Southeast Asia and northern Australia are the main

endemic foci for melioidosis. In areas of Southeast Asia where this bacterium is endemic, infection by *B. pseudomallei* has been estimated to be responsible for 20% to 30% of mortality due to septicaemia and 40% of sepsis-related mortality. Nevertheless, recent case reports of melioidosis have expanded the endemic zone to southern China (Ma *et al.*, 2009), Taiwan (Chen *et al.*, 2010), most of the Indian subcontinent (Chugh, 2008) and Papua New Guinea (Warner *et al.*, 2008). In addition to endemic melioidosis, there are several documented situations where melioidosis has become established throughout the world. Sporadic cases have occurred in Brazil (Rolim *et al.*, 2005), elsewhere in the Americas (Inglis *et al.*, 2006) and in island communities such as New Caledonia in the Pacific Ocean (Le Hello *et al.*, 2005) and Mauritius in the Indian Ocean (Issack *et al.*, 2005) (Fig. 2). Of note, the incidence of melioidosis has increased incrementally over the past 20 years particularly in the hot spots of Northern Territory of Australia (Currie *et al.*, 2010) and Northeast Thailand (Limmathurotsakul *et al.*, 2010b).

## CLINICAL FEATURES

### Modes of acquisition

There are several established modes of transmission within the patient population. Inhalation was initially thought to be the primary mode of acquisition, based on studies on American



**Fig. 2.** Global map showing the categories of distribution of melioidosis and *B. pseudomallei*. Adapted from Currie *et al.* (2008) with permission.

soldiers in Vietnam, where it was noted that helicopter crews seemed to have a high incidence of the disease (Howe *et al.*, 1971). Nevertheless, there is no evidence to support direct human-to-human transmission via the respiratory route. It is now recognized that human and animal melioidosis is thought to occur by inoculation from contaminated soil through skin lesions, based on the observations that people at high risk included rice paddy farmers who spend most of the working day wading in mud and surface water during planting and harvesting seasons (Cheng and Currie, 2005). The number of registered cases of infection also increases with rainfall (Sam and Puthuchery, 2007). Rare cases of nosocomial infections have arisen in intensive care units (Raja *et al.*, 2005) while vertical transmission at childbirth and sexual transmission have also been reported (Abbink *et al.*, 2001). However, zoonotic episodes are rare but highly probable as *B. pseudomallei* has an extremely broad host range (Sprague and Neubauer, 2004).

### Clinical syndromes

Limmathurotsakul and Peacock (2011) have provided an excellent overview of the clinical presentation of melioidosis. Generally *B. pseudomallei* can cause disease in apparently healthy individuals although most infections are asymptomatic. Once infected, the infection can remain quiescent for long periods and become active after months, years or decades. The longest latent period documented to date is 62 years after exposure in a Vietnam War veteran (Ngauy *et al.*, 2005). The incubation period from defined inoculating events to onset of melioidosis was previously ascertained as 1-21 days (Currie *et al.*, 2000). The disease is characterised by abscess formation (Wong *et al.*, 1995) where it may be localized or disseminated to various organ systems such as lungs, skin and subcutaneous tissues, bones and joints, liver, spleen, pancreas, kidneys, bladder, prostate, genital organs, brain and meninges, parotid glands, lymph nodes and pericardium (Ip *et al.*, 1995).

The clinical spectrum of melioidosis is extremely diverse, consisting of four forms of disease: acute fulminant septicaemia, subacute illness, chronic infection and subclinical disease. In acute form, death can occur within 24-48 hours of the onset of symptoms (Ip *et al.*, 1995). Subacute melioidosis is described as prolonged febrile illness characterized by multiorgan involvement, systemic abscess formation and bacteraemia where clinical manifestations can last for weeks to months or years. Chronic melioidosis is the most common presentation of this disease. It generally remains undiagnosed until activated by a traumatic event or post-mortem examination of the tissues (Brett and Woods, 2000) whereas subclinical melioidosis is an

infection with no symptoms resulting in a chronic carrier state. However, if decrease in immune competence occurs, one of the clinical forms of the disease can occur. Recurrent disease due to reactivation of the original infecting strain (relapse) or different strain (re-infection) after apparent successful treatment is well described (Maharjan *et al.*, 2005). Re-activation of melioidosis from a latent focus can present as fulminant or less severe melioidosis but it is still an important cause of morbidity and mortality in patients who survive a primary episode of melioidosis.

## DIAGNOSIS AND MANAGEMENT

### Diagnosis

Rapid diagnostics are needed for melioidosis as the clinical presentation is nonspecific and treatment requires specific antibiotics. Isolation of *B. pseudomallei* from blood, urine, sputum, skin lesions and throat swab samples remains the “gold standard” of diagnosis (Limmathurotsakul *et al.*, 2010a). For non-sterile specimens, Ashdown’s selective media is commonly used but, identification of *B. pseudomallei* can also be made by combining the commercial API 20NE or 20E biochemical kit (Kiratisin *et al.*, 2007) with a simple screening system involving Gram stain, the oxidase reaction, typical growth characteristic and resistance to gentamicin and colistin (Lowe *et al.*, 2002).

Melioidosis can also be diagnosed presumptively using serological tests in the absence of isolated *B. pseudomallei* in the specimen. A variety of antigen detection methods have been developed for use on direct specimens or in blood culture supernatant. For example, direct immunofluorescence microscopy of infected sputum, urine and pus (Wuthiekanun *et al.*, 2005) or a latex agglutination test based on specific monoclonal antibodies to LPS for definitive identification from blood culture (Ekpo *et al.*, 2007). The indirect hemagglutination (IHA) test detects antibodies against *B. pseudomallei* that appear in the blood within 1-2 weeks after infection and reach a maximal titre in 4 to 5 months (Tiyawisutsri *et al.*, 2005). IHA remains the most widely used serological test for the diagnosis of melioidosis despite its poor sensitivity and specificity. The use of enzyme linked immunosorbent assay (ELISA) to detect specific IgG and IgM antibodies against *B. pseudomallei* in serum specimens (Chantratita *et al.*, 2007c) has improved the diagnostic accuracy of tests based on antibody detection.

Molecular biology techniques such as polymerase chain reaction (PCR) and dot immunoassay are also used for diagnosis of melioidosis.

Several studies demonstrated that nucleic acid-based methods using PCR are recommended for rapid, reliable and accurate identification of *B. pseudomallei* with less biological hazard risks for technical personnel (Gal *et al.*, 2005; Chantratita *et al.*, 2007b). For instance, PCR-based techniques previously evaluated are PCR targeting the 16S rRNA (Ruppitsch *et al.*, 2007) and real-time PCR targeting type III secretion system (TTSS3) genes (Meumann *et al.*, 2006). Nevertheless, PCR-based assays are not routinely used as the sensitivity is lower on blood cultures compared to conventional techniques, probably due to low bacterial concentrations (Chantratita *et al.*, 2008).

### Antimicrobial therapy

Generally, disease management is based on either an intensive intravenous phase of treatment and/or a prolonged eradication phase of treatment. Ceftazidime is the drug of choice for initial intensive therapy and when the response is not encouraging, carbapenem is administered (Cheng *et al.*, 2004). Following initial intensive treatment, subsequent eradication therapy is considered necessary for preventing recrudescence or later relapses of melioidosis. Currently, the most effective eradication therapy is a combination of trimethoprim and sulfamethoxazole for 12 – 20 weeks (Limmathurotsakul *et al.*, 2006). In the wake of increasing reports of *B. pseudomallei* resistance towards conventional antibiotic therapy, new approaches are being taken to identify new antimicrobials against this pathogen. Recent preliminary reports on the isolation and characterisation of antimicrobial peptides that inhibit the growth of *B. pseudomallei* include the LL-37 antimicrobial peptide (Kanthawong *et al.*, 2012), polysaccharides derived from the Acai berry (Skyberg *et al.*, 2012) and bovine lactoferrin antimicrobial peptides (Puknun *et al.*, 2013).

### VIRULENCE FACTORS OF *B. pseudomallei*

A number of potential virulence factors have been described and characterized in *B. pseudomallei*. The factors described here have been grouped according to the current data indicating the known role in virulence for other pathogens or virulence in experimental models (reviewed in Lazar Adler *et al.* (2009) and Wiersinga and van der Poll (2009).

#### Secreted factors

Previous studies have deduced that *B. pseudomallei* secretes numerous soluble factors into the external milieu via the type II general secretory pathway (Gsp) (DeShazer *et al.*, 1999) and these include several proteases, phospholipase C,

haemolysin, lecithinase, lipase and cytotoxic exolipid that cause tissue necrosis, haemolysis, cytolysis and death in the host (Ashdown and Koehler, 1990). Nevertheless, whilst transposon mutagenesis of the Gsp resulted in a failure of secretion, the pathogen was not attenuated in an animal model (Brett and Woods, 2000) suggesting that these exoproducts may only play a minor role in virulence of *B. pseudomallei*. Many reports have alluded to the potential role of proteases in *B. pseudomallei* pathogenicity. The serine MprA protease has been implicated as a possible virulence factor (Lee and Liu, 2000) causing extensive damage to mammalian physiological proteins involved in circumventing the detrimental effects of bacterial secreted proteases (Chin *et al.*, 2007).

A recent finding has discovered a highly potent toxin named *Burkholderia lethal factor 1 – BLF1*, which interferes with the protein translation elongation factor eIF4A of the host. Furthermore, this toxin was demonstrated to act as a potent cytotoxin in eukaryotic cells and mice (Cruz-Migoni *et al.*, 2011). Aside from the exoproducts, the ability to acquire iron from host sources is a prerequisite for the successful establishment and maintenance of most bacterial infections. Most bacteria including *B. pseudomallei* have the ability to produce and secrete iron chelator molecules, siderophores (malleobactin) during growth under iron-deficient conditions, to scavenge iron from both lactoferrin and transferrin at acidic pH (Yang *et al.*, 1991).

#### Quorum sensing

Quorum sensing (QS) is a mode of bacterial communication used by multiple bacterial species to regulate processes such as antibiotic synthesis, biofilm formation and virulence factor expression as a function of population density (Miller and Bassler, 2001). In many Gram negative bacteria, quorum sensing is mediated by acyl-homoserine-lactones (AHLs) that are synthesized and recognized by quorum sensing circuits composed of LuxI and LuxR homologs for the coordination of extracellular virulence factor expression. The *B. pseudomallei* genome has been reported to have three LuxI and five LuxR homologues. *B. pseudomallei* mutants of the LuxIR quorum sensing homologues showed a significant increase in the LD<sub>50</sub> (lethal dose 50%) in a Syrian hamster melioidosis model and displayed reduced colonization in the BALB/c mouse model, delaying time to death (Ulrich *et al.*, 2004). Furthermore, the extracellular secretion of *B. pseudomallei* QS AHLs is highly dependent on the *B. pseudomallei* BpeAB-OprB efflux pump (Chan *et al.*, 2007). This efflux pump is notorious for conferring antimicrobial resistance to amino glycosides and macrolides, optimal production of

siderophores and phospholipase C as well as biofilm formation. In addition, BpeAB mutants displayed reduced levels of invasion and cytotoxicity of human lung epithelia (A549) and human macrophage (THP-1) cells (Chan and Chua, 2005).

### Type III secretion system

The type III secretion system (TTSS) is a syringe-like apparatus that facilitates the injection of deleterious proteins called effectors into the target-cell cytosol. Depending on the species, TTSS effectors have been implicated in perturbing a variety of host cellular processes including cytoskeletal dynamics, gene expression, cell cycle progression and apoptotic cell death programs (Galan and Wolf-Watz, 2006). *B. pseudomallei* has three type III secretion system (TTSS) gene clusters. One of these clusters (the TTSS3 cluster) is very similar to that of the human pathogens *Salmonella typhimurium* and *Shigella flexneri* (Rainbow *et al.*, 2002; Stevens *et al.*, 2002). This gene cluster (termed bsa, *Burkholderia* secretion apparatus) encodes proteins predicted to be required for the synthesis of both the secretion apparatus and the effector proteins. Mutants of the bsa secretion and translocation apparatus have been shown to have impaired intracellular survival and are unable to escape from endocytic vacuoles, replicate or form actin tails in the first 8 hours after infection of J774.2 macrophage cells (Stevens *et al.*, 2002). In addition, a separate study corroborated the findings by showing that mutations involving the TTSS3 (as opposed to TTSS1 and TTSS2) attenuate virulence in a Syrian hamster model and mutants exhibited delays in vacuolar escape, multi-nucleated giant cell (MNGC) formation and actin-mediated intracellular motility (Warawa and Woods, 2005). Recently, the presence of a new secretion system, designated T6SS, was reported. Six T6SS clusters have been identified in the *B. pseudomallei* K92643 genome (Holden *et al.*, 2004), of which at least one was implicated in macrophage invasion (Shalom *et al.*, 2007).

### Surface polysaccharide and LPS

*B. pseudomallei* expresses several surface polysaccharides. Capsular polysaccharides (CPS) such as O-PS I enable bacteria to evade host defense mechanisms by inhibiting complement activation and phagocytic mediated killing (Reckseidler-Zenteno *et al.*, 2005). Inoculation with *B. pseudomallei* mutants of the O-PS I biosynthesis locus genes resulted in reduced survival in mice (Atkins *et al.*, 2002). In addition, another polysaccharide, LPS (formerly O-PS II), expressed by *B. pseudomallei* has been suggested to play a role in internalization and intracellular survival of the bacteria (Arjcharoen *et al.*, 2007). In addition

to CPS and LPS, two other surface polysaccharides, termed type III and IV O-PS have been identified and shown to play important roles in virulence of *B. pseudomallei* (Sarkar-Tyson *et al.*, 2007). A number of previous studies have confirmed that *B. pseudomallei* is capable of synthesizing outer membrane proteins (OMP) implicated in adhesion to and invasion of host cells, resistance to phagocytosis and the killing of host cells. These surface associated proteins are also important in maintaining structural integrity and in enabling pathogens to adapt to differing environments within the host (Harding *et al.*, 2007).

### Flagella and pili

Adhesion is a fundamental requirement for many bacteria in order to colonize the host and cause disease. The *B. pseudomallei* K96243 genome encodes eight type IV pili-associated loci. However, only one type IV A pilin gene, *pilA*, is present on the genome. A study has shown that *pilA* is required for efficient adherence of *B. pseudomallei* onto cultured cells and for virulence *in vivo* (Essex-Lopresti *et al.*, 2005). The importance of flagella in cell invasion of phagocytic and non-phagocytic cells has been demonstrated in a number of studies (Chua *et al.*, 2003; Chuaygud *et al.*, 2008). Furthermore, in a mouse model of systemic infection, flagella-deficient *B. pseudomallei* is less virulent compared with the wild type strain (Chuaygud *et al.*, 2008).

### INTRACELLULAR ADAPTATION OF *B. pseudomallei*

Our group undertook a study on comparing expression profiles obtained from intracellular *B. pseudomallei* and the transcriptome of control bacteria grown in cell culture medium (Chieng *et al.*, 2012). A majority of the bacterial genes were down-regulated throughout the infection period (1–6 hours) when compared to bacteria grown *in vitro*. Adaptation of *B. pseudomallei* within macrophages occurred rapidly with most of the changes occurring as early as 1 hour post-infection. With time, the number of significantly expressed genes gradually decreased, suggesting that *B. pseudomallei* had become well adapted to the intracellular environment and displayed behaviour similar to *in vitro* grown bacteria. Functional classification of intracellularly modulated bacterial genes at any time point showed that most of these genes encoded core functions such as metabolism, cell envelope, regulatory functions and transport and binding, as well as unknown and hypothetical proteins. Only 25 genes were constantly up-regulated and were those involved in cellular functions such as biosynthesis

of flagella and capsule, energy metabolism and regulatory systems. Therefore, *B. pseudomallei* adapts rapidly to the intracellular environment by relying on a strategy that includes regulation of intracellular bacteria metabolism and growth kinetics and the possibility of host cell immune response avoidance through shutdown of known virulence factors.

### HOST-PATHOGEN INTERACTIONS IN *B. pseudomallei* INFECTION

Host response to infectious disease can be modeled by exposing a host to a pathogen(s) and monitoring the molecular changes that occur over time. The availability of host model systems for melioidosis, such as *in vitro* cell-based models and *in vivo* models including animals and invertebrates has boosted pathogenesis studies.

#### *In vitro* cell-based models

*In vitro* studies have established that *B. pseudomallei* is capable of surviving and multiplying intracellularly within professional phagocytes, including macrophages, monocytes and neutrophils (Jones *et al.*, 1996; Stevens and Galyov, 2004) or non-phagocytic cells, including respiratory epithelial cells (Brown *et al.*, 2002; Wongprompitak *et al.*, 2009) although the exact mechanism of invasion and colonization remains unknown. When *B. pseudomallei* is taken up by macrophage-like cells, it is capable of escaping from endocytic vacuoles into the cytoplasm, where it can replicate by lysing the endosome membrane (Harley *et al.*, 1998). It also appears to be able to evade phagosome-lysosome fusion and reside within phagolysosomes, making use of its ability to survive and grow in acidic environments. Moreover, *B. pseudomallei* can be propelled by inducing continuous polymerization of actin resulting in the formation of membrane protrusions in host cells that may project into an adjacent cell, facilitating cell-to-cell spread of the bacteria (Kespichayawattana *et al.*, 2000). As a result of intracellular motility, cell fusion leads to MNGC formation (Wong *et al.*, 1995).

Eventually, the ability of intracellular *B. pseudomallei* to survive and replicate in macrophages is in part due to the suppression of macrophage killing mechanisms, including the production of the free radical species nitric oxide (NO). In macrophages, NO production results from active inducible nitric oxide synthase (iNOS), an enzyme essential for the generation of reactive nitrogen intermediates (RNIs), which regulate survival and multiplication of intracellular bacteria.

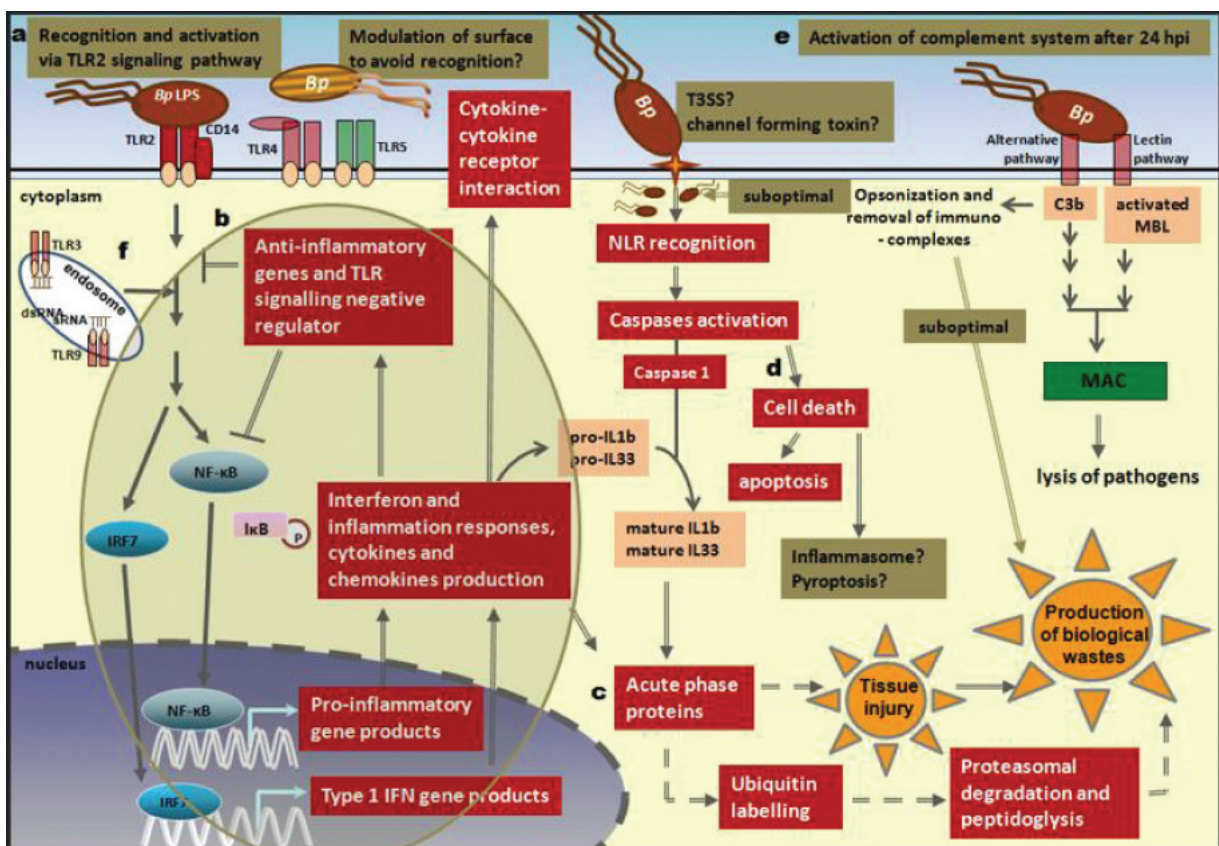
However, macrophages infected with *B. pseudomallei* do not activate iNOS expression (Utainsincharoen *et al.*, 2004). A recent report has also indicated that *B. pseudomallei* might evade killing by macrophages and dendritic cells through the induction of caspase-1-dependent host-cell death that resembles oncosis, similar to the caspase-1-dependent death described for *Salmonella* and *Shigella* spp. (Sun *et al.*, 2005).

#### *In vivo* models

While *in vitro* studies are of practical importance to provide insight into the pathogenesis of *B. pseudomallei*, it is critical to understand host response to infection using *in vivo* models which reflect multiple cell types, tissues and interactions, in addition to signaling events and expression cascades (reviewed in Warawa (2010)). Rodents are routinely used as an *in vivo* model of *B. pseudomallei* infection. The more susceptible BALB/c and the relatively resistant C57BL/6 inbred mouse strains have been used extensively in studies of host responses to *B. pseudomallei* to mimic the acute and chronic disease in humans (Hoppe *et al.*, 1999). Following infection with *B. pseudomallei*, there is a rapid influx and activation of neutrophils. The importance of neutrophils in innate immunity is well recognized and further demonstrated with the establishment of an acute *B. pseudomallei* infection in C57BL/6 mice depleted of neutrophils (Easton *et al.*, 2007). Furthermore, myeloid differentiation primary response gene 88 (MyD88) knockout mice demonstrated increased susceptibility to *B. pseudomallei* infection as a result of reduced neutrophil recruitment and activation. This revealed that MyD88, as the key signaling adaptor for most TLRs, the IL-1-receptor and IL-18 receptor, is involved in protective neutrophil recruitment (Wiersinga *et al.*, 2008). In addition, the pro-inflammatory cytokine interferon (IFN)- $\alpha$  has been shown to have an important role in early resistance against *B. pseudomallei* infection with additional vital roles played by tumor necrosis factor (TNF)- $\alpha$ , (IL)-12 and IL-18 in the T<sub>H</sub>1 cell-mediated immune response (Easton *et al.*, 2007; Wiersinga *et al.*, 2007). The role of T<sub>H</sub>1 cell-mediated immune response in protecting against melioidosis was evident from studies demonstrating that T<sub>H</sub>1-response-prone C57BL/6 mice are relatively resistant to *B. pseudomallei* when compared with the T<sub>H</sub>2-response-prone BALB/c mice (Hoppe *et al.*, 1999). However, levels of proinflammatory cytokines were found to be significantly elevated in patients with melioidosis, resulting in excessive inflammation which fails to control the infection but contributes to tissue destruction and multiple organ failure (Gan, 2005; Wiersinga *et al.*, 2007).

The use of the murine model of melioidosis has provided a detailed picture of how *B. pseudomallei* affects a whole range of host mediator systems, including toll-like receptor (TLR) signaling and coagulation during a systemic infection. The interaction of *B. pseudomallei* with TLR2, TLR4 and CD14 was confirmed by reduced tumor necrosis factor (TNF)- $\alpha$  expression in leucocytes from knockout mice. Following *B. pseudomallei* infection, TLR4 knockout mice demonstrated wild-type mortality, whereas both TLR2 and CD14 knockout mice demonstrated reduced mortality, bacterial loads and inflammation. In addition, both TLR4 and TLR2 have been shown to detect the LPS of *B. pseudomallei* and the macrophage cytokine response to LPS is totally dependent on binding to TLR4 (West *et al.*, 2008) although TLR2 has been identified as an agonist (Wiersinga *et al.*, 2007). Chin *et al.* (2010) investigated the gene expression profile of *B. pseudomallei* induced immunity by

microarray-based expression profiling of a murine acute-phase melioidosis model and highlighted the crucial link between innate and adaptive immunity in responses to *B. pseudomallei* infection. They also demonstrated that TLR2 was induced to initiate an inflammatory response to the acute *B. pseudomallei* infection. Nevertheless, the overwhelmed inflammatory response to infection as well as tissue injury leads to metabolic disturbances and homeostatic imbalance which is detrimental to the host, corroborating the notion that development of acute disease is not due to a lack of but rather, an excess of inflammation (Gan, 2005). More importantly, this study has also demonstrated that suboptimal activation and function of the downstream complement system promotes uncontrolled spread of the bacteria, eventually leading to death of the infected host (summarised in Fig. 3). The strongest risk factor associated with severe melioidosis is diabetes. In a separate study using a diabetic mouse



**Fig. 3.** A summary of host immune responses to *B. pseudomallei* acute infection

Upon encountering *B. pseudomallei*, the host responds with a multitiered defense approach to protect itself. a) TLR2 and TLR4 are responsible for initiation of host defense responses to *B. pseudomallei*; b) Many of the highly elevated inflammatory genes are “alarm signals” commonly seen in general inflammation infection as represented in the shaded area; c) High inflammation responses lead to occurrence of tissue injury, that ultimately activates the ubiquitin-mediated proteolysis; d) Activation of the caspase and cell death programs to reduce a favorable niche for the intracellular pathogen; e) Suboptimal activation and function of the downstream complement system promotes uncontrolled spread of intracellular pathogen. f) Recognition of endogenous signals that resulted from tissue injury by TLRs amplifies the inflammatory responses. Arrow represents activator connection; lines ending in bars represent inhibitory connection; solid lines represent direct activation; dashed lines represent indirect activation. The boxes highlighted in red depict the up-regulated biological processes, while the box highlighted in green depicts the down-regulated biological processes.



model, Chin *et al.* (2011) demonstrated that the presence of elevated glucose levels impairs the host innate immune system by delaying the identification and recognition of *B. pseudomallei* surface structures, resulting in delayed activation of various inflammatory and immune responses as well as the general “alarm signal” of infection. This could explain the increased susceptibility of diabetic patients to this fatal disease.

A novel approach to studying the pathogenesis of *B. pseudomallei* using an invertebrate model *Caenorhabditis elegans* was developed by Jeddloh and colleagues (O’Quinn *et al.*, 2001). They found that nematode killing appears to be mediated by a toxin which was shown to be UV-labile but gamma-ray resistant. Additionally, they also showed that a number of characterized mutations, including those affecting the Amr multidrug efflux system, LPS O-antigen synthesis, the general protein secretion machinery (*gspD*) and flagella (*fliC*) had little effect on this toxin-based killing. A separate study has also reported the use of *C. elegans* to study pathogenesis of *B. pseudomallei*, particularly on the perspective of identifying pathogen virulence determinants in *B. pseudomallei* by initially screening for bacterial mutants attenuated in virulence towards *C. elegans* (Gan *et al.*, 2002). Our group has recently also developed the *B. pseudomallei* – *C. elegans* pathogenesis model system. We demonstrated that direct prolonged interaction between the host (*C. elegans*) and the pathogen (*B. pseudomallei*) is required for a complete lethal effect as the killing of worms requires living or proliferating bacteria to continuously produce toxins in order to mediate the full killing effect (Lee *et al.*, 2007; Ooi *et al.*, 2012). To understand the host defense response to *B. pseudomallei* infection within the context of a whole organism, we performed a genome-wide transcriptome analysis on infected *C. elegans*. The use of a microarray-based platform technology has successfully deciphered the *C. elegans* immune response especially the signalling pathways that are triggered and the defense arsenal that is deployed, in response to *B. pseudomallei* infection. Interestingly, the analysis revealed a previously undescribed mechanism by which *B. pseudomallei* suppresses the host immune defense by specifically targeting an intestinal transcription factor, GATA/ELT-2, hence reducing its availability and subsequently repressing the expression of ELT-2 transcriptional targets which are intestinally-expressed host defense effectors. Our findings suggest that *B. pseudomallei* employs a mechanism distinct from other pathogens in causing a more detrimental and malicious assault upon the host (Lee *et al.* in preparation).

## CONCLUSION

Much progress on melioidosis research has been made in recent years, especially with respect to genetics and genomics, epidemiology, diagnosis and treatment, identification of virulence factors and pathogenesis. Despite the vast advances in our understanding of important virulence factors as well as the molecular and cellular basis of the host response to *B. pseudomallei* infection, the pathogenic factors and specific mechanisms of intracellular survival still remain an enigma. Thus, continued research on identifying critical virulence factors in *B. pseudomallei* is crucial to uncover a new and effective vaccine against melioidosis. Nonetheless, more efforts have to be devoted to understanding the complex interaction between host and pathogen factors that not only shed light on pathogenic mechanisms but aid in discovering specific protein targets for drug development to combat this disease.

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