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Abstract Number: 059 (B)
Control Number: 173

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Natural History of *Bacillus anthracis* Ames in Cynomolgus Macaques Following Inhalation Challenge

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Abstract

Background
The use of *Bacillus anthracis* Ames (BAA) as a bioweapon has necessitated the continued research of therapeutic agents. Understanding the pathogenesis of inhalation anthrax in animal models will facilitate this research. The purpose of this study was to evaluate the natural history of inhalation anthrax in cynomolgus macaque nonhuman primates (NHP) following lethal challenge of aerosolized BAA spores.

Methods
Three groups cynomolgus macaques were challenged with target inhaled doses of 100, 200, and 400 LD_{50} using a novel oro-nasal inhalation exposure system. Blood was collected daily for microbiological, clinical chemistry and hematological analyses. Various tissue samples, including lung, liver, spleen, lymph node, and brain, were collected at necropsy for macroscopic and microscopic pathological evaluation.

Results
The NHPs received 142 ± 74, 294 ± 60, and 391 ± 178 LD_{50} of BAA (n = 4). NHPs showed few clinical signs until one to two hours prior to death. All NHPs died or were euthanized within 10 days. The onset of bacteremia started at 12 hours in 17% of NHPs, but peaked at 100% bacteremia at 48 hours. NHPs challenged with BAA by inhalation exposure showed marked inter-individual variability in hematology and clinical chemistry responses with no clear dose-response relationship in incidence or magnitude of observable changes. Macroscopic and microscopic lesions observed in the animals in this study were consistent with previously reported lesions of inhalation anthrax in non-human primates and other species.

Conclusions
In conclusion, the natural history of anthrax in cynomolgus macaques following inhalation challenge was evaluated. Blood collections for hematology, clinical chemistry, and bacteremia verified infection while gross and microscopic pathological analyses confirmed pathogenesis. The cynomolgus macaque is an appropriate model for the study of inhalation anthrax.

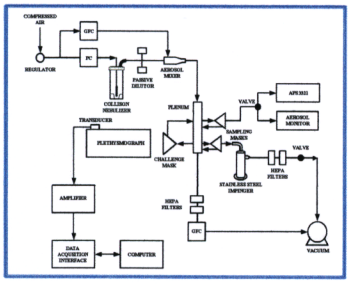
Introduction

The cynomolgus macaque (*Macaca fascicularis*) has been recognized as an alternative species to the rhesus macaque for therapeutic and vaccine efficacy research against inhalation anthrax.[1] To facilitate this research, the goal of this project was to evaluate the natural history of inhalation anthrax in cynomolgus macaques following lethal challenge of aerosolized *Bacillus anthracis* Ames spores.

Methods

Three groups of four male and female cynomolgus macaque nonhuman primates (NHP) were challenged by oro-nasal inhalation. The NHPs were anesthetized and positioned in a supine orientation in a custom designed head-out plethysmograph. A schematic of the challenge system is presented in Figure 1. All NHPs were challenged with a polydisperse aerosol of *Bacillus anthracis* Ames (BAA) spores with a particle size distribution of 1.2 µm, 1.7 (MMAD, GSD). The total inspired volume for Groups 1, 2, and 3 NHPs was 6.0 L, 12.9 L, and 23.0 L resulting in inhaled doses of 142 ± 74, 294 ± 60, and 391 ± 178 LD_{50}, respectively (1 LD50 = 6.2E+04 CFU).[2] Beginning on the day of challenge, all NHPs were observed approximately every six hours for clinical signs of disease. Body weights and temperatures were collected daily. Blood samples were collected for hematological and microbiological analysis at six hour intervals post challenge for two days and then daily through Day 10. Sections of lung, liver, spleen, lymph nodes, and brain were collected for macroscopic and microscopic histopathological evaluation.

Figure 1. NHP Oro-Nasal Inhalation Challenge/Plethysmography System

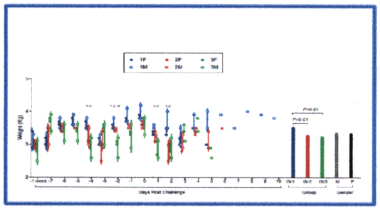


Results

Body Weight

Individual body weight across gender and groups is presented in Figure 2. Animal weights were higher for NHPs in Group 1 than in Group 2 and Group 3 (t(6) = 3.82; P = 0.0088, t(6) = 4.42; P = 0.0088. No significant differences in weight were observed between male and female NHPs.

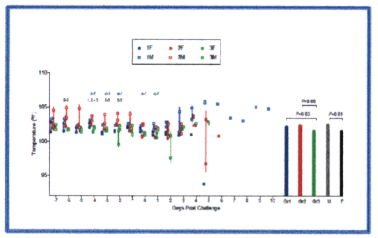
Figure 2. Individual and Group Body Weight



Body Temperature

Individual and group body temperature data is presented in Figure 3. Body temperatures were higher in Group 1 and Group 2 NHPs than Group 3 NHPs. There were no significant differences in body temperatures between Group 1 and Group 2. Overall, body temperatures for male NHPs were higher than for female NHPs (t(6) = -486; P = 0.0028).

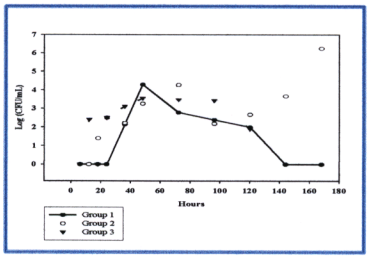
Figure 3. Individual and Group Body Temperature



Microbiological Analysis

The mean bacterial concentration of BAA in the blood of Groups 1, 2, and 3 NHPs collected from 6 to 240 hours is presented in Figure 4. At no one time collection point were all blood samples bacteremic. Bacterial concentration ranged from 30 CFU/mL to 1.80E+06 CFU/mL

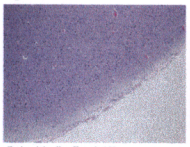
Figure 4. Average Concentration of *Bacillus anthracis* in the Blood



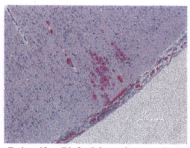
Microscopic Histopathology

Microscopic lesions consistent with BAA infection were observed in various organs of the NHPs but were most frequently observed in the lymph nodes, spleen, brain, and meninges. These lesions consisted of necrosis, lymphoid apoptosis, acute inflammation, hemorrhage, edema, congestion, and the presence of rod shaped bacteria. Microscopic photographs of tissue slides prepared from minimally and acutely affected organs are presented in Figures 5-9 below.

Figure 5. Brain

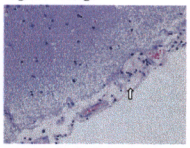


Brain minimally affected. 100x

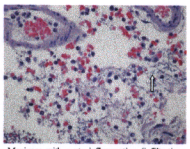


Brain with mild focal hemorrhage, moderate perivascular hemorrhage, moderate vascular necrosis, minimal focal necrosis, mild congestion, and presence of rod bacteria. 100x

Figure 6. Meninges

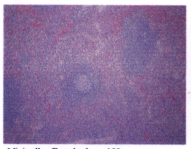


Meninges minimally affected. Note occasional rod bacteria present. 600x

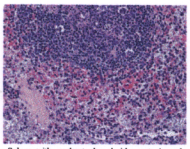


Meninges with acute inflammation (infiltration of neutrophils) and rod bacteria (arrow). 600x

Figure 7. Spleen

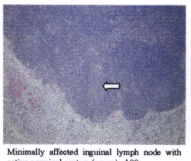


Minimally affected spleen. 100x

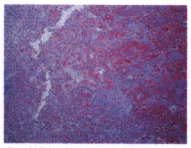


Spleen with moderate lymphoid apoptosis and numerous rod bacteria in the red pulp. 400x

Figure 8. Lymph Node

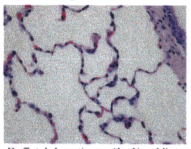


Minimally affected inguinal lymph node with active germinal centers (arrow). 100x

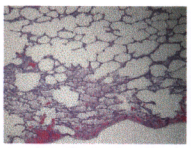


Bronchial lymph node with marked necrosis, mild acute inflammation, marked hemorrhage, marked ectasia, and rod bacteria. 100x

Figure 9. Lung



Unaffected lung tissue with thin, delicate alveolar walls, clear alveolar lumen and ciliated cuboidal cells lining the small bronchus. 600x

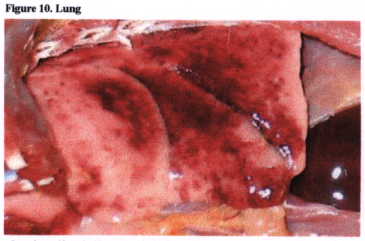


Lung tissue with eosinophilic proteinaceous material within the alveolar lumen and rod bacteria. 400x

Macroscopic Histology

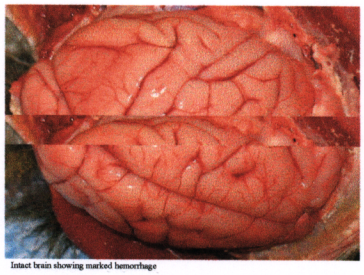
Macroscopic lesions observed in the NHPs of this study were consistent with BAA infection. Lesion were observed in the lymph nodes, spleen, lung, brain, stomach, small and large intestine, heart, kidney, adrenal glands, and various other organs. Macroscopic lesion were observed most frequently in the lymph nodes; however, lesions in all organs consisted of perivascular hemorrhage, edema, inflammation and necrosis. Examples of macroscopic lesion observed in the lung and brain are shown in Figures 10 and 11.

Figure 10. Lung



Intact lung with marked hemorrhage and inflammation.

Figure 11. Brain



Intact brain showing marked hemorrhage

Conclusions

The rhesus macaque (*Macaca mulatta*) has historically been used as the primary nonhuman primate model for inhalation anthrax. However, the rhesus macaque is becoming less available. Thus, this study sought to confirm that the cynomolgus macaque was an acceptable alternative. The natural history of inhalation anthrax in cynomolgus macaques following BAA challenge was demonstrated. Infection with BAA was confirmed by hematology, clinical chemistry, and bacteremia. Pathology was confirmed with macroscopic and microscopic analyses. The cynomolgus macaque is an appropriate model for inhalation anthrax that can be used for future efficacy testing.

References

1. Leffel EK and Pitt LM. *Anthrax;* Biodefense Research Methodology and Animal Models; Taylor and Francis: Boca Raton, Florida, 2006; Chapter 6; pp 77-94. Edited by Swearengen JR

2. Vasconcelos D, Barnwell, R, Babin M, Hunt R, Estep J, Neilsen C, Carnes R, Carney J (2003). Pathology of Inhalation Anthrax in Cynomolgus Monkeys (*Macaca fascicularis*). Laboratory Investigation. 83(8), pp 1201-1209.

Acknowledgements

This contract was funded by the National Institutes of Health, National Institute of Allergy and Infectious Disease, Contract N01-AI-30063.