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Recent Advances in *Burkholderia mallei* and *B. pseudomallei* Research

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Abstract *Burkholderia mallei* and *Burkholderia pseudomallei* are Gram-negative organisms, which are etiological agents of glanders and melioidosis, respectively. Although only *B. pseudomallei* is responsible for a significant number of human cases, both organisms are classified as Tier 1 Select Agents and their diseases lack effective diagnosis and treatment. Despite a recent resurgence in research pertaining to these organisms, there are still a number of knowledge gaps. This article summarizes the latest research progress in the fields of *B. mallei* and *B. pseudomallei* pathogenesis, vaccines, and diagnostics.

Keywords Glanders · Melioidosis · *B. mallei* · *B. pseudomallei* · Vaccines · Diagnostics · Pathogenesis

Introduction

Burkholderia mallei and *Burkholderia pseudomallei* are Gram-negative bacilli which share over 99 % genetic homology. *B. mallei* is an obligate mammalian pathogen and the causative agent of glanders, a disease that primarily affects solipeds (horses, mules, and donkeys). Glanders is endemic to parts of Africa, Asia, Middle East, Central, and South America [1]. Naturally acquired human cases typically occur among persons with prolonged contact with solipeds, and the major routes of transmission are cutaneous and respiratory. Clinical manifestations are route dependent and include abscesses, fever, pneumonia, dissemination to the liver and spleen causing necrotizing abscesses, and bacteremia [2]. *B. pseudomallei* is a saprophyte of wet soils and are endemic to tropical and subtropical areas around the world [3]. This organism can infect various mammals and is the causative agent of melioidosis in humans. Melioidosis symptoms are similar to those described for glanders, but in addition to cutaneous and respiratory routes of infection, melioidosis is also acquired by the gastrointestinal route [4]. *B. pseudomallei* is a leading cause of sepsis in Northern Australia [5] and bacterial pneumonia in Thailand [6]. Both glanders and melioidosis have high mortality rates (up to 50 %), even when aggressive antimicrobial therapy is employed [7]. Additionally, both species are classified as Tier 1 Select Agents by the US Federal Select Agent Program because of their biothreat potential and intrinsic characteristics, including low infectious doses, high morbidity and mortality rates, multi-drug antibiotic resistance, and amenability to aerosolize [8]. Although *B. mallei* and *B. pseudomallei* research has seen significant advancements, there are still large gaps in the understanding of these pathogens and their interactions with the host. This report highlights recent progress made regarding in the areas of pathogenesis, vaccinology, and diagnostics (Fig. 1).

Pathogenesis

B. mallei and *B. pseudomallei* possess multiple secretion systems that facilitate invasion and replication within host cells. These systems play a major role in pathogen virulence, and provide novel targets for the development of vaccines and therapeutics.

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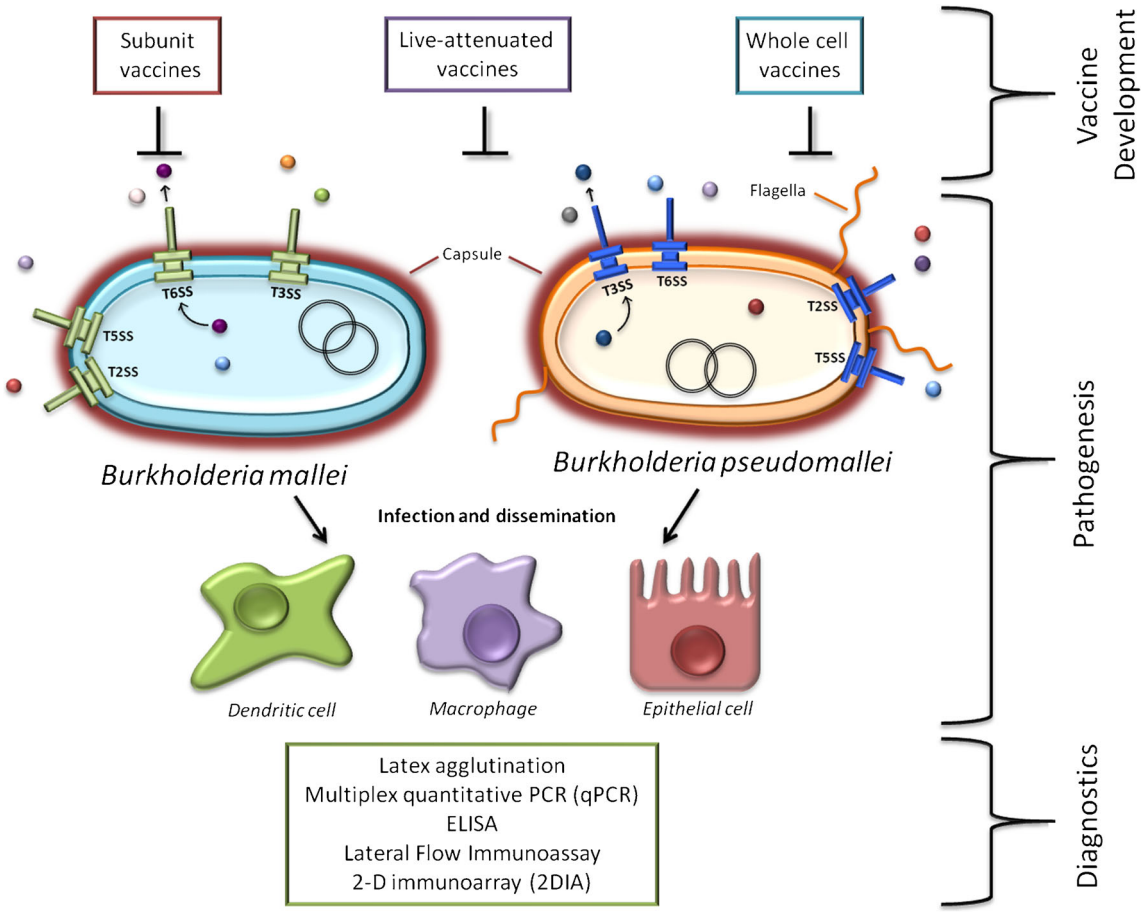

Fig. 1 Graphic representation of *Burkholderia mallei* and *B. pseudomallei* pathogenesis, vaccine development, and emerging diagnostic tools. T2SS, T3SS, T5SS, and T6SS refer to secretion systems that contribute to pathogenesis in both organisms

Type 2 Secretion System

Until recently, the type 2 secretion system (T2SS) has been poorly characterized in *B. pseudomallei*. Burtnick et al. carried out one of the first analyses of the T2SS proteome [9]. The T2SS relies on the ATPase GspE and the outer membrane channel GspD for function. Construction and analysis of in-frame deletion mutants of *gspE* and *gspD* allowed the identification of ~50 proteins secreted by the T2SS. Among these were numerous hydrolytic enzymes and the deubiquitinase TssM, which has previously been shown to be immunogenic via reaction with human melioidosis patient sera [10]. Unexpectedly, the current study revealed that deletion of the T2SS slightly increases mutant virulence, as demonstrated by a decreased LD_{50} and time to death in a BALB/c mouse model [9]. The mechanism behind this increase in virulence remains to be elucidated.

Type 3 Secretion System

A functional type 3 secretion system (T3SS) is essential for *B. pseudomallei* escape from the phagolysosome following invasion. Virulence factors such as BopA, BipB, and BipD have previously been reported to play a pivotal role in phagosomal escape, multi-nucleated giant cell (MNGC) formation, and intracellular replication. Kang et al. have characterized another *Burkholderia* invasion protein, BipC, demonstrating its role in virulence [11]. A *bipC* mutant was constructed from *B. pseudomallei* K96243 and evaluated in human lung epithelial cells. The mutant displayed decreased invasion, adherence, and intracellular survival in vitro. Additionally, the *bipC* mutant exhibited a delay in actin-based motility and phagosomal escape. Similar findings were observed in vivo, confirming the decreased virulence of the mutant by increased survival and healthier overall appearance of i.p. challenged BALB/c mice. Therefore, BipC appears to play an essential role in the overall pathogenicity and virulence of *B. pseudomallei*.

Type 5 Secretion System

Autotransporters comprise the largest family of proteins secreted by the type 5 secretion system (T5SS). The functions of autotransporters are diverse, including adhesion, invasion,

serum resistance, intracellular survival, and biofilm formation. Among the well-described *Burkholderia* autotransporters are BimA and BoaA/BoaB, which function in actin polymerization and adhesion, respectively. Further characterization of BpaC, BcaA, and BcaB autotransporters has recently been reported. A study by Lafontaine et al. demonstrated that BpaC plays an important role in adhesion [12]. In vitro analysis of *bpaC*-transformed *Escherichia coli* revealed increased adhesion to human lung, laryngeal, and bronchial epithelial cells. Similar studies were conducted with *bpaC* mutants of *B. mallei* ATCC 23344 and *B. pseudomallei* DD503. Interestingly, while the *B. mallei* mutant showed decreased adhesion in all three cell types, the *B. pseudomallei* mutant portrayed decreased adhesion only to the bronchial epithelium. However, changes in adhesion did not appear to affect virulence of the mutants in vivo, as indicated by equivalent LD_{50} values and bacterial burden in an aerosol mouse model of infection [12].

Another study by Campos et al. demonstrated that *B. pseudomallei* strain 340 *bcaA* and *bcaB* mutants displayed impaired invasion and decreased plaque formation in lung epithelial cells [13]. Furthermore, a BALB/c i.n. model of acute infection showed significantly fewer bacteria recovered from the spleen of mice infected with Δ*bcaA*, suggesting that BcaA may play a role in dissemination to the spleen.

Type 6 Secretion System

Type 6 secretion system (T6SS) is necessary for MNGC formation and plays an important role in intracellular growth and actin-based motility. Hemolysin co-regulated protein-1 (Hcp1), located within T6SS cluster-1, and it has been well characterized for its role in MNGC formation and intracellular survival. By altering the divalent ion concentrations in minimal media, it was shown that the addition of iron and zinc negatively regulates in vitro expression of T6SS cluster-1 genes. This negative regulation was demonstrated by significant reduction in *hcp1* expression in *B. mallei* SR1A and *B. pseudomallei* K96243 and 1026b [14••]. By optimizing growth conditions, the highest expression of Hcp1 by both *Burkholderia* was observed in minimal salts media lacking iron and zinc and supplemented with 0.4 % glucose and 0.5 % bacto-casamino acids. Furthermore, it was determined that VirG positively regulates expression of both *Burkholderia* T6SS-1 systems, as demonstrated by negligible production of Hcp-1 when a *virG* mutant was analyzed.

Hopf et al. performed further characterization of the T6SS-1 in a study that described the T6SS-1 gene, BPSS1504, and its apparent effect on Hcp1 secretion. A BPSS1504 mutant showed no difference in *hcp1* mRNA levels; however, secreted Hcp1 protein was unable to be detected. In addition to abrogated Hcp1 secretion, the mutant exhibited decreased virulence in vivo and in vitro. These findings suggest that BPSS1504 may play a role in the functionality of the T6SS-1 apparatus [15].

A member of the *vgr* family, VgrG5, has been reported to play a role in the virulence of *B. pseudomallei*. VgrG5 was thoroughly characterized by Toesca et al. and Schwarz et al. [16, 17]. Both confirmed that VgrG5 functions in MNGC in vitro formation and reported that the C-terminal domain (CTD) of VgrG5 is essential for its function. Additionally, both groups constructed *Burkholderia thailandensis vgrG5* mutants for evaluation in vitro and reported that they were unable to induce membrane fusion. In order to further characterize the role of CTD, Schwarz et al. performed in vivo studies in a C57BL/6 mouse model via aerosol challenge of *B. thailandensis* CTD mutant. All mice challenged with ΔCTD survived >14 days post-infection, whereas mice infected with wild-type (WT) succumbed to infection between days 2–4. Additionally, Toesca et al. demonstrated high conservation of VgrG5 between *Burkholderia* species. By cross-complementing a *B. pseudomallei vgrG5* mutant with *vgrG5* alleles from *B. mallei* ATCC 22344, *B. pseudomallei* MSHR668, *B. oklahomensis* C6786, and *B. thailandensis* E264, this group was able to restore membrane fusion capability and MNGC formation in vitro.

Surface Polysaccharides

Capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are the best well-described surface antigens expressed by both *Burkholderia* species. Because passive immunization with CPS and LPS has previously been shown to provide partial protection from infection, current vaccine studies focus on incorporating LPS or CPS for enhanced immunity. Although *B. pseudomallei* LPS has been considered weakly antigenic in experimental models, a recent study by Chantratita et al. suggests that LPS plays an important role in stimulating the human innate immune response to *B. pseudomallei* infection [18]. Stimulation of human monocytes with heat-killed *B. pseudomallei* 1026b or K96243 in the presence of LPS antagonist polymyxin B or TLR4 neutralizing antibody showed dramatic decrease in TNF-α production. Additionally, stimulation of whole blood with purified *B. pseudomallei* LPS generated a greater cytokine response than *E. coli* LPS. Taken together, these data suggest an important role for *B. pseudomallei* LPS in initiating innate cytokine production [18].

Quorum Sensing

The role of quorum sensing (QS) in the pathogenesis of *B. mallei* remains controversial. Ulrich et al. reported that *B. mallei* QS mutants showed severe attenuation both in vitro and in vivo [19]. However, a recent report by Majerczyk et al. suggests that QS is not essential for acute

infection [20]. In order to more fully elucidate the QS role in *B. mallei* infection, the horse-passaged strain GB8 was used to construct unmarked, in-frame deletions of the homoserine lactone (HSL) synthases genes *bmaI1* and *bmaI3*. Additionally, the *B. mallei* strain ATCC 23344 was used to generate an acyl-homeoserine lactone (AHL) lactonase-producing mutant. In vitro experiments were performed using murine macrophage-like RAW 264.7 cells. Intracellular replication of GB8Δ*bmaI1*, GB8Δ*bmaI3*, and GB8Δ*bmaI1*/Δ*bmaI3* were indistinguishable from WT parent strain. Similar results were observed in a BALB/c mouse model, with comparable survival times seen between WT and mutant-challenged mice. Additionally, the *B. mallei* AHL-lactonase-producing strain CM4 demonstrated WT virulence in vivo. In order to assess the functionality of the *bmaI1* promoter, BALB/c mice were infected with the luminescent fusion strain CM4. High fluorescence was visible in the lungs at disease onset, indicating that the *bmaI1* gene is active during infection. These results suggest that QS is not required for acute *B. mallei* infection, but its role in chronic infection has not yet been characterized.

Likewise, the role of quorum sensing in closely related *B. pseudomallei* is poorly understood. Recently, reports suggest that QS may play a role in MNGC formation by *B. pseudomallei*. Horton et al. developed AHL-synthase mutants of the *bpsI1*, *bpsI2*, and *bpsI3* genes in strain MSHR520 [21]. Interestingly, the triple mutant *ΔbpsI123* showed significant increase in MNGC formation compared to WT. However, no significant difference was seen in the intracellular survival or replication of the mutants. Additionally, comparable virulence was observed between Δ*bpsI123* and WT, as determined by CFU counts of blood, spleen, lungs, and liver tissues of challenged BALB/c mice.

Host–Pathogen Interaction

During infection, the release of stress hormones functions to regulate many physiological responses including inflammation and metabolic activity. Intarak et al. describe the role of epinephrine on *B. pseudomallei* pathogenicity in vitro [22]. Epinephrine concentration, ranging from 50 to 200 μM, was shown to have a positive effect on bacterial growth, which was significantly increased over untreated cultures. Because catecholamines, like epinephrine, are believed to function in the release of free iron, the relationship of iron to bacterial growth was analyzed in vitro. The addition of $FeCl_3$ was shown to increase *B. pseudomallei* growth, whereas administering the iron-chelating agent deferoxamine (DFO), in the presence of epinephrine, dramatically decreased growth. Additionally, treatment with 50 μM epinephrine resulted in increased radial motility and expression of flagellar genes, including *fliC*, *fliA*, *flhD*, and *motA*. These results suggest that epinephrine contributes to increased growth, gene expression, and motility in vitro.

Williams et al. showed that dendritic cells facilitate dissemination of *B. pseudomallei* NCTC 13179 during acute infection [23•]. *B. pseudomallei* was shown to induce maturation of bone marrow-derived dendritic cells (DCs) and stimulate in vitro migration. Importantly, DCs were shown to retain intracellular *B. pseudomallei* during migration, indicating a potential for trafficking. In both in vitro and in vivo studies, *B. pseudomallei* infection resulted in a more potent stimulation of DCs than LPS, as indicated by increased expression of maturation markers and increased migration. The ability of DCs to facilitate spread of *B. pseudomallei* was confirmed in vivo in a C57BL/6 mouse model. This study confirms that DCs are able to traffic *B. pseudomallei* from the site of cutaneous infection to the spleen and lungs and thereby, facilitating bacterial dissemination.

Mulye et al. also demonstrated the importance of the innate immune system by elucidating the role of serum opsonins in the uptake and killing of *B. pseudomallei* by neutrophils and macrophages [24]. Increased complement deposition was shown to occur on the *B. pseudomallei* acapsular mutant SZ210. Additionally, *B. pseudomallei*, its acapsular mutant, and *B. thailandensis* all showed resistance to direct killing by complement, even when incubated in high concentrations of serum. While serum opsonization resulted in increased uptake of *B. pseudomallei* by both neutrophils and macrophages, intracellular killing by macrophages remained unaffected. In contrast, decreased intracellular survival within neutrophils was observed when bacteria were opsonized with ≥5 % serum. These data suggest that opsonization functions to increase uptake and may play a role in intracellular killing by neutrophils.

Vaccine Development

Based on the high incidence of melioidosis in endemic areas, the potential for re-emergence of glanders, and both diseases potential for bioterrorist use, development of effective vaccines against these diseases is an ideal approach to prevent infection. Currently, no vaccine candidates have been selected for glanders or melioidosis human or veterinary prevention trials. Numerous vaccine reviews and studies suggest that both cellular and humoral immune responses are required for complete protection against these pathogens [25–28]. Therefore, a summary of the recently evaluated *B. mallei* and *B. pseudomallei* vaccine candidates is described.

Live Attenuated Vaccines

Scott et al. looked at the ability of *B. thailandensis* E555, a non-pathogenic strain producing *B. pseudomallei*-like mannoheptose capsule, to protect against melioidosis. The study demonstrated complete bacterial clearance in the lungs, liver,

and spleen by day 3 in BALB/c mice vaccinated prior to challenge with *B. pseudomallei* K96243. Sera from BALB/c mice receiving either *B. thailandensis* or a non-encapsulated control strain showed that animals exposed to E555 produced higher *B. pseudomallei*-specific IgG. Finally, mice immunized with E555 prior to challenge with *B. pseudomallei* K96243 exhibited increased survival rates over mice vaccinated with non-encapsulated control [29].

Mott et al. constructed an unmarked *in-frame* deletion in the *B. mallei* ATCC 23344 *tonB* gene (BMAA1801), creating an iron transport-deficient strain. BALB/c mice intranasally immunized with the Δ*tonB* strain at doses of 1.5×10^5 and 1.5×10^4 CFU and subsequently challenged with WT *B. mallei* had survival rates of 100 and 75 % respectively. A cross-protection assay of mice vaccinated with Δ*tonB* vaccine and challenged with the WT *B. pseudomallei* K96243 strain demonstrated that mice had a survival rate of 75 % up to 36 days post-infection. Although highly protective against both pathogens, persistence was noted both by the appearance of splenic abscesses and recovery of *B. mallei* Δ*tonB* strain from the spleens of surviving mice. Despite persistence, the ability of the *B. mallei tonB* mutant to provide cross-protection against both *B. mallei* and *B. pseudomallei* makes it a potential candidate for further vaccine development and optimization [Mott et al., personal communication].

Silva et al. demonstrated that subcutaneous vaccination can also protect against murine melioidosis. A highly attenuated *purM* mutant of *B. pseudomallei* strain 1026b (Bp82) was used to immunize BALB/c and C57BL/6 mice prior to WT challenge. Results indicated that BALB/c and C57BL6 mice vaccinated with the Bp82 showed 100 and 60 % survival, respectively. Post-immunized sera contained a significantly higher level of IgG and IgM in comparison to controls; likewise, significantly higher levels of IL-17 and IFN-γ were also detected. Mice deficient in B cells ($uMT^{-/-}$) or T cells ($CD4^{-/-}$ and $CD8^{-/-}$) were analyzed in response to vaccination and challenge, and those lacking B cells exhibited only 50 % survival. Immune sera transferred to naïve mice resulted in 38 % survival. In contrast, mice deficient in T cells exhibited survival equivalent to that of vaccinated WT. This study identified correlates of immune protection, specifically highlighting the role of humoral immunity in protecting against *B. pseudomallei* infection [30•].

Killed Vaccines

Puangperch et al. evaluated the protective effects of i.m. administration of heat-killed (HK) or paraformaldehyde-killed (PK) *B. pseudomallei* to BALB/c mice prior to challenge with WT *B. pseudomallei*. The PK treatment provided increased protection against *B. pseudomallei* over HK (60 % survival vs 0 %) Additionally, it was shown that PK-immunized mice had decreased bacterial counts in their blood and increased *B. pseudomallei*-specific IgG and IFN-γ serum levels in comparison to HK immunized mice [31].

Subunit Vaccines

Outer membrane vesicles (OMVs) are non-infectious particles that contain LPS, CPS, and a milieu of membrane and periplasmic proteins. OMVs have been isolated from *B. pseudomallei* 1026b and have previously been demonstrated as an effective vaccine platform, which provides significant protection (60 % survival) against respiratory murine melioidosis [32]. Nieves et al. showed that s.c. immunization with OMVs also provided protection against lethal septic BALB/c mouse infection, as demonstrated by increased survival rates compared to unvaccinated controls [33]. However, splenic persistence in surviving animals from both studies indicated that OMV vaccination only provided partial bacterial clearance.

Petersen et al. have demonstrated the safety and immunogenicity of OMV vaccination in rhesus macaque non-human primates (NHP). In this study, NHPs received either three doses of adjuvant alone (control) or three escalating doses of OMVs plus adjuvant. Safety was evident by lack of pathology at the injection site and normal blood chemistry and hematology results in NHPs. Immunogenicity of the OMVs was demonstrated by significant increases in OMV-specific, LPS-specific, and CPS-specific IgG titers in OMV-vaccinated NHPs compared to controls [34•].

Other studies have investigated the use of *B. mallei* and *B. pseudomallei* glycoconjugate vaccines. Scott et al. evaluated a conjugate vaccine comprised of covalently joined LPS and tetanus toxin H_c ($TetH_c$) in a melioidosis mouse model. The conjugate vaccine provided partial protection, resulting in slightly increased survival in comparison to only LPS or unconjugated $TetH_c$ and LPS. Additionally, although there was no significant difference in splenic colonization between the groups, mice receiving the conjugate had significantly higher titers of IgG1 and IgG2a compared to the other groups [35]. Scott et al. also evaluated a series of unconjugated and conjugated *O*-polysaccharide (OPS) and CPS vaccine preparations combined with cationized bovine sheep albumin (cBSA) and LolC. Both conjugate vaccines were more protective than their unconjugated polysaccharide and cBSA counterparts. The conjugate vaccine containing CPS was more protective than the OPS conjugate, with median time to death of 29.5 and 10 days, respectively. The addition of LolC to the CPS conjugate resulted in increased protection, with immunized mice showing 70 % survival in contrast to 50 % survival with the CPS conjugate alone. The incorporation of LolC resulted in lower bacterial counts in the lungs, livers, and spleens [36].

Gregory et al. developed and evaluated the protective capability of gold nanoparticles (AuNPs) conjugated to purified LPS (AuNP-LPS) and covalently linked to recombinant

carrier proteins (TetHc, Hcp1, or FliC). Different combinations of the AuNPs were delivered i.n. to BALB/c mice prior to a lethal challenge with *B. mallei* ATCC 23344. At a low challenge dose (~2 LD_{50}), LPS, AuNP-LPS, and AuNP-LPS provided similar levels of protection; however, at the high challenge dose (6.5 LD_{50}), the groups receiving AuNP showed increased protection and had significantly lower splenic bacterial burdens. Furthermore, sera from the protein-coupled nanoglycoconjugate groups had significantly higher LPS-specific IgG and IgM levels than controls [37].

Torres et al. also evaluated the protective capacity of AuNP-LPS coupled with FliC (AuNP-LPS-FliC) in a NHP model of respiratory glanders. AuNP-LPS-FliC-vaccinated NHPs demonstrated increased survival over the PBS control group, with survival rates of 50 and 33.3 % in the groups, respectively. While bacterial burdens were detected in infected tissues of surviving control animals, no detectable bacteria were present in organs (spleen, liver, and lungs) of vaccinated animals. Serum LPS-specific IgG titers were significantly increased in vaccinated NHPs than in controls. Overall, results showed that AuNP-LPS-FliC vaccine is immunogenic and provides protection against aerosolized glanders infection [38••].

Diagnostics

The current "gold standard" diagnostic tests for melioidosis and glanders have long turnaround times and are prone to misdiagnosis due to low sensitivity and specificity. A definitive glanders or melioidosis diagnosis via routine bacterial culture typically takes approximately 7 days, and the poor sensitivity of this methodology increases the risk of undetected cases. Conventional serological testing of humans in melioidosis endemic areas is unreliable due to high seroprevalence rates. Additionally, diagnostic shortcomings have been identified in the mallein and compliment fixation test (CFT), which are used to identify glanders-positive solipeds. The mallein test has a reported sensitivity of only 75.7 % [39] and the CFT, while more sensitive (97 %), has been implicated in false-positive results [1]. We next summarize novel detection methods that may address these gaps.

Latex Agglutination Test

Duval et al. evaluated the latex agglutination test on its ability to accurately detect *B. mallei* and *B. pseudomallei* from culture. The detection reagent in this assay is comprised of latex particles coated with 4B11 monoclonal antibody to *B. mallei* and *B. pseudomallei* CPS, which, when combined with a single isolated *B. mallei* or *B. pseudomallei* colony on a ringed glass slide and rocked briefly, will agglutinate. Using a panel of 110 *B. pseudomallei and B. mallei* isolates and a panel of 36 clinically relevant and phylogenetically related *Burkholderia* spp., the assay had sensitivity of 99.1 % and specificity of 97.2 % [40]. Despite the requirement of bacteria to be cultured and isolated prior to identification, the accuracy, ease of use, relatively low cost per test, and negligible requirements for additional equipment make it a potentially invaluable diagnostic tool, particularly in resource-poor areas.

Molecular Diagnostics

Janse et al. developed a single-reaction quadruplex quantitative real-time PCR for the detection and differentiation of *B. pseudomallei* and *B. mallei* from both bacterial culture lysates and various patient samples. The assay includes BuMC, a sequence unique to both *B. mallei* and *B. pseudomallei*; *psu*, a sequence unique to *B. pseudomallei*; and *mau*, a sequence specific to *B. mallei*. Additionally, primer and probe sets are included in the assay to act as an internal DNA extraction control. This assay was validated via a panel of 10 *B. mallei* clinical isolates, 19 *B. pseudomallei* isolates, 16 negative bacterial controls, positive autopsy tissue samples (lung, liver, spleen, and prostate) from a melioidosis patient, and 10 negative multi-species blood and tissue samples. The assay correctly identified all negative and *B. mallei*-positive samples. Although the assay detected *psu* in all 19 *B. pseudomallei* samples, it failed to detect BuMC in three of the *B. pseudomallei* isolates. Although no sensitivity or specificity data was provided, the limit of detection of the assay was estimated to be <1 genome equivalent per reaction [41].

Hsu et al. developed a 15-plex assay for the identification of 11 pathogens of clinical and biothreat importance including *B. mallei* and *B. pseudomallei*. This Luminex-based assay was validated by performing blind testing with bacterial culture colonies of 57 ATCC reference strains and 1 clinical *Brucella* spp. isolate, and it correctly identified individual pathogens with 100 % accuracy. This method is able to identify all agents present when mixtures of up to six different bacterial DNAs were assayed. The limit of detection for this assay is between 5 and 100 genomic copies for each of the 11 pathogens [42].

Serological Diagnostics

Singha et al. developed an indirect ELISA method which was able to identify TssB, a highly antigenic protein of the *B. mallei* T6SS. The assay, evaluated with a panel of 49 known glanders-positive and 40 negative equine serum samples, was able to diagnose with 100 % accuracy. Comparison with the complement fixation test on 1811 equine serum samples

indicated that 4 of the 1811 samples were false positive by ELISA, but none were false negative. The sensitivity and specificity of this TssB indirect ELISA was determined to be 100 and 99.7 %, respectively [43].

Houghton et al. developed and evaluated the Active Melioidosis Detect Lateral Flow Immunoassay (AMD-LFI). Using a single colony of 77 *B. pseudomallei* isolates, 33 *B. mallei* isolates, and 36 near-neighbor common clinical isolates, the test showed a sensitivity of 98.7 % (76/77) for *B. pseudomallei* and 90.9 % (30/33) for *B. mallei*, with an overall specificity of 97.2 % (35/36). The limit of detection for this test was calculated to be 0.2 ng/ml of CPS. Finally, the assay was optimized for its use with a variety of direct patient specimens, including serum, pus, urine, sputum, and pleural fluid [44••].

Indirect hemagglutination assay (IHA) is used in Australia as serodiagnostic test with poor sensitivity. Sorenson et al. developed and evaluated a two-dimensional immunoarray (2DIA) designed to detect the three most common *Burkholderia* LPS types (A, B, and B2). Using a panel of 60 serum samples, they found that 2DIA had a much higher sensitivity than IHA (100 vs. 58.6 %), giving positive results for all melioidosis culture-positive/IHA-negative serums, but a lower specificity (87.1 vs. 100 %) [45].

Conclusion

Understanding *B. mallei* and *B. pseudomallei* pathogenesis has opened the door to the development of more specific diagnostics and therapies. The advancements in the understanding of host–pathogen interaction, in addition to the identification and purification of bacterial virulence factors, have increased the speed and accuracy of diagnostics and identification of immunogenic vaccine targets. However, identification of vaccine candidates that provide long-term and complete protection as well as sterilizing immunity remains an elusive goal requiring further attention.

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Compliance with Ethics Guidelines

Conflict of Interest Christopher L. Hatcher, Laura A. Muruato, and Alfredo G. Torres declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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• Of importance
•• Of major importance

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