



Innate immune response to *Burkholderia mallei*

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Purpose of review

Burkholderia mallei is a facultative intracellular pathogen that causes the highly contagious and often the fatal disease, glanders. With its high rate of infectivity via aerosol and recalcitrance toward antibiotics, this pathogen is considered a potential biological threat agent. This review focuses on the most recent literature highlighting host innate immune response to *B. mallei*.

Recent findings

Recent studies focused on elucidating host innate immune responses to the novel mechanisms and virulence factors employed by *B. mallei* for survival. Studies suggest that pathogen proteins manipulate various cellular processes, including host ubiquitination pathways, phagosomal escape, and actin–cytoskeleton rearrangement. Immune-signaling molecules such as Toll-like receptors, nucleotide-binding oligomerization domain, myeloid differentiation primary response protein 88, and proinflammatory cytokines such as interferon-gamma and tumor necrosis factor- α , play key roles in the induction of innate immune responses. Modifications in *B. mallei* lipopolysaccharide, in particular, the lipid A acyl groups, stimulate immune responses via Toll-like receptor4 activation that may contribute to persistent infection.

Summary

Mortality is high because of septicemia and immune pathogenesis with *B. mallei* exposure. An effective innate immune response is critical to controlling the acute phase of the infection. Both vaccination and therapeutic approaches are necessary for complete protection against *B. mallei*.

Keywords

Burkholderia mallei, cellular immunity, immune signaling, Innate Immune response, vaccine

INTRODUCTION

Burkholderia mallei are the etiological agent of a highly contagious, acute, or chronic, usually fatal disease of solipeds, known as glanders. This obligate mammalian, facultative intracellular pathogen is a Gram-negative, nonmotile, nonspore-forming bacillus which is widely regarded as a host-adapted deletion clone of *Burkholderia pseudomallei*, an environmental saprophytic pathogen that causes the disease melioidosis. Although horses, donkeys, and mules constitute the only known natural reservoirs for *B. mallei*, humans and other mammalian hosts [e.g., camels, nonhuman primates (NHPs), goats, dogs, cats, rabbits, hamsters, guinea pigs, and mice] are susceptible to infection and display similar disease progression and disease [1–7]. Glanders transmits amongst animals via respiratory secretions and exudates from skin lesions. In human infections, the primary modes of *B. mallei* transmission are via direct contact with damaged skin, invasion of mucous membranes, and deposition into the lung. Depending on the route of exposure, the disease course of glanders infection can range from acute to chronic and manifest in multiple

forms, such as localized, pulmonary, disseminated, and septicemic. The clinical and pathological presentation of *B. mallei* infections bare a striking resemblance to *B. pseudomallei* infections, including their ability to remain quiescent and persist in the host following apparent clinical resolution [8]. Owing to the reasons above, in addition to their highly infectious nature as an aerosol, both pathogens are classified as Tier 1 select agents by the federal select agent program. Currently, no licensed vaccines are

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KEY POINTS

- *B. mallei* proteins interact with multifunctional host proteins that have large number of interacting partners to broadly influence host cellular mechanisms such as ubiquitin-mediated proteolysis and focal adhesion.
- Compared with other *Burkholderia* spp., *B. mallei* displays unique manipulation of host-signaling architectures and mechanisms of evading host innate immune responses.
- The biological activity of *B. mallei* LPS is directly correlated with the acylation status of its lipid A molecule.
- *MyD88*-targeted postexposure therapy may be a potential strategy against *B. mallei* infection.
- Live attenuated *B. mallei* mutants may be promising candidates for vaccine development against acute glanders.

available for either disease, and medical therapeutic options are limited.

Both *B. pseudomallei* and *B. mallei* thrive intracellularly via modulation of host immune responses, which attributes to their resilience against current medical countermeasures. Despite the characterization of many *B. pseudomallei* virulence factors, its strategies for circumventing intracellular host defenses remain ill defined. Comparatively, even less is known for *B. mallei*. Limited understanding of these survival tactics poses a major challenge in the development of effective therapeutics. Thus, delineating the specific molecular mechanisms utilized by these pathogens to dysregulate host immune responses is paramount. The majority of research and review articles are focused on host immune responses to *B. pseudomallei*. This review will concentrate on recent advances in characterizing *B. mallei*-specific host immune responses, specifically innate immune responses.

HOST-PATHOGEN INTERACTIONS AND INNATE IMMUNE RECOGNITION OF *BURKHOLDERIA MALLEI*

Although mechanisms can vary among *Burkholderia* spp., adhesion and invasion of host epithelial cells are vital steps during infection and appear to contribute to the overall virulence [9[■]]. For successful infection of host cells, *B. mallei* depend on the strategic utilization of a multitude of virulence factors and mechanisms to manipulate many host processes and pathways. Recently, a combined computational and experimental approach was utilized

to systemically assess nine *B. mallei* virulence factors and their interactions with host proteins to elucidate mechanisms of *B. mallei* pathogenicity [10[■]]. Topological analyses of *B. mallei*-host protein-protein interactions suggest that *B. mallei* targets multifunctional intracellular host proteins, host proteins that interact with each other, and proteins with a large number of interacting partners. Host processes broadly influenced by these protein-protein interactions include the ubiquitination degradation system and focal adhesion pathways [10[■]]. These results are consistent with the previous work that reported TssN protein interactions with the polyubiquitin-B protein and with the cullin-1a protein. These host proteins interact with tumor necrosis factor (TNF) receptor-associated factor 6 and IκB inhibitor-α, components central to Toll-like receptor (TLR) signaling [11]. These studies provide some insights into *B. mallei* pathogenesis, and on the proposed hypothesis that *B. mallei* modulate innate immune responses by interfering with host ubiquitination directly or in combination with other pathogen proteins.

A comprehensive assessment of murine macrophages infected with a diverse panel of *Burkholderia* spp. resulted in the uniform production of cytokines interleukin 1β (IL-1β), tumor necrosis factorα (TNFα), and murine keratinocyte-derived protein chemokine, a murine homolog of human IL-8 [12]. Compared with *B. pseudomallei*-infected macrophages, *B. mallei*-infected macrophages secreted significantly higher levels of IL-6 and IL-10, which suggest these two pathogens differentially modulated host signaling cascades. Additionally, macrophages expressed IL-1β, IL-10, TNF receptor superfamily member 1B, and IL-36α mRNA, at significantly higher levels when infected with *B. mallei* compare with the other *Burkholderia* spp. [12], suggesting the existence of gene-based differences in the host inflammatory response that is unique to *B. mallei*.

Infected macrophages further assessed for changes in their host-signaling dynamics showed increased phosphorylation of adenosine monophosphate-activated protein kinase; regulators of nuclear factor-kappa B signaling pathway (e.g., IκB inhibitor-α, Glycogen synthase kinase (GSK)3β, Src, and *STAT1*) and mitogen-activated protein kinases (e.g., p38, Extracellular-signal regulated kinase 1/2, and c-Myc) [13[■]]. The degrees in which target host proteins or processes are modulated correlated to the differences in pathogenicity observed amongst *Burkholderia* species. In infected macrophages, *B. mallei* were a stronger inducer of Inducible nitric oxide synthase expression and interferon-gamma (IFNβ) production compared with *B. pseudomallei*. Based on these data, in addition to current

knowledge of signaling transduction, a representative network of signaling pathways and axes was constructed to illustrate the activation of signaling cascades in response to *Burkholderia* spp infection [13[■]]. Based on canonical pathways downstream of TLR4, induction of phosphorylated forms of adenosine monophosphate-activated protein kinase- α 1, GSK3 β , and Src play key roles in regulating the inflammatory response of *Burkholderia* spp. infections.

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, and a potent stimulator of host innate immune responses. Structure–activity relationship studies of TLR4 agonist suggest the biological activity of LPS correlates with the composition of its lipid A moiety [14]. Evaluation of *B. mallei* LPS showed the acylation of lipid A had a greater effect on its biological activity than their length [15[■]]. Thus, overall differential macrophage activation may be related to *B. mallei* LPS, which is similar to the *B. pseudomallei* LPS and bears a penta-acylated lipid A with 4-amino-4-deoxyarabinose in almost half of its molecules, and appears to be a weaker macrophage activator as compared with enterobacterial LPS. Consistent with this, a significant reduction in mRNA expression or secretion of IL-6, TNF α , and IL-1 β is exhibited when stimulated with purified *B. mallei* LPS compared with *E. coli* LPS-treated macrophages. Compared with *E. coli*-infected macrophages, *B. mallei*-infected macrophages also produce reduced levels of both IFN-dependent genes and mediators (IFN β and Nitric oxide) and cytokines [TNF α , IL-6, IL-10, Granulocyte-macrophage colony stimulating factor (GM-CSF), and regulated on activation normal T cell expressed and secreted (RANTES)].

B. mallei must overcome a gamut of antibacterial mechanisms and products (e.g., Adenosine monophosphates and reactive oxygen and nitrogen species) critical to innate immunity to establish persistent infection. *B. mallei* Frederick Memorial Hospital (FMH) isolates collected from mice spleens 60 days postinfection showed attenuated abilities to replicate and induce cytotoxicity in macrophage assays [16]. One *B. mallei* isolate displayed a change in its LPS phenotype, from smooth to rough, resulting from the loss of its O-polysaccharide (OPS) during the course infection [16]. These phenotypic changes were conceived to stem from the infection shifting from an acute to a chronic or subclinical form, which is less prone to stimulate host immune responses. Earlier studies highlighted that genetic and phenotypic characteristics potentially associated with persistence of both *B. pseudomallei* and *B. mallei* [17,18]. Further studies, including sequencing the OPS biosynthetic gene cluster of

this *B. mallei* FMH strain may provide insight into the genetic basis for the loss of OPS. Intriguingly, OPS modification and loss is a hallmark of chronic *Pseudomonas aeruginosa* infection [19].

CYTOKINES AND CHEMOKINE REGULATING INNATE IMMUNITY TO *B. MALLEI* INFECTION

Highlighting the susceptibility of *B. mallei* to cell-mediated immune responses, previous studies compared the survival rates of infected BALB/c and IFN γ knockout mice. BALB/c mice survived more than 37 days longer than IFN γ knockout mice and showed significantly lower levels of bacterial colonization, which illustrates the importance of IFN γ -mediated immunity for control of infection [20]. Macrophages and human pulmonary alveolar type II cells contribute to innate immunity by secreting inflammatory cytokines during *B. mallei* infection [21]. When exposed to heat-killed *B. mallei*, primary Peripheral blood Mononuclear Cells (PBMCs) from NHPs and humans elicit the strong production of IFN γ , TNF α , IL-6, and IL-1 β [22[■]]. Cytokine responses varied among the NHPs, in which the African green monkey appears to be most responsive, compared with Rhesus or Cynomolgus species, suggesting the inflammatory responses vary within mammalian species [22[■]]. Similar results were observed with aerosol exposure of *B. mallei* FMH 23344 strain to NHPs conducted at USAMRIID, where most of the African green monkeys died but all Rhesus or Cynomolgus species survived (Personal communication). The immune-signaling mechanism for the strong cellular response demonstrated that myeloid differentiation primary response protein 88 (*MyD88*)-mediated signaling contributes to proinflammatory cytokine responses [22[■]]. These results were consistent with earlier reports which showed that *MyD88*^{-/-} mice were highly susceptible to pulmonary challenges with *B. mallei* and had significantly short survival time, increased bacterial burdens, and severe organ pathology compared with wild-type mice [23]. Recruitment of inflammatory monocytes and Dendritic Cells to the lungs and local production of IL-12, followed by Natural killer cell production of IFN γ , are the key cellular responses required for early protection from *B. mallei* infection.

LACK OF AUTOPHAGY AND PERSISTENCE OF *B. MALLEI*

B. pseudomallei demonstrate an ability to escape autophagosomes in host phagocyte *in vitro* as well as in murine models and human cases of

melioidosis, thus avoiding immune responses [24]. The recurring illness of melioidosis patients in endemic areas can potentially be because of relapse or reinfection. Bacteria can become quiescent and subclinical to avoid host immune mechanisms of clearance. An earlier report indicated that nonfunctional mutations in *BPSS0180*, a type VI cluster-associated gene capable of inducing autophagy in both phagocytic and nonphagocytic mammalian cells, resulted in significant colocalization of *B. pseudomallei* with autophagy marker light chain3 and impaired intracellular survival [25]. A recent report suggests that *B. pseudomallei* evade autophagy [26]. Consistent with these earlier reports, recent results from our laboratory also suggest that lack of autophagy correlate with intracellular persistence of bacteria with aerosol exposure not only of *B. pseudomallei* but also *B. mallei* in spleens of BALB/c and C57/BL6 mice with chronic infection (Alam *et al.* 2016; manuscript submitted). Memisevic *et al.* [10¹¹] suggests that multiple *B. mallei* virulence factors such as *BMAA1865*, *BMAA0728* (*TssN*), and *BMAA0553* influence critical host processes related to modulation of host ubiquitination, phagosome escape, interference with host cytoskeleton rearrangement, and focal adhesion and a means to modulate and adapt the host cell environment to advance infection. Further studies may shed light on whether any of these *B. mallei* proteins are directly or indirectly linked in the evasion of host autophagy processes.

POTENTIAL THERAPEUTIC AND PREVENTIVE STRATEGY TO GLANDERS

Antibiotic resistance associated with *Burkholderia* infection is on the rise [27]. Even with optimal antibiotic treatment, the mortality from acute severe melioidosis is high (30–50% in Thailand, 19% Australia) and mortality rates can be as high as 40% for cases of glanders [28–30]. Recently, Waag [31] reported that mice experimentally exposed to *B. mallei* suggest that although antibiotics can be efficacious after prolonged interval between exposure and treatment, but only if the animals were previously vaccinated. Thus, it is likely that both vaccination against *B. mallei* and postexposure therapeutic approaches would be required for complete protection against *B. mallei* exposure.

THERAPEUTIC STRATEGY: *MyD88*-TARGETED THERAPY IN PREVENTING PERTURBED INFLAMMATION AND SEPTICEMIA

Primary cellular responses by analyses of IL-1 β and other inflammatory cytokine responses by

comparison with *E. coli* LPS, African green monkeys appears to be most responsive to *B. mallei* or *B. pseudomallei* than Cynomolgus or Rhesus [22¹¹]. Characterization of the immune-signaling mechanism for cellular inflammatory response revealed that *MyD88*-mediated signaling contributed to the *B. mallei* and *B. pseudomallei* induced proinflammatory responses. Notably, *B. mallei*, *B. pseudomallei*, or purified LPS from these pathogens induced *MyD88*-mediated reporter activity was inhibited and inflammatory cytokine production was attenuated by a *MyD88* inhibitor [22¹¹]. In the scenario of dysregulating inflammatory responses with established *B. mallei* infection that often leads to septicemia and immune pathogenesis, thus *MyD88*-targeted therapeutic intervention may be a potential strategy for therapy.

VACCINE STRATEGY: VACCINE MODULATION OF INNATE IMMUNITY

For complete protection against *Burkholderia* pathogens, previous vaccine efforts focused on inducing both cellular and humoral immune responses [32]. Possible candidates include whole-cell killed, subunit glycoconjugate, and live attenuated vaccines, as recently reviewed by Aschenbroich *et al.* [33]. These vaccines showed limited efficacy that resulted in partial protection and bacterial dissemination in murine models of infection. Live attenuated recombinant *Salmonella* expressing *B. mallei* LPS O antigen showed protection in a murine infection model of *B. thilandensis*, a surrogate for biothreat *Burkholderia* spp., and suggest a promising platform for vaccine development [34].

Recently, two live attenuated *B. mallei* strains consisting of mutations in ubiquitination and phagosomal escape (Δ *tssN*) or iron transport (Δ *tonB*) show protection against lethal challenges in models of murine glanders [35¹¹,36¹¹]. Analysis of the immune responses observed in vaccination-challenge studies was performed to understand how these mutants modulate immune responses. BALB/c mice surviving exposure to aerosolized Δ *tssN* showed elevated expression of proinflammatory cytokines and chemokines: IL-1 α , IL-1 β , IL-2, IL-4, IL-10, IL-12, MIG, macrophage inhibitory protein-1 α , and TNF α , and Vascular endothelial growth factor [35¹¹]. This modulation of host responses showed Δ *tssN* capable of inducing prolonged innate immunity despite its high degree of attenuation. Mice immunization with Δ *tssN* demonstrated 67% survival rates at 21 days postwild-type challenge [35¹¹]. Authors suggested the partial protection afforded by Δ *tssN* immunization was mainly driven by innate immunity as BALB/c mice failed to show increased

expression of proinflammatory cytokines and chemokines after Δ tssN prime and boost regimens.

BALB/c mice immunized with Δ tonB provided up to 100% survival at 21 days postwild-type challenge [36^{***}]. Compared with controls, immunized mice expressed moderated inflammatory cytokine/chemokine profiles with significant reductions reported in IL-6, GM-CSF, monocyte chemoattractant protein -1, and RANTES [36^{***}]. Authors correlated these results with reduced immune-mediated tissue damage observed in immunized mice. In cross-protection studies, Δ tonB-immunized mice challenged with *B. pseudomallei* K96243 demonstrated 75% survival 36 days postinfection [36^{***}]. Although these studies displayed protection and resulted in wild-type clearance, Δ tonB immunization was noted to result in persistence infection of the live attenuated mutant in the spleens of surviving mice. Despite persistence, the *B. mallei* tonB mutant shows potential as a candidate for further vaccine development and optimization.

CONCLUSION

B. mallei target intracellular host immune-signaling pathways for intracellular survival. Recent studies provide some understanding of pathogen–host protein interactions, dysregulation of macrophage activation, and immune evasion by *B. mallei*. Still, considerable gaps exist regarding the understanding of specific *B. mallei* protein(s) and signaling pathways that likely contribute to intracellular survival and evasion of host immune effector mechanisms. More focused research in delineating the molecular basis for host inability or dysregulation of the host immune effector mechanism manipulated by this pathogen is needed. This may limit persistent infection, and likely provide direction toward developing medical countermeasures.

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Conflicts of interest

There are no conflicts of interest.

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