MINIREVIEW

Glanders: off to the races with *Burkholderia mallei*

Gregory C. Whitlock[1], D. Mark Estes[1,2,3] & Alfredo G. Torres[1,3,4]

[1]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA; [2]Department of Pediatrics, University of Texas Medical Branch, Galveston, TX, USA; [3]Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX, USA; and [4]Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA

Correspondence: Alfredo G. Torres, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1070, USA. Tel.: +00 1 409 747 0189; fax: +00 1 409 747 6869; e-mail: altorres@utmb.edu

Received 13 August 2007; accepted 14 September 2007.
First published online 26 October 2007.

DOI:10.1111/j.1574-6968.2007.00949.x

Editor: Ian Henderson

Keywords
Burkholderia; *Burkholderia mallei*; glanders; virulence factors; immune responses.

Abstract

Burkholderia mallei, the etiologic agent of the disease known as glanders, is primarily a disease affecting horses and is transmitted to humans by direct contact with infected animals. The use of *B. mallei* as a biological weapon has been reported and currently, there is no vaccine available for either humans or animals. Despite the history and highly infective nature of *B. mallei*, as well as its potential use as a bio-weapon, *B. mallei* research to understand the pathogenesis and the host responses to infection remains limited. Therefore, this minireview will focus on current efforts to elucidate *B. mallei* virulence, the associated host immune responses elicited during infection and discuss the feasibility of vaccine development.

History and clinical importance

Burkholderia mallei, the etiologic agent of glanders disease, is a Gram-negative, nonmotile, facultative intracellular bacterium. Most known members of the family *Burkholderiaceae* are resident in the soil; however, *B. mallei* is an obligate mammalian pathogen. Horses are highly susceptible to infection and are considered to be the natural reservoir for infection, although mules and donkeys are susceptible as well (Neubauer *et al.*, 2005). Glanders maintains a lengthy history dating back to Aristotle in *c.* 350 BC when the disease was given the name 'melis'. Identification of the etiologic agent *B. mallei* was described in 1882 by isolating an organism from the infected liver and spleen of a horse (Schadewaldt, 1975). Since its discovery, the pathogen has been classified as *Loefflerella mallei*, *Pfeifferella mallei*, *Malleomyces mallei*, *Actinobacillus mallei*, *Corynebacterium mallei*, *Mycobacterium mallei*, *Pseudomonas mallei* and *Bacillus mallei*. The current genus *Burkholderia* was a result of DNA–DNA homology values, cellular lipid and fatty acid composition and phenotypic characteristics, as well as 16S rRNA gene typing in 1992 (Yabuuchi *et al.*, 1992). Members of this genus include *Burkholderia pseudomallei*, the causative agent of melioidosis (Dance, 1990), *Burkholderia cepacia*, an important pathogen in cystic fibrosis patients (Burns *et al.*, 1996), and *Burkholderia thailandensis*, a relatively low-virulence bacterium (Glass *et al.*, 2006). Clinically, *B. mallei* infected solipeds can present with either a chronic (horses) or an acute (mules and donkeys) form. Although eradication has been successful in the United States, glanders is endemic among domestic animals in Africa, Asia, the Middle East and Central and South America. The primary route of equine infection is most likely the consumption of feed or water contaminated with nasal discharges of infected animals, although a cutaneous form also exists, known as farcy. Chronically infected animals present a variety of signs and symptoms dependent on the route of infection including mucopurulent nasal discharge, lung lesions and nodules involving the liver and spleen. Acute infection results in high fever and emaciation, with ulceration of the nasal septum, accompanied by mucopurulent to hemorrhagic discharge. Pathological changes are limited in gut-associated lymphatic tissues, with the majority of pathology occurring in the lungs and airways (Schlater, 1992). Human infection with *B. mallei* has occurred rarely and sporadically among laboratory workers and those in direct contact with infected animals (Srinivasan *et al.*, 2001). Person-to-person spread of *B. mallei* is extremely rare. The bacteria usually enter the

body through the eyes, nose, mouth or breaks in the skin. In humans, glanders is characterized by initial onset of fever, rigors and malaise, culminating in a rapid onset of pneumonia, bacteremia, pustules and abscesses, leading to death in 7–10 days without antibiotic treatment. The course of infection is dependent on the route of exposure. Direct contact with the skin can lead to a localized cutaneous infection. Inhalation of aerosol or dust containing *B. mallei* can lead to septicemic, pulmonary or chronic infections of the muscle, liver and spleen. The disease has a 95% case fatality rate for untreated septicemia infections and a 50% case fatality rate in antibiotic-treated individuals (Mandell *et al.*, 1995).

Burkholderia mallei organisms are highly infectious as an aerosol, and infection requires few organisms, offering the potential for intentional release as a biological threat agent. The use of *B. mallei* as a biological warfare agent during the American Civil War, World Wars I and II and the Russian invasion of Afghanistan has been reported, and, as such, is now considered a select agent due to its potential use as a bio-weapon (Wheelis, 1998; Rotz *et al.*, 2002; Horn, 2003). As a result of this potential threat, research involving *B. mallei* is confined to biosafety level 3 facilities in the United States and other countries worldwide. Despite the history and highly infective nature of *B. mallei*, as well as its potential use as a bio-weapon, *B. mallei* research to understand the pathogenesis and the host responses to infection remains limited. Therefore, this minireview will focus on current efforts to elucidate *B. mallei* virulence; the associated host immune responses elicited during infection; and discuss the feasibility of vaccine development.

Burkholderia mallei pathogenic determinants

The *B. mallei* strain ATCC 23344, isolated in 1944 from postmortem human cultures of blood and secretions, was sequenced to reveal two circular chromosomes comprising a total of *c.* 5.8 Mb and a G/C content of *c.* 69% (Nierman *et al.*, 2004). A total of 5535 predicted protein-encoding ORFs have been identified in the genome. Genomic comparisons with the closely related species *B. pseudomallei* and *B. thailandensis* reveal significant homology, being *c.* 99% identical between conserved genes in *B. pseudomallei*, although *B. mallei* contains roughly 1.41 Mb less DNA than *B. pseudomallei* (Godoy *et al.*, 2003; Nierman *et al.*, 2004). Comparative analysis of *B. mallei* with virulence-associated genes found in pathogenic bacteria has revealed genes encoding an exopolysaccharide capsule, a lipopolysaccharide, type IV pili, as well as type III (plant- and animal-like) and type VI secretion systems (Fig. 1). Interestingly, most of the secretion systems and virulence-associated genes described so far are located in chromosome 2, while genes involved in metabolism, capsule production and lipopolysaccharide biosynthesis are found in chromosome 1.

Initial reports suggested that *B. mallei* lacked a capsule (Jennigs, 1963; Kovalev, 1971). However, subsequent studies have demonstrated that a polysaccharide capsule is present in *B. mallei*, and this extracellular structure is a virulence determinant (Popov *et al.*, 1995, 2000; Fritz *et al.*, 1999, 2000; DeShazer *et al.*, 2001). Using subtractive hybridization, DeShazer *et al.* identified a polysaccharide gene cluster in *B. mallei* ATCC 23344 antigenically similar to *B. pseudomallei*. Further molecular characterization indicated *wcbF* as an essential gene for the production of capsular polysaccharide. Additionally, demonstration of a surface capsule on *B. mallei* ATCC 23344 by immunogold electron microscopy has been noted (Fritz *et al.*, 2000; DeShazer *et al.*, 2001). Challenge with the wild type and the capsule negative mutant showed a $>10^5$-fold difference in virulence in Syrian hamsters and a $>10^3$-fold difference in BALB/c mice when infected by the intraperitoneal and aerosol route respectively, demonstrating that the capsular polysaccharide is a key virulence factor (DeShazer *et al.*, 2001). Similar studies with *B. pseudomallei* have identified genes responsible for the production of capsular polysaccharides demonstrating comparable virulence association (Reckseidler *et al.*, 2001).

Another surface structure in *B. mallei* is the lipopolysaccharide, which is predicted to possess a lipid component (Lipid A), a core region and cell wall antigens (O antigens). Analysis of lipopolysaccharide profiles of *B. mallei* strains has helped aid the identification of the gene cluster responsible for O polysaccharide biosynthesis in *B. mallei* ATCC 23344 (Burtnick *et al.*, 2002). Sequence analysis revealed a 19.9-kb contiguous sequence containing the entire *B. mallei* O polysaccharide biosynthetic gene cluster. Sequence alignment of the *B. pseudomallei* and *B. mallei* O polysaccharide biosynthetic regions revealed 99% identity at the nucleotide level, with nomenclature consistent with that of *B. pseudomallei*. Resistance to the bactericidal effects of normal human serum (NHS) has been demonstrated for both *B. pseudomallei* and *B. mallei* ATCC 23344, while those strains lacking intact O polysaccharide moieties (NCTC 120 and ATCC 15310) were completely killed following incubation with 30% NHS, implicating lipopolysaccharide as a potential virulence determinant (DeShazer *et al.*, 1998; Burtnick *et al.*, 2002). Furthermore, *B. mallei* lipopolysaccharide has been shown to activate hTLR4 complexes on HEK 293-hTLR4/MD2-CD14-expressing cells as well as stimulate cytokine and chemokine responses by antigen-presenting cells (Brett *et al.*, 2007).

It is well known that the interaction of bacteria with host cell membranes is a prerequisite step for the infectious process to occur. The adherent capability of *Burkholderia* species has received little attention, with the exception of *B. pseudomallei*, *B. thailandensis* and *B. cepacia* (Chiu *et al.*,

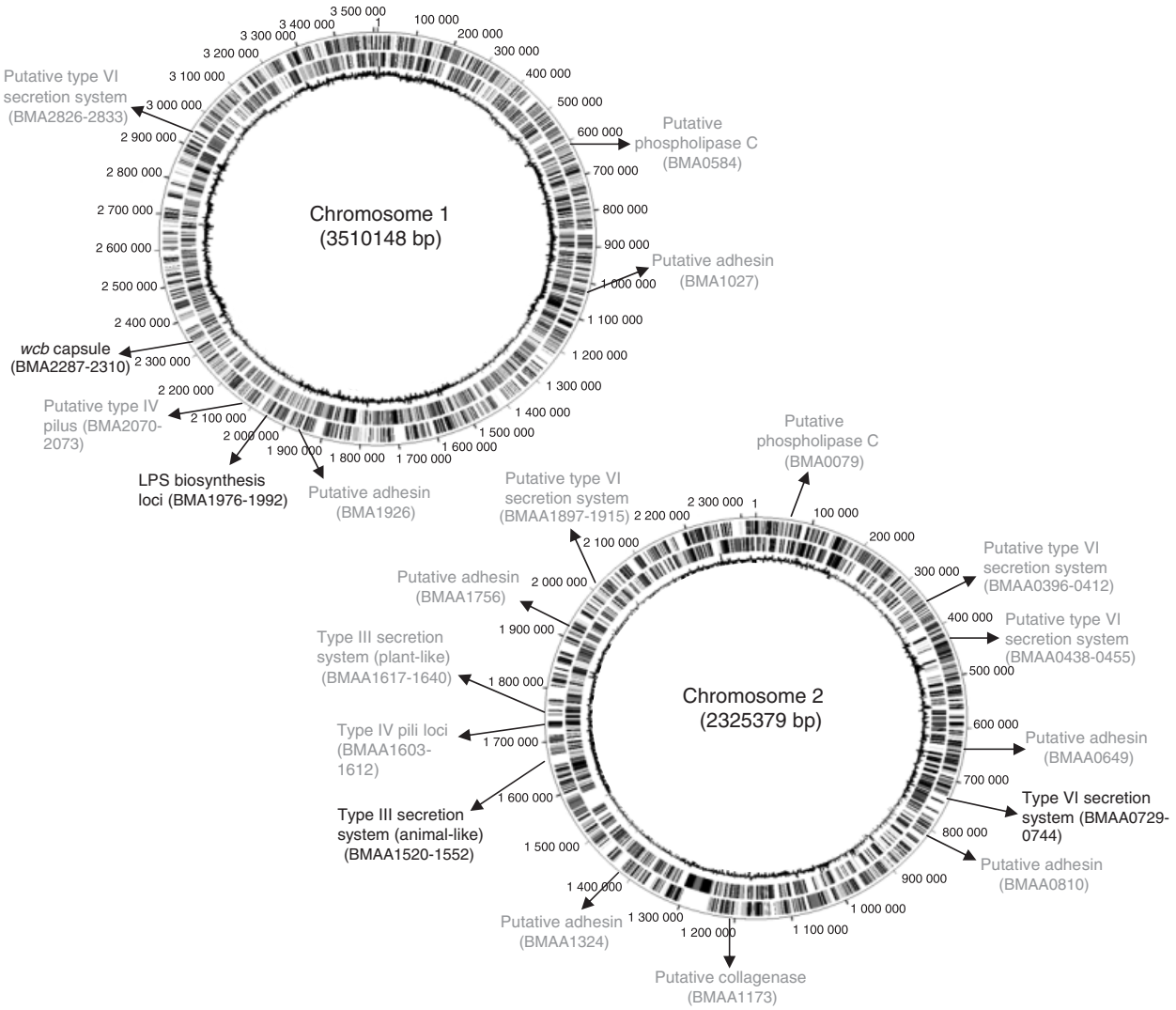

Fig. 1. Circular diagrams of chromosome 1 and chromosome 2 in *Burkholderia mallei*. Locations of selected genes encoding known virulence factors (black letters) and potential virulence-associated elements (gray letters) as displayed in PATHEMA (http://pathema.tigr.org/tigr-scripts/Burkholderia/PathemaHomePage.cgi). The size of the two chromosomes and the locus tag number for each one of the genes are also depicted.

2001; Brown *et al*., 2002; Kespichayawattana *et al*., 2004). Investigations of temperature regulation on adherence to eukaryotic cells *in vitro* using the clinical isolate *B. pseudomallei* 08 revealed increased adherence when grown at 30 vs. 37 °C (Brown *et al*., 2002). Additionally, adherence demonstrated a statistically significant increase when experiments were conducted with stationary-phase cultures as opposed to those grown to the log phase (Brown *et al*., 2002). Conversely, temperature failed to modulate the direct adherence of *B. pseudomallei* K96243 to eukaryotic cells (Boddey *et al*., 2006). *Burkholderia pseudomallei* K96243 contains a type IV pilin protein, PilA, which contributes to adherence to cultured respiratory cell lines, implicating this pili as a potential mediator in the pathogenic process (Essex-Lopresti *et al*., 2005). The ability to colonize the eukaryotic monolayer may involve multiple cellular interactions, depending on the strain: bacterium–cell interactions (adherence) and bacterium–bacterium interactions (microcolonies). To the authors' knowledge, no adherence or microcolony studies exist in the literature for *B. mallei*, although a type IV pilin protein has been identified, showing no protective characteristics following active or passive immunization (Fernandes *et al*., 2007). The lack of protection offered by type IV pili immunization may, in part, be due to a lack of pili production during infection. Although antipilin antibody titers were detected in immunized animals, it is possible that these antibodies were the result of preformed pili in the inoculating dose.

An additional feature common to *B. mallei*, *B. pseudomallei* and *B. cepacia* is the localization of infection to the lungs and respiratory airways. Aerogenic exposure of

B. mallei to BALB/c mice includes localization of the pathogen in the upper and lower sections of the respiratory tract and transportation of bacteria within alveolar macrophages to regional lymph nodes, suggestive of intracellular survival (Lever *et al.*, 2003). Additionally, certain species of *Burkholderia* are capable of intracellular invasion in both phagocytic and nonphagocytic cell lines, such as RAW 264.7, J774.2, J774.1, A549, HeLa and Vero cells (Jones *et al.*, 1996; Harley *et al.*, 1998; Pilatz *et al.*, 2006; Ribot & Ulrich, 2006). Investigations into the intracellular characteristics of *B. mallei* have predominantly focused on the type III secretion system (TTSS) as a possible mechanism responsible for the invasion, escape from intracellular compartments and survival in host cells. *Burkholderia mallei* carries two chromosomal regions encoding for TTSSs: one with similarity to animal-like TTSS and a second TTSS similar to that found in plant pathogens (Fig. 1). The *B. mallei* ATCC 23344 animal-like TTSS encodes 30 ORFs, and it is genetically similar to that of *B. pseudomallei* (Hueck, 1998). These *B. mallei* TTSS genes are clustered in a distinct region of chromosome 2, sharing homology with both *Salmonella inv/spa/prg* and *Shigella ipa/mxi/spa* TTSSs (Table 1). Correlations of the *B. mallei* TTSS and pathogenicity have been established. A functional animal-like TTSS is required for full pathogenicity of *B. mallei* ATCC 23344 in the BALB/c mouse and Syrian hamster models of infection (Ulrich & DeShazer, 2004). Mutations in the *B. mallei bsaQ* and *bsaZ* genes (homologues of *Salmonella invA* and *spaS* genes) demonstrated decreased virulence as evident by survival, clinical signs and bacterial burden of the lungs, liver and spleen (Ulrich & DeShazer, 2004). Although *B. mallei* TTSS mutants display attenuated virulence, the protection offered by these mutants has been shown to be suboptimal, suggesting that vaccine candidates may exist within the TTSS, e.g. the TTSS effector molecules or the TTSS needle cap protein (G.C. Whitlock & A.G. Torres, unpublished data). The requirement of a competent TTSS for intracellular survival of *B. mallei* within phagocytic cell lines has been documented; however, the experimental approach adopted was limited to the contribution of a functional TTSS apparatus by disruption of protein delivery (Ribot & Ulrich, 2006). Therefore, identification of putative effector molecules is essential to decipher the molecular mechanisms associated with the *B. mallei* TTSS. *In silico* analysis reveals a second gene cluster encoding a plant-like TTSS in *B. mallei*, which displays 99% sequence identity to *B. pseudomallei*, and in a lesser degree to plant TTSS analogs (Rainbow *et al.*, 2002). Identification of a plant host for *B. mallei* has not been determined, although the presence of an apparently intact plant-like TTSS within the *B. mallei* genome (Table 2) suggests that a possible contribution of this secretion system to bacterial virulence may exist.

A genomic search of *B. mallei* ORFs assigned to conserved components of the highly regulated type VI secretion systems (T6SSs) revealed four putative T6SSs located on chromosome 2 and one truncated on chromosome 1 (BMA2826–2833) (Schell *et al.*, 2007). Three of these T6SS gene clusters (clusters 1–3) possess >80% sequence conservation with *B. pseudomallei* and *B. thailandensis*, while cluster 4 (BMAA1897–1915) is exclusive to pathogenic *B. mallei* and *B. pseudomallei*. Cluster 1 (BMAA0729–0744) appears to be transcriptionally regulated by the VirAG two-component system and AraC-type regulator BMAA1517. A hemolysin-coregulated protein (Hcp) family member encoded in cluster 1 is secreted *in vitro* by this T6SS and displays immunogenic characteristics in mice, horses and humans (Schell *et al.*, 2007). In addition, overexpression of VirAG or BMAA1517 results in enhanced secretion of the T6SS encoded protein Hcp1. Syrian hamsters injected intraperitoneal with *B. mallei* T6SS mutants demonstrated 100% survival compared with *B. mallei* ATCC 23344 wild-type challenged animals up to 14 days (Schell *et al.*, 2007). Further, immunoglobulin recognition of Hcp1 in multiple infected hosts demonstrates the ability of Hcp1 expression *in vivo*, lending to its potential use as a candidate vaccine.

Host immune responses to *B. mallei* infections

Currently, there is no available animal or human vaccine against *B. mallei* infection. Development of an effective vaccine will require an animal model capable of predicting immune components necessary to induce immunity while exhibiting pathogenesis seen in human disease. Among potential rodent model species for vaccine development, guinea-pigs and hamsters are the most susceptible to *B. mallei* infection. Mice are relatively intermediate in susceptibility on par with humans and are thus an ideal model for development of vaccine targets and immune functional studies. Mouse strains vary in the relative levels of susceptibility, with BALB/c mice ranking among the more susceptible strains and C57BL6 as the more resistant by both intraperitoneal and aerosol methods of infection (Fritz *et al.*, 2000; Lever *et al.*, 2003). Immune correlates of protection are not fully developed for *B. mallei* although its capacity to replicate inside the host cell suggests that type 1-mediated cellular immune responses will be linked to a protective response. Recent vaccine studies utilizing various nonviable *B. mallei* strains (encapsulated or not) have demonstrated that a mixed type 1/type 2 response is not protective. These studies evaluated both antigen-specific isotype (IgG1-type 2 vs. IgG2a-type 1) and cytokine responses to recall stimulation as a readout system (Amemiya *et al.*, 2002). Following intraperitoneal inoculation of *B. mallei* ATCC 23344, BALB/c mice demonstrate a strong

Table 1. *Burkholderia mallei* Type III secretion (animal-like) system

Protein or gene name	Similarity to *Burkholderia* species	Similarity to other species	Predicted function and characteristics
BicP	BicP (*B. pseudomallei*)	Tir (*Shewanella baltica*)	Type III secretion chaperone
BopA	BopA (*B. pseudomallei*)	IcsB (*S. dysenteriae*)	Putative type III secreted intercellular spread protein
BMAA1522	BURPS1106A_A2066 (*B. pseudomallei*)		Unknown
BopE	BopE (*B. pseudomallei*)	SopE (*S. enterica*)	Type III secreted G-nucleotide exchange factor
BapC	BapC (*B. pseudomallei*)	Putative transglycosylase (*Ralstonia eutropha*)	Putative exported and invasion protein
BapB	BapB protein (*Burkholderia thailandensis*)	ACP (*Thermotoga maritima*)	Acyl carrier protein of unknown function
BapA		ISS (*Ostreococcus tauri*)	Putative protein disulfide-isomerase
BprD	BipD (*B. pseudomallei*)	IpaD (*S. dysenteriae*)	Type III secretion needle tip protein (IpaD/SipD/SspD family)
BprA	BprA (*B. pseudomallei*) and HNS (*B. vietnamiensis*)		Putative HNS-like regulatory protein
BipC	BipC (*B. pseudomallei*)	IpaC (*S. flexneri*)	Type III secretion effector (IpaC/SipC family)
BipB	BipB protein (*B. pseudomallei*)	SipB (*S. enterica*)	Type III secreted cell invasion protein
BicA	BicA (*B. pseudomallei*)	SicA (*Salmonella enterica*)	Type III secretion low calcium response chaperone (LcrH/SycD family)
BsaZ	BsaZ (*B. pseudomallei*)	SpaS (*S. typhimurium*)	Type III secretion apparatus protein (YscU/HrpY family)
BsaY	BsaY (*B. pseudomallei*)	YsaT (*Y. enterocolitica*)	Type III secretion apparatus protein (SpaR/YscT/HrcT family)
BsaX	BsaX (*B. pseudomallei*)	YsaS (*Y. enterocolitica*)	Type III secretion apparatus protein
BsaW	BsaW (*B. pseudomallei*)	YsaR (*Y. enterocolitica*)	Type III secretion apparatus protein (YscR/HrcR family)
BsaV	BsaV (*B. pseudomallei*)	SpaO (*S. enterica*)	Type III secretion system apparatus protein (YscQ/HrcQ family)
BsaU	BsaU (*B. pseudomallei*)		Putative surface presentation of antigens protein
BsaT	BsaT (*B. pseudomallei*)		Putative surface presentation of antigens protein
BsaS	BsaS (*B. pseudomallei*)	SpaL (*S. enterica*)	Type III secretion system ATPase
BsaR	BsaR (*B. thailandensis*)	SpaK (*S. enterica*)	Type III secretion system protein
BsaQ	BsaQ (*B. thailandensis*)	YsaV (*Y. enterocolitica*)	Type III secretion system apparatus protein
BsaP	BsaP (*B. pseudomallei*)	MxiC (*S. flexneri*)	Type III secretion regulator (YopN/LcrE/InvE/MxiC family)
BsaO	BsaO (*B. pseudomallei*)	InvG (*S. enterica*)	Type III secretion outer membrane pore (YscC/HrcC family)
BsaN	BsaN (*B. pseudomallei*)	InvF (*S. enterica*)	Type III secretion system transcriptional regulator (AraC family)
BMAA1546	BURPS1106A_A2090 (*B. pseudomallei*)		Conserved hypothetical protein of unknown function
BsaM	BsaM (*B. pseudomallei*)	PrgH (*S. enterica*)	Type III secretion system needle protein (PrgH/EprH family)
BsaL	BsaM (*B. pseudomallei*)	EprI (*E. coli* O157:H7)	Type III secretion system needle protein
BsaK	BsaM (*B. pseudomallei*)	PrgJ (*S. typhimurium*)	Needle complex minor subunit
BsaJ	BsaM (*B. pseudomallei*)	EprK (*E. coli* O157:H7)	Type III secretion apparatus lipoprotein (YscJ/HrcJ family)
OrgA	OrgA (*B. pseudomallei*)	OrgA (*S. typhimurium*)	Oxygen-regulated type III secretion system protein (OrgA/MxiK family)
BMAA1552	Bpse17_02004987 (*B. pseudomallei*)	OrgB (*S. typhimurium*)	Oxygen-regulated type III secretion apparatus protein (HrpE/YscL family)

IFN-γ response, accompanied by IL-18, IL-12, IL-27 and IL-6 expression within 24 h after infection (Rowland *et al.*, 2006). Stimulation of Toll-like receptor 9 (TLR9) with a CpG-containing oligodeoxynucleotide exhibited elevated IL-12 and IFN-γ levels following *B. mallei* aerosol challenge (Waag *et al.*, 2006). Additionally, BALB/c mice pretreated intraperitoneal with CpG motifs showed increased survival and lower levels of *B. mallei* in the lungs and spleens (Waag *et al.*, 2006). Aerogenic exposure to *B. mallei* auxotrophs shows enhanced production of whole bacillus-specific IgG2a over IgG1, demonstrating the potential in protection against aerosol challenge, in addition to studies that have used heat-killed or irradiated bacilli and the type 1 inductive cytokine IL-12 (Ulrich *et al.*, 2005; Amemiya *et al.*, 2006). IL-12, in combination with nonviable *B. mallei*, has been shown to be superior in inducing protection relative to killed bacteria alone. Much of the enhancement is thought to occur via increased production of IFN-γ and activation of macrophages although the cellular source of IFN-γ [conventional αβ T cells, natural killer (NK) cells, NK T cells or

Table 2. *Burkholderia mallei* Type III secretion (plant-like) system

Protein or gene name	Similarity to *Burkholderia* species	Similarity to other species	Predicted function and characteristics
BMAA1617	BPSS1607 (*B. pseudomallei*)	HrpB1 (*Xanthomonas oryzae* pv. oryzicola)	Type III secretion system protein
BMAA1618	Bpse17_02005433 (*B. pseudomallei*)	RimL (*Anabaena variabilis*)	Putative acetyltransferase
BMAA1619	BURPS305_2288 (*B. pseudomallei*)		Hypothetical protein of unknown function
BMAA1620	BURPS1106A_A2187 (*B. pseudomallei*)		Hypothetical protein of unknown function
HrpB	HrpB (*B. pseudomallei*)	HrpB (*Ralstonia solanacearum*)	Type III secretion transcriptional regulator (AraC/XylS family)
BMAA1622	BPSS1611 (*B. pseudomallei*) and HpaB (*B. cenocepacia*)		Type III secretion chaperone
BMAA1623	BURPS1710b_A0670 (*B. pseudomallei*)		Hypothetical protein of unknown function
BMAA1624	Bpse110_02005542 (*B. pseudomallei*) and BTH_II0759 (*B. thailandensis*)		Putative type III secretion protein
BMAA1625	Pseudogene		Authentic frameshift of a type III secretion inner membrane protein
BMAA1626	BpseP_03005479 (*B. pseudomallei*) and BTH_II0756 (*B. thailandensis*)		Putative type III secretion protein
SctS (EscS)	Hrp (*B. pseudomallei*)	HrcS (*Xanthomonas oryzae*)	Type III secretion inner membrane protein (HrpO family)
SctR (EscR)	SctR (*B. pseudomallei*) and YscR (*B. cepacia*)		Type III secretion apparatus protein (YscR/HrcR family)
SctQ	SctQ (*B. pseudomallei*) and SPOA (*B. thailandensis*)		Type III secretion inner membrane protein (YscQ/HrcQ family)
SctV (EscV)	HrcV (*B. pseudomallei*) and EscV (*B. dolosa*)		Type III secretion inner membrane protein (HrcV family)
BMAA1631	BpseP_03005485 (*B. pseudomallei*)	HrcU protein (*Xanthomonas oryzae*)	Type III secretion inner membrane protein (YscU/HrpY family)
BMAA1632	BPSS1622 (*B. pseudomallei*)	HrpB1 (*Xanthomonas oryzae*)	Type III secretion protein (HrpB1/HrpK family)
BMAA1633	HrpB2 (*B. pseudomallei*)	HrpB2 (*Xanthomonas oryzae*)	Type III secretion protein
BMAA1634	Pseudogene		Authentic frameshift of a type III secretion inner membrane protein
BMAA1635	HrpB4 (*B. pseudomallei*)	HrpB4 (*Xanthomonas campestris*)	Type III secretion protein
SctL	SctL (*B. pseudomallei*)	HrpF (*Ralstonia solanacearum*)	Type III secretion inner membrane protein (HrpE/YscL family)
SctN	SctN (*B. pseudomallei*) BMAA_BmtmRNA3	HrpB6 (*Xanthomonas oryzae*) sRNA	Type III secretion system ATPase
SctT	SctT (*B. pseudomallei*)	HrcT (*Acidovorax avenae*)	Type III secretion inner membrane protein (SpaR/YscT/HrcT family)

γδ T cells] is not well defined for *B. mallei*. In naive mice, IFN-γ production has been shown to be essential for protection from *B. pseudomallei* infection in the first 24 h (Santanirand *et al*., 1999; Haque *et al*., 2006). The studies of *B. pseudomallei* and T cell-mediated immunity suggest that IFN-γ-expressing cells should be the targets for an effective vaccination strategy. *Burkholderia mallei* heat-killed vaccinated BALB/c mice, depleted of TNF-α or IFN-γ, show 100% mortality following intraperitoneal challenge with *B. mallei* ATCC 23344 (G.C. Whitlock, R.A. Lukaszewski, B.M. Judy, S. Paessler, A.G. Torres & D.M. Estes, unpublished data). The effector role for IFN-γ in mediating protection against *B. mallei* may include both immunoregulatory and nonregulatory functions. Regardless, the requirement of IFN-γ, as demonstrated by administration of neutralizing antibody before infection, indicates that stimulation of IFN-γ response is a desirable goal for a *B. mallei* vaccine. Moreover, BALB/c mice with an established *B. mallei* chronic infection rapidly lose the ability to control the growth of the bacillus upon neutralization of TNF-α (G.C. Whitlock, R.A. Lukaszewski, B.M. Judy, S. Paessler, A.G. Torres & D.M. Estes, unpublished data), demonstrating a role for sustained TNF-α production in the maintenance of host survival throughout the course of *B. mallei* infection. Following depletion of $B220^{+}$ cells in heat-killed vaccinated BALB/c mice, survival rates decrease as much as 100% relative to nondepleted controls when challenged intraperitoneal. Additionally, relatively resistant

C57BL/6 mice deficient in mature B-cells (μMT) succumb to similar mortality rates (G.C. Whitlock, R.A. Lukaszewski, B.M. Judy, S. Paessler, A.G. Torres & D.M. Estes, unpublished data). Both antibody responses (IgG2a/IgG1 ratios) and passive immunotherapy with monoclonal antibodies to *B. mallei* lipopolysaccharide have demonstrated a role for antibody in protection to infection (Trevino *et al.*, 2006). BALB/c mice injected intraperitoneal with anti-*B. mallei* monoclonal antibodies 18 h before whole-body aerosol challenge with 20 lethal doses 50% (LD_{50}; 1.9×10^4 CFU) of *B. mallei* ATCC 23344 demonstrated 100% survival (Trevino *et al.*, 2006). In contrast, administration of anti-*B. mallei* monoclonal antibodies 18 h postchallenge was ineffective in providing protection (Trevino *et al.*, 2006). Although protection was achieved in the above study, experiments were limited to 14 days postchallenge. The bacterial burden of harvested spleens demonstrated significant numbers of *B. mallei* indicating a lack of sterilizing immunity, suggestive of an antibody-induced reduction of infecting organisms, perhaps below the lethal threshold. Clearance and/or limitation of available pathogen in the initial phase of infection would presumably limit the number of internalized organisms by host cells, thus circumventing the immune-evasive characteristics of an intracellular pathogen. In addition to humoral immunity, a specific cell-mediated immunity will most likely prove advantageous in the development of an efficacious vaccine regimen.

Future challenges: the *B. mallei* vaccine

Burkholderia infections are difficult to treat with antibiotics and as indicated before, no vaccine exists. Aerogenic vaccination with a *B. mallei* auxotroph promoted a Th1-like Ig response, suggesting that live-attenuated strains might serve as promising vaccine candidates (Ulrich *et al.*, 2005). Further, *B. mallei* is susceptible to cell-mediated immune responses, promoting expression of type 1 cytokines, which suggests that the development of an effective vaccine should target the production of IFN-γ (Rowland *et al.*, 2006). The use of these and other approaches relies on a reasonable understanding of the protective innate and adaptive host response, which, at present, is relatively unresolved for *B. mallei* and also in part for *B. pseudomallei*.

Acknowledgements

The authors thank Scott T. Moen for critically reading the manuscript. *Burkholderia* research performed in the authors' labs is supported in part by the UTMB John Sealy Memorial Endowment Fund for Biomedical Research (to A.G.T.), by NIH grant U54 AI057156 (to D.M.E.), and by NIH/NIAID, Task Order 19 (Part-C) Contract No: N01-AI-30065. G.C.W. was funded by a fellowship from the UTMB Sealy Center for Vaccine Development.

References

Amemiya K, Bush GV, DeShazer D & Waag DM (2002) Nonviable *Burkholderia mallei* induces a mixed Th1- and Th2-like cytokine response in BALB/c mice. *Infect Immun* **70**: 2319–2325.

Amemiya K, Meyers JL, Trevino SR, Chanh TC, Norris SL & Waag DM (2006) Interleukin-12 induces a Th1-like response to *Burkholderia mallei* and limited protection in BALB/c mice. *Vaccine* **24**: 1413–1420.

Boddey JA, Flegg CP, Day CJ, Beacham IR & Peak IR (2006) Temperature-regulated microcolony formation by *Burkholderia pseudomallei* requires *pilA* and enhances association with cultured human cells. *Infect Immun* **74**: 5374–5381.

Brett PJ, Burtnick MN, Snyder DS, Shannon JG, Azadi P & Gherardini FC (2007) *Burkholderia mallei* expresses a unique lipopolysaccharide mixture that is a potent activator of human Toll-like receptor 4 complexes. *Mol Microbiol* **63**: 379–390.

Brown NF, Boddey JA, Flegg CP & Beacham IR (2002) Adherence of *Burkholderia pseudomallei* cells to cultured human epithelial cell lines is regulated by growth temperature. *Infect Immun* **70**: 974–980.

Burns JL, Jonas M, Chi EY, Clark DK, Berger A & Griffith A (1996) Invasion of respiratory epithelial cells by *Burkholderia* (*Pseudomonas*) *cepacia*. *Infect Immun* **64**: 4054–4059.

Burtnick MN, Brett PJ & Woods DE (2002) Molecular and physical characterization of *Burkholderia mallei* O antigens. *J Bacteriol* **184**: 849–852.

Chiu CH, Wong S, Hancock RE & Speert DP (2001) Adherence of *Burkholderia cepacia* to respiratory tract epithelial cells and inhibition with dextrans. *Microbiology* **147**: 2651–2658.

Dance DAB (1990) Melioidosis. *Rev Med Microbiol* **1**: 143–150.

DeShazer D, Brett PJ & Woods DE (1998) The type II O-antigenic polysaccharide moiety of *Burkholderia pseudomallei* lipopolysaccharide is required for serum resistance and virulence. *Mol Microbiol* **30**: 1081–1100.

DeShazer D, Waag DM, Fritz DL & Woods DE (2001) Identification of a *Burkholderia mallei* polysaccharide gene cluster by subtractive hybridization and demonstration that the encoded capsule is an essential virulence determinant. *Microb Pathog* **30**: 253–269.

Essex-Lopresti AE, Boddey JA, Thomas R *et al.* (2005) A type IV pilin, PilA, Contributes To Adherence of *Burkholderia pseudomallei* and virulence *in vivo*. *Infect Immun* **73**: 1260–1264.

Fernandes PJ, Guo Q, Waag DM & Donnenberg MS (2007) The type IV pilin of *Burkholderia mallei* is highly immunogenic but fails to protect against lethal aerosol challenge in a murine model. *Infect Immun* **75**: 3027–3032.

Fritz DL, Vogel P, Brown DR & Waag DM (1999) The hamster model of intraperitoneal *Burkholderia mallei* (glanders). *Vet Pathol* **36**: 276–291.

Fritz DL, Vogel P, Brown DR, Deshazer D & Waag DM (2000) Mouse model of sublethal and lethal intraperitoneal glanders (*Burkholderia mallei*). *Vet Pathol* **37**: 626–636.

Glass MB, Gee JE, Steigerwalt AG *et al.* (2006) Pneumonia and septicemia caused by *Burkholderia thailandensis* in the United States. *J Clin Microbiol* **44**: 4601–4604.

Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R & Spratt BG (2003) Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. *J Clin Microbiol* **41**: 2068–2079.

Haque A, Easton A, Smith D *et al.* (2006) Role of T cells in innate and adaptive immunity against murine *Burkholderia pseudomallei* infection. *J Infect Dis* **193**: 370–379.

Harley VS, Dance DA, Drasar BS & Tovey G (1998) Effects of *Burkholderia pseudomallei* and other *Burkholderia* species on eukaryotic cells in tissue culture. *Microbios* **96**: 71–93.

Horn JK (2003) Bacterial agents used for bioterrorism. *Surg Infect (Larchmt)* **4**: 281–287.

Hueck CJ (1998) Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* **62**: 379–433.

Jennigs WE (1963) *Glanders*. Charles C. Thomas, Springfield, IL.

Jones AL, Beveridge TJ & Woods DE (1996) Intracellular survival of *Burkholderia pseudomallei*. *Infect Immun* **64**: 782–790.

Kespichayawattana W, Intachote P, Utaisincharoen P & Sirisinha S (2004) Virulent *Burkholderia pseudomallei* is more efficient than avirulent *Burkholderia thailandensis* in invasion of and adherence to cultured human epithelial cells. *Microb Pathog* **36**: 287–292.

Kovalev GK (1971) Glanders (review). *Zh Mikrobiol Epidemiol Immunobiol* **48**: 63–70.

Lever MS, Nelson M, Ireland PI *et al.* (2003) Experimental aerogenic *Burkholderia mallei* (glanders) infection in the BALB/c mouse. *J Med Microbiol* **52**: 1109–1115.

Mandell GL, Bennett JE & Dolin R (1995) *Pseudomonas Species (Including Melioidosis and Glanders)*. Churchill Livingstone, New York.

Neubauer H, Sprague LD, Zacharia R *et al.* (2005) Serodiagnosis of *Burkholderia mallei* infections in horses: state-of-the-art and perspectives. *J Vet Med B Infect Dis Vet Public Health* **52**: 201–205.

Nierman WC, DeShazer D, Kim HS *et al.* (2004) Structural flexibility in the *Burkholderia mallei* genome. *Proc Natl Acad Sci USA* **101**: 14246–14251.

Pilatz S, Breitbach K, Hein N *et al.* (2006) Identification of *Burkholderia pseudomallei* genes required for the intracellular life cycle and *in vivo* virulence. *Infect Immun* **74**: 3576–3586.

Popov SF, Kurilov V & Iakovlev AT (1995) *Pseudomonas pseudomallei* and *Pseudomonas mallei* – capsule-forming bacteria. *Zh Mikrobiol Epidemiol Immunobiol* **5**: 32–36.

Popov SF, Tikhonov NG, Piven NN, Kurilov V & Dement'ev IP (2000) The role of capsule formation in *Burkholderia mallei* for its persistence *in vivo*. *Zh Mikrobiol Epidemiol Immunobiol* **3**: 73–75.

Rainbow L, Hart CA & Winstanley C (2002) Distribution of type III secretion gene clusters in *Burkholderia pseudomallei*, *B. thailandensis and B. mallei*. *J Med Microbiol* **51**: 374–384.

Reckseidler SL, DeShazer D, Sokol PA & Woods DE (2001) Detection of bacterial virulence genes by subtractive hybridization: identification of capsular polysaccharide of *Burkholderia pseudomallei* as a major virulence determinant. *Infect Immun* **69**: 34–44.

Ribot WJ & Ulrich RL (2006) The animal pathogen-like type III secretion system is required for the intracellular survival of *Burkholderia mallei* within J774.2 macrophages. *Infect Immun* **74**: 4349–4353.

Rotz LD, Khan AS, Lillibridge SR, Ostroff SM & Hughes JM (2002) Public health assessment of potential biological terrorism agents. *Emerg Infect Dis* **8**: 225–230.

Rowland CA, Lertmemongkolchai G, Bancroft A *et al.* (2006) Critical role of type 1 cytokines in controlling initial infection with *Burkholderia mallei*. *Infect Immun* **74**: 5333–5340.

Santanirand P, Harley VS, Dance DA, Drasar BS & Bancroft GJ (1999) Obligatory role of gamma interferon for host survival in a murine model of infection with *Burkholderia pseudomallei*. *Infect Immun* **67**: 3593–3600.

Schadewaldt H (1975) Discovery of glanders bacillus. *Dtsch Med Wochenschr* **100**: 2292–2295.

Schell MA, Ulrich RL, Ribot WJ *et al.* (2007) Type VI secretion is a major virulence determinant in *Burkholderia mallei*. *Mol Microbiol* **64**: 1466–1485.

Schlater LK (1992) *Glanders, in Current Therapy in Equine Medicine*. W.B. Suanders, St Louis, MO.

Srinivasan A, Kraus CN, DeShazer D *et al.* (2001) Glanders in a military research microbiologist. *N Engl J Med* **345**: 256–258.

Trevino SR, Permenter AR, England MJ, Parthasarathy N, Gibbs PH, Waag DM & Chanh TC (2006) Monoclonal antibodies passively protect BALB/c mice against *Burkholderia mallei* aerosol challenge. *Infect Immun* **74**: 1958–1961.

Ulrich RL & DeShazer D (2004) Type III secretion: a virulence factor delivery system essential for the pathogenicity of *Burkholderia mallei*. *Infect Immun* **72**: 1150–1154.

Ulrich RL, Amemiya K, Waag DM, Roy CJ & DeShazer D (2005) Aerogenic vaccination with a *Burkholderia mallei* auxotroph protects against aerosol-initiated glanders in mice. *Vaccine* **23**: 1986–1992.

Waag DM, McCluskie MJ, Zhang N & Krieg AM (2006) A CpG oligonucleotide can protect mice from a low aerosol challenge dose of *Burkholderia mallei*. *Infect Immun* **74**: 1944–1948.

Wheelis M (1998) First shots fired in biological warfare. *Nature* **395**: 213.

Yabuuchi E, Kosako Y, Oyaizu H *et al.* (1992) Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol Immunol* **36**: 1251–1275.