Onset of Bacteremia in Mice Following Nose-Only Inhalation of Bacillus anthracis Ames Spores

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Abstract

Background
Bacillus anthracis is the etiological agent of the disease anthrax in humans and animals. The 90% lethal dose (LD90) in mice has been described in several animal species. However, the onset of bacteremia following nose-only inhalation exposure has not been well described. The accurate timing of onset of bacteremia is critical for experimental design when testing antimicrobial and therapeutic agents for post-exposure prophylaxis to prevent an ensuing fatal disease. Methods
Six groups of BALB/c mice, consisting of five male and five female mice, were exposed by nose-only inhalation to an average inhaled dose of 3.0E+05 CFU of B. anthracis Ames strain spores. Mice were euthanized at 0, 1, 3, 6, 12, 24, 36, and 48 hours post-exposure. Blood samples were obtained, and the percentage of viable B. anthracis spores was determined by colony counts on TSA plates. Results
Bacteremia was absent in mice of all ages immediately after exposure. Subsequently, bacteremia rapidly developed to 0.0% by 12 hours and remained at 0.0% at 24 hours. By 48 hours, 100% of mice were positive for B. anthracis bacteremia. Bacterial load analysis of blood demonstrated high bacterial loads (= 5.0E+05 CFU/ml) in nearly all 24 hours post-exposure. Conclusions
These results suggest that initiating antimicrobial treatment may be necessary earlier than 24 hours post-exposure for successful prevention of anthraxosis.

Introduction
Bacillus anthracis, the etiological agent of anthrax, is a Gram-positive, aerobic endospore-forming, rod-shaped bacterium. Spores are produced under nutrient starvation, and are highly resistant to a variety of harsh environmental and chemical conditions. Spores are capable of long-term survival in the environment and can germinate and outgrow into vegetative cells following favorable conditions such as a mammalian host. The bacteria causes cutaneous, gastrointestinal, and inhalational anthrax disease, with the inhalational form being the most lethal to humans. Following deposition in the lungs, spores are phagocytized by alveolar macrophages and rapidly begin producing the lethal endotoxin (LPS). The spores may cause fatal infection disease regardless of antibiotic therapy due to the presence of the toxins in the spore. This feature provides the anthrax bacteria with the ability to escape the immune system.

Methods

Exposure System
A model used in this study was an inhalation chamber that consisted of a Collison Jet nebulizer, passive air diluter, radial aerosizer, stainless steel delivery line, and radial nose-only exposure plenum. The exposure system used in this study was an inhalation chamber that consisted of a Collison Jet nebulizer, passive air diluter, radial aerosizer, stainless steel delivery line, and radial nose-only exposure plenum. The exposure system used in this study was an inhalation chamber that consisted of a Collison Jet nebulizer, passive air diluter, radial aerosizer, stainless steel delivery line, and radial nose-only exposure plenum.

Results

Bacteremia was analyzed at time of scheduled euthanasia, time of death, or time of morbidity. Bacterial load levels were up to 5.0E+05 CFU/ml in bacteremic animals. Initial group. However, results of bacteremia analyses were interpreted based on actual time of blood collection. The final group sizes and percent bacteremia are listed in Table 1. Onset of bacteremia is presented in Figure 3.

Conclusions
The MMWG for male and female mice was 2.0E+05 CFU and 1.6E+05 CFU, respectively. Bacteremia following nose-only inhalation exposure is an immediate follow-up. The presence of vegetative cells was first observed at 1.24%. Because administration of antibiotic treatment after the onset of bacteremia may not effectively control anthraxosis, this study suggests that it may be necessary to begin antibiotic therapy earlier than 24 hours post-exposure.

References

Acknowledgements
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Table 1. B. anthracis Ames Particle Size Distribution

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<th>Number</th>
<th>Concentration (µm)</th>
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