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# Immune profiling of the progression of a BALB/c mouse aerosol infection by *Burkholderia pseudomallei* and the therapeutic implications of targeting HMGB1☆

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SUMMARY

*Introduction:* The role of damage-associated molecular pattern HMGB1 signalling in a murine BALB/c model of severe respiratory melioidosis (*Burkholderia pseudomallei* infection) was explored in this study.
*Methods:* Time course experiments were performed.
*Results:* It was established that HMGB1 was released in concert with increasing weight of organs and increasing concentration of liver enzymes in the blood a short time after cytokine release. Differences in the cytokine response between organs were observed, where the lungs contained higher concentrations of chemokines and interleukin 17, while the spleen produced more interferon-gamma, which is essential in the host defence against *B. pseudomallei*. This is evidence as to why the disease is seemingly more severe in the respiratory form. The effect of depleting HMGB1 using an antibody was also evaluated. It was found that this treatment significantly reduced bacterial load in the liver, spleen, and, to a greater degree, in the lungs. Cytokine quantification indicated that this reduction in bacterial load is likely due to the treatment reducing the release of a variety of pro-inflammatory cytokines.
*Conclusion:* It is concluded that anti-HMGB1 treatment would be effective alongside other therapeutics, where it would reduce the characteristic over-inflammation associated with late stage infection.


## 1. Introduction

The Gram-negative bacterial species *Burkholderia pseudomallei* is the causative agent of melioidosis. Melioidosis is one of the leading causes of septicaemia in certain Southeast Asian countries.[1] From the environment, *B. pseudomallei* can enter the host via different routes, and the most common routes are believed to be through the drinking of contaminated water and through cuts and abrasions of the skin.[1] It is also recognized that *B. pseudomallei* can cause particularly unpleasant primary and secondary pneumonia,[2] and this contributes to the argument that *B. pseudomallei* represents a credible bio-threat agent.[3] Melioidosis can be modelled in the laboratory in mice.[4–7] One complication of melioidosis is that *B. pseudomallei* has intrinsic tolerance to a variety of antibiotic drugs, making treatment complex and difficult,[8] and this indicates the requirement for novel therapeutics to treat the disease.

When considering the immune response to *B. pseudomallei*, much murine work has been performed to understand the infectious process, and some of this has been supported by clinical studies. The inability of the immune response to control infection in two specific ways results in sepsis and death. Firstly, it is known that neutrophils are important in limiting the morbidity of the disease. Murine neutrophils can be observed taking up the bacteria in in vivo models of respiratory disease,[9] mice that have had neutrophils ablated are hyper-sensitive to infection by *B. pseudomallei*,[10] and human melioidosis is often linked with disorders that impair neutrophil function.[11] However, neutrophils are not a ubiquitously beneficial cell and it has been demonstrated that the activity of neutrophils can contribute to the severe damage associated with disease.[12] Interferon-gamma (IFN-γ) signalling is also known to be essential in the control of *B. pseudomallei*. A plethora of work has been done to understand the cytokine response,[13–19] which has resulted in an understanding that *B. pseudomallei* seems to prevent pyroptosis, therefore reducing IFN-γ, interleukin (IL)-12, and IL-18 secretion; this results in

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increased IL-1 signalling, which is likely to contribute to the sepsis.[20]

The role of damage-associated molecular pattern (DAMP) signalling is diverse and has started to be elucidated.[21] Specifically, there is a growing body of work aimed at understanding the role of high-mobility group B protein 1 (HMGB1) signalling in a variety of inflammatory conditions.[22] Until recently, little was known about whether DAMP signalling may contribute to the disease process of melioidosis. Charoensup et al. first established that increased levels of HMGB1 in human melioidosis patients correlated with a poor prognosis and secondly explored the therapeutic potential of targeting HMGB1 in a murine model of melioidosis.[23] They found that knockdown of HMGB1 had no effect on survival independent of other treatments, but had a modest effect when administered in combination with ceftazidime.

Our study group also has an interest in HMGB1 as a target to treat infectious disease; in a previous study, it was found that HMGB1 had therapeutic benefit when used against infection by the bacterium *Francisella tularensis*.[24] In the present study, the therapeutic potential of anti-HMGB1 was investigated in a mouse model of melioidosis in which BALB/c mice were infected by aerosol route.

## 2. Materials and methods

### 2.1. Mice

Six to eight-week-old female BALB/c mice (Charles River, UK) were transferred to a high-containment class III rigid isolator, where they were assigned randomly to groups and given unlimited access to food and water. Mice were challenged with *B. pseudomallei* strain K96243 by aerosol, as described previously, using a Henderson-type apparatus[25] and a Collison nebulizer.[26] Bacteria were grown in Luria broth at 37 °C on a rotary platform. Mice were checked twice daily and scored for clinical signs. Mice were culled at predetermined humane endpoints. Survival times were recorded for some mice, and others were culled for analysis of tissues at different time points. All procedures and housing were in accordance with the United Kingdom Animal (Scientific Procedures) Act (1986). Shinotest anti-HMGB1 and isotype control IgY were obtained from Oxford Biosystems Cadama (UK). A 600-μg dose of either antibody was administered by intraperitoneal route at 24 h and 48 h post-infection to the appropriate mice.

### 2.2. Post-mortem analysis

Whole blood was micro-centrifuged at 7000 rpm for 20 min, and the serum was removed and stored at −80 °C. Serum HMGB1 was measured with an ELISA kit (Shinotest, supplied through Oxford Biosystems Cadama, UK) in accordance with the manufacturer's instructions. Serum was also tested using a 'dry-slide' technology biochemistry analyser (VetTest; IDEXX Laboratories) in accordance with the manufacturer's instructions.

The lung, liver, spleen, and blood were all processed at less than 1 h post-mortem. All organs were weighed and collected in 2 ml phosphate buffered saline (PBS) and then disrupted through a 40-μm cell sieve, and the resulting homogenate was collected. Subsequently, 100-μl aliquots of the cell suspension were used for enumeration of bacteria on agar following serial dilution in PBS and plating out on Luria agar. For cytokine analysis, 200-μl aliquots of cell suspension were centrifuged for 5 min at 2000 rpm. Supernatants were removed for cytokine analysis and stored at −80 °C. The levels of cytokine were measured via 23-plex murine Luminex array (Bio-Rad), used in accordence with the manufacturer's instructions. In addition, a magnetic plate washer

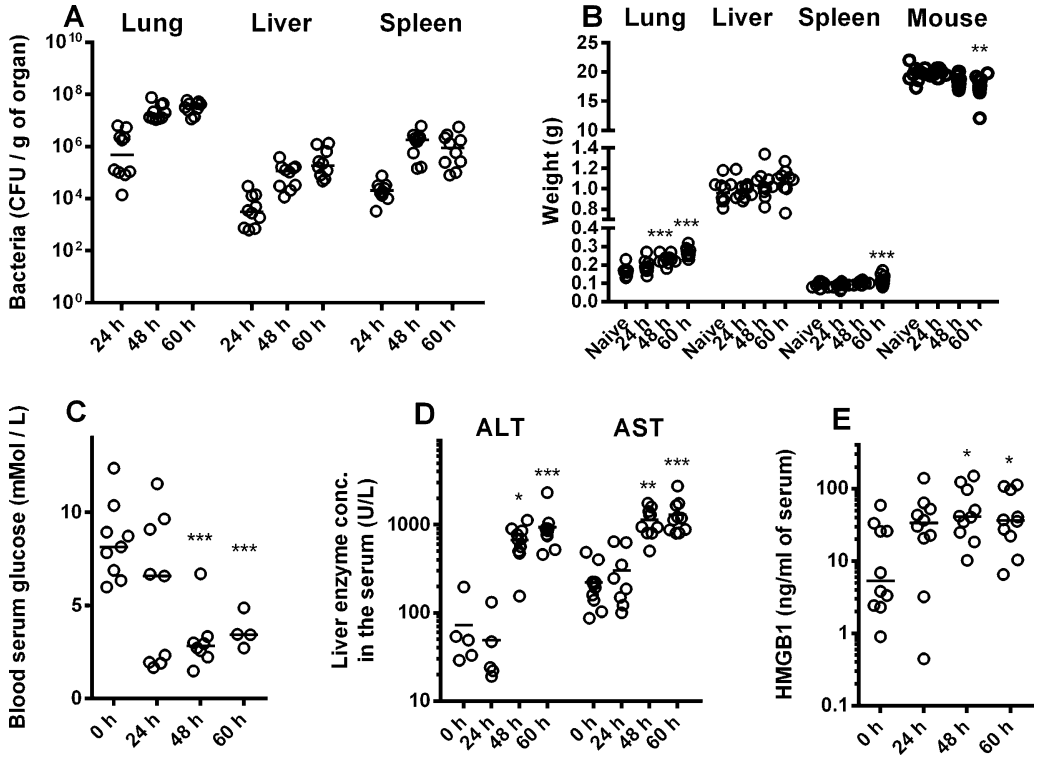


**Figure 1.** Organ bacterial load, organ weight, blood glucose, blood liver enzyme, and HMGB1 in BALB/c mice at time points post infection with *Burkholderia pseudomallei* strain K96243 by the aerosol route. Data from two experiments are shown. Groups of five mice were culled before, or 24 h, 48 h or 60 h post infection. Estimated challenge doses for the two experiments were 75 and 217 CFU. Values for each mouse are shown with symbols and the median values are included as lines. Panel A shows organ weights (lung, liver, spleen, and whole mouse). Panel B shows the bacterial load in CFU/organ (lung, liver, and spleen). Panel C shows blood serum glucose. Panel D shows liver enzyme concentrations in the blood serum (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)). Panel E shows blood serum HMGB1 concentrations. Significance markers are indicative of Bonferroni post-test comparisons to naïve mice ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).