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Detection of low-level animal-to-animal transmission in a mouse model of inhalational melioidosis.

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Abstract

Burkholderia pseudomallei, the causative agent of melioidosis, has been demonstrated to have two phases of infection. The acute phase occurs shortly after infection and is most often associated with bacterial sepsis, potentially leading to death, while the chronic phase occurs when the infection persists symptomatically or is asymptomatic for many months or years. The BALB/c mouse model is routinely used to demonstrate the acute phase of infection. However, BALB/c mice continue to succumb to infection after the acute phase is considered complete. While *B. pseudomallei* is not known for being transmitted from mouse to mouse, we hypothesized that recovered mice could potentially become re-infected from mice with an enduring chronic infection. In this study, we tested this hypothesis by co-housing naïve mice with mice challenged by aerosol exposure to *B. pseudomallei* K96243 and followed the disease outcome for 99 days post-exposure. The infected mice were housed in static or ventilated caging with uninfected mice that were introduced one hour or 48 hours following exposure to aerosolized *B. pseudomallei* K96243. The delayed introduction by 48 hours attempted to account for potential bacteria on the fur (due to whole body challenge) that would likely be removed by grooming. Two of the 48 naïve mice (approximately 4%) became infected with *B. pseudomallei* following exposure to infected mice, contaminated bedding, or other caging surfaces. Both naïve mice that were found to be infected originated in the same ventilated cage and were introduced to the exposed mice 48 hours post-exposure to aerosolized bacteria. All other mice were clear of colonizing bacteria in their spleens and lungs and showed no antibody titers to irradiated whole-cell antigens, suggesting that those naïve mice remained free from *B. pseudomallei* infection. The results of this study showed that transmission of *B. pseudomallei* is possible mouse-to-mouse at a low rate. We conclude that although the chance of re-infection is low among mice housed in the same cage, this possible scenario should be considered when interpreting data from long-duration melioidosis mouse models.

Introduction

Burkholderia pseudomallei is a gram-negative bacterium that can be found in most tropical and sub-tropical areas throughout the world. Very recently, *B. pseudomallei* was isolated from the Gulf-Coast region of Mississippi, USA and was associated with at least three confirmed human infections. A significant amount of research in both the public health and the biodefense research communities has been conducted but many important questions remain unanswered, including the mechanisms of recrudescence and persistence in animal infection models. In these studies, we wanted to explore the possibility of re-infection in a highly controlled laboratory setting using the BALB/c mouse model after exposure to aerosolized *B. pseudomallei* or after receiving *B. pseudomallei* via an intraperitoneal injection. While, infrequent, we have documented that clinically affected mice can infect naïve mice when co-housed. While it is impractical to avoid co-housing laboratory rodents, the fact that ill animals can either infect naïve animals or animals that had previously resolved the infection due to the administration of an experimental medical countermeasure must at least be considered when interpreting in vivo data, particularly from long-duration therapeutic studies.

Methods

Aerosol exposure: Female BALB/c mice were exposed to aerosolized *B. pseudomallei* K96243 in a whole-body exposure chamber and inhaled doses was calculated from CFU determinations from the AGI using Guyton's formula. The mice inhaled a dose of approximately 106 CFU. Exposed mice (n=4 per cohort) were then co-housed with naïve mice (n=4 per cohort) in static or ventilated cages (< 0.2m/sec, 70 air changes per h, exhaust air -55% air exhaust) starting at approximately 1 h or 48 h after exposure to aerosolized bacteria. There were three cages for each parameter tested for a total of 24 mice in each cohort. The differential times used to initiate co-housing would account for bacteria on the fur that would normally be removed by grooming or bacterial "die-off" within 48 h. The cages were changed every 7 days. The mice were followed at least daily for 99 days (approximately 60 days after the last exposed mouse had succumbed to disease or was euthanized in accordance with early endpoint criteria). All exposed mice and 2 naïve mice had succumbed to disease or were euthanized before the end of study. The naïve mice that succumbed had blood and organs checked for bacterial growth.

Intraperitoneal exposure: Female BALB/c mice were infected with approximately $3.8x10^4$ CFU of *B. pseudomallei* K96243 via intraperitoneal injection. The experimental design was as described above, without the cohort that was co-housed together 48 h after infection since there was little to no potential for bacteria to be on exposed fur. Mice in this study were clinically observed at least daily for 140 days post-infection. All (but 2 infected mice that survived the infection) and 2 naïve mice had succumbed to disease or were euthanized before the end of study. Subsets of mice had blood and select organs checked for bacterial growth.

Results

It is possible for mice exposed to aerosolized *B. pseudomallei* to spread the bacterium to naïve mice. While possible, this mouse-to-mouse transmission occurs at very low frequency. In this study a total of 48 naïve mice were co-housed with mice that were overtly showing clinical signs of melioidosis and only 2 became appreciably affected within 99 days (transmission rate of 4.2%) (**Figure 1**). Interestingly, the naïve mice that became ill were house in a ventilated cage and the mice were not co-housed until 48 h after exposure to aerosolized bacteria. The two naïve mice that eventually succumbed to disease or were euthanized had bacteria in the spleen and lungs at high levels (**Table 1**). The remaining surviving mice had no detectable bacteria in the lungs and spleens and no evidence of an antibody response to *B. pseudomallei* at the end of the study.

Mice infected intraperitoneally with *B. pseudomallei* have significant bacterial burdens in their lungs despite never having been exposed to aerosolized bacteria. To understand possible scenarios as to how the 2 naïve mice became infected, we wanted to evaluate the lung burden after parenteral infection. As described in **Table 2**, mice (*n*=13) that were infected with *B. pseudomallei* via intraperitoneal injection had significant bacterial burden in the lung, which could have resulted either from re-aerosolized bacteria or hematogenous spread.

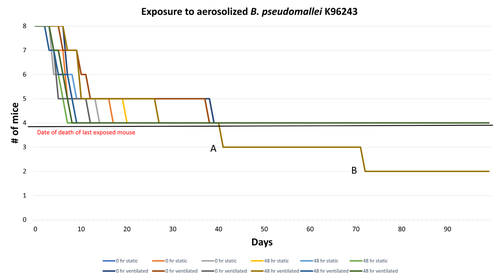

Figure 1. Survival curves of BALB/c mice exposed to aerosolized *B. pseudomallei* K96243 and then co-housed with naïve BALB/c mice. Infected mice were introduced immediately after exposure to aerosolized bacteria or 48 h later. All mice that were exposed to aerosolized bacteria succumbed to disease or were euthanized in accordance with early endpoint euthanasia by day 39. Naïve mouse A succumbed on day 41 and naïve mouse B was euthanized on day 72.

Table 1: Bacterial burden identified in naïve mice that became infected after being co-housed with mice exposed to *B. pseudomallei*

Cohort	Naive Mouse Death	Route of Exposure	Days after final Death of challenged mouse	Organ	CFU/g
48 hr Ventilated Cage	A	Aerosol	14 days	Spleen	$3.08x10^8$
48 hr Ventilated Cage	A	Aerosol	14 days	Lung	$3.80x10^7$
48 hr Ventilated Cage	B	Aerosol	45 days	Spleen	$2.76x10^6$
48 hr Ventilated Cage	B	Aerosol	45 days	Lung	$6.28x10^4$
Ventilated Cage	A	Intraperitoneal	14 days	Spleen	$5.92x10^7$
Ventilated Cage	A	Intraperitoneal	14 days	Lung	$2.02x10^8$
Ventilated Cage	B	Intraperitoneal	17 days	Spleen	$2.49x10^8$
Ventilated Cage	B	Intraperitoneal	17 days	Lung	$1.85x10^8$

It is possible for mice that were exposed to *B. pseudomallei* via intraperitoneal injection to spread the bacterium to naïve mice. To understand if this mouse-to-mouse transmission was only associated with inhalational melioidosis, we completed a study that co-housed intraperitoneally infected mice with naïve mice. In this study, the results were nearly identical to the inhalational model experiment. Two naïve mice became infected during the study. These mice were housed in the same ventilated cage. In this study there was a total of 24 naïve mice and 2 became infected within 140 days (transmission rate of 8.3%). The two naïve mice that eventually succumbed to disease or were euthanized had bacteria in the spleen and lungs at high levels (**Table 1**). The remaining surviving mice had no appreciable bacterial colonization in the lungs and spleens and no evidence of an antibody response to irradiated whole-cells of *B. pseudomallei* K96243 at the end of the study.

Table 2: Bacterial burden identified in mice after injection with *B. pseudomallei*

Organ	CFU/g
Spleen	$1.14x10^8$ ($2.52x10^3$-$7.42x10^8$)
Lung	$2.45x10^5$ (0-$2.67x10^6$)

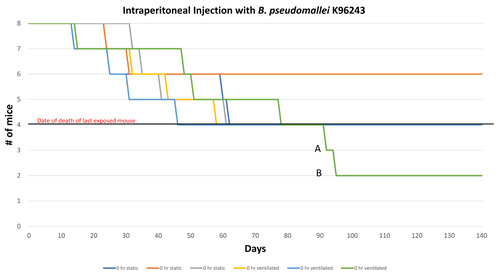

Figure 2. Survival curves of BALB/c mice injected intraperitoneally with *B. pseudomallei* K96243 and then co-housed with naïve BALB/c mice. Infected mice were introduced immediately after injection. All but two mice that were exposed to bacteria succumbed to disease or were euthanized in accordance with early endpoint euthanasia by day 39. Naïve mouse A succumbed on day 92 and naïve mouse B succumbed on day 95.

SUMMARY

1. Mouse-to-mouse transmission of melioidosis is infrequent but possible.
2. While not enough naïve mice became infected for robust statistical analyses, in both studies, transmission was observed in mice housed in ventilated cages. Could this mechanism of ventilation be aerosolizing bacteria excreted by infected mice ?
3. Other routes of transmission are possible. These include eating or inhaling contaminated fecal material or urine. While cannibalism was not observed in these cases, it is another possible for route of infection.
4. This low-level of transmission is unlikely to impact the results of vaccination experiments performed with C57BL/6 mice due to their increased resistance to *B. pseudomallei,* but this was not investigated.
5. This low-level of transmission should be considered for long-duration therapeutic studies, although the significance of this is equivocal, but measures to prevent this may be considered.

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Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense Health Agency.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011. The facility where this research was conducted is fully Accredited by the Association for Assessment and Accreditation Of Laboratory Animal Care International.

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