INFECTION AND IMMUNITY, July 2005, p. 4420–4422 Vol. 73, No. 7
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.7.4420–4422.2005

Complement Depletion Renders C57BL/6 Mice Sensitive to the *Bacillus anthracis* Sterne Strain

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Received 18 October 2004/Returned for modification 10 January 2005/Accepted 16 February 2005

Concerns regarding safety and control of virulent *Bacillus anthracis* have created substantial hurdles to the study of anthrax. The Sterne strain is considered relatively safe to study, but this acapsular strain has a defect in normal mice and is often studied in A/J mice. A/J mice are highly susceptible to the Sterne strain, due to a defect in the Hc locus, which encodes complement factor 5 (C5). Here we show that normally resistant C57BL/6 mice become highly susceptible to the Sterne strain upon complement depletion with cobra venom factor. This generalizable approach should allow the virulence of anthrax to be studied under relatively safe conditions and using a wide variety of mouse strains.

Bacillus anthracis, the etiological agent of anthrax, is a gram-positive, aerobic, spore-forming, rod-shaped bacterium (2). Dormant spores are highly resistant to adverse environmental conditions and are able to reestablish vegetative growth in the presence of favorable environmental conditions (6). Fully virulent strains of *B. anthracis* carry two large plasmids, pXO1 and pXO2, which carry the genes encoding anthrax toxin production and capsule formation, respectively. The roles of these factors in pathogenesis have been extensively studied (for review see references 1, 3, 5, and 6). Bioterrorism concerns have spurred increased interest in *B. anthracis* and in efforts to improve vaccines and treatments against anthrax. However, advances are limited by an incomplete understanding of the biology of infection, bacterial growth, pathology, and protective immunity. Unfortunately, only a handful of laboratories are equipped to perform animal experiments with the virulent bacterium. The utility of less virulent strains is dependent on an understanding of the defect(s) associated with each strain and how this defect(s) influences interactions with host immunity. Ideally, an experimental model would involve a defined strain with increased sensitivity to a specific host factor that rendered it avirulent in normal animals but left it fully virulent in animals lacking that factor. Such a model would approximate natural infection under conditions achievable in many laboratories without the biocontainment concerns associated with the use of the virulent strain. If such a model could be achieved in mice, the extensive tools available in this organism could be applied to dissect the genetic and immunological aspects of the bacterium-host interaction.

The most widely studied strain of *B. anthracis* is the Sterne strain, which harbors plasmid pXO1, which encodes the toxin, but lacks plasmid pXO2 and therefore lacks the capsule. Importantly, use of this strain eliminates concerns about the toxic effect of capsule observed in mice (12). Welkos et al. demonstrated that two strains of mice, A/J and DBA/2J, were susceptible to the Sterne strain when delivered by the intraperitoneal or subcutaneous route (11). They also observed that both strains of mice had greatly reduced levels of C5 enzymatic activity relative to strains of mice resistant to infection with the Sterne strain (10). This work indicates that the susceptibility of the A/J and DBA/2J strains of mice to the Sterne strain is due to complement deficiency, although it does not rule out the possibility that other genetic factors confer or contribute to that sensitivity. We hypothesized that if the major role of capsule was to protect *B. anthracis* from the effects of complement, the acapsular Sterne strain would be virulent in mice from a resistant background with a specific lack of complement activity. We observed that when *B. anthracis*-resistant (C57BL/6) mice were depleted of complement, they became as highly susceptible to pulmonary exposure with the Sterne strain as the sensitive (A/J) mice. The implications of this finding to future studies of *B. anthracis* pathogenesis are discussed.

To examine whether complement is involved in the control of the acapsular Sterne strain, C57BL/6 mice were given intraperitoneal injections of either phosphate-buffered saline (PBS) (vehicle control) or PBS containing cobra venom factor (CVF) as described previously (4, 8). CVF enzymatically degrades complement and has been shown to result in nearly complete depletion of complement activity (9). Control mice were mock inoculated with PBS, and naturally sensitive A/J mice were included in the study as a comparator group. Groups of mice were then exposed for 90 min to aerosolized spores prepared from *B. anthracis* strain 7702 (7×10^9 to 8×10^9 spores per ml in distilled H_2O) (7). The spore aerosol was generated with a 6-jet Collison nebulizer equipped with a precious fluids jar (BGI Incorporated, Waltham, MA) using a nose-only exposure system (CH Technologies, Westwood, NJ). Prior to exposure, mice were supplied with fresh air for 10 min to allow respiratory rates to normalize. One hour following exposure, two to four mice were euthanized, and their lungs were homogenized

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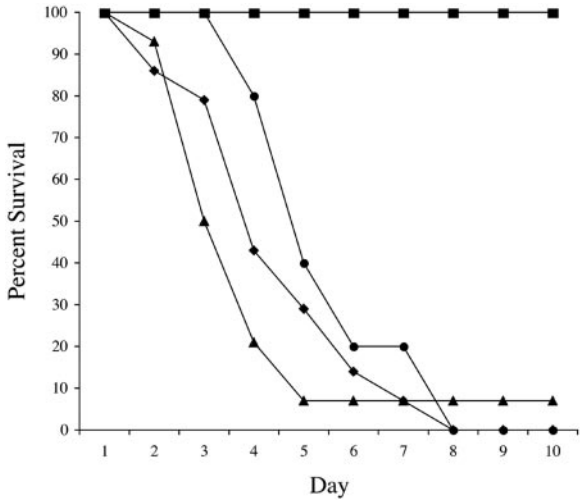

FIG. 1. Survival of mice challenged with the *B. anthracis* Sterne strain. Mice were exposed to aerosols of spores prepared from *B. anthracis* strain 7702 as described in the text. The survival of groups of mice with a retained dose after challenge between 1×10^6 and 5×10^6 spores from three sequential experiments is shown: A/J (diamonds), CVF-treated A/J (circles), C57BL/6 (squares), CVF-treated C57BL/6 (triangles). For A/J, C57BL/6, and CVF-treated C57BL/6 mice, the cumulative mortality of three independent challenges is represented in the graph ($n = 15$). For CVF-treated A/J mice, the cumulative mortality of two independent challenges is represented in the graph ($n = 10$).

using a Stomacher (Seward Inc., Thetford, Norfolk, United Kingdom) and plated to determine the number of organisms that were retained in the lungs (average retained dose). In all of the challenge studies presented in this report, the retained dose in the lungs on day zero was between 1×10^6 and 5×10^6 CFU per mouse. A subset of mice was observed for 10 days to assess survival. At various time points following exposure, mice were sacrificed, and lungs and spleens were removed, homogenized, and plated on brain heart infusion agar to determine the number of CFU. A/J mice began to succumb to anthrax within 2 days following this challenge, with 100% of mice dying with an average time to death of 4.6 ± 1.8 days (Fig. 1). C57BL/6 mice survived without any apparent signs of disease. In contrast, when CVF-treated (complement-deficient) C57BL/6 mice were challenged with strain 7702, mice began to succumb to disease within 2 days, with 93% of mice dying with an average time to death of 4.0 ± 1.3 days (Fig. 1). Depleting complement from A/J mice only minimally affected susceptibility to *B. anthracis* if at all. Relative to untreated A/J mice, CVF treatment of A/J mice resulted in the same overall mortality and a similar time to death (5.6 ± 1.5 days) (Fig. 1). These results indicate that depletion of complement alone is sufficient to render the resistant C57BL/6 strain highly susceptible to the Sterne strain. A/J mice were not affected by CVF treatment, consistent with their susceptibility to Sterne being due primarily to a natural complement deficiency. Together, these results suggest that the avirulence of the Sterne strain in normal mice (and humans) is due to its increased sensitivity to complement, and that the Sterne strain is virulent in mice lacking complement.

To determine whether the Sterne strain kills complement-deficient mice by overgrowth and systemic spread, as is observed in fulminant anthrax, mice were exposed to anthrax as described above. On day 5 postinfection, mice were sacrificed, and lungs and spleens were removed, homogenized, and plated

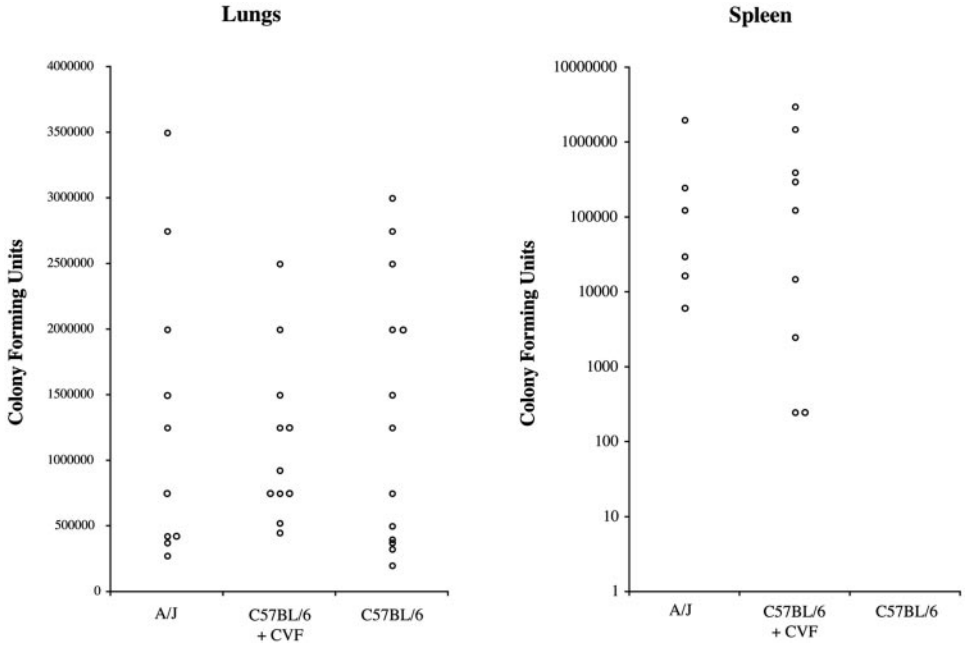

FIG. 2. Infection of lungs and spleens in mice challenged with the *B. anthracis* Sterne strain. A/J, C57BL/6, and CVF-treated C57BL/6 mice were exposed to aerosols of spores prepared from *B. anthracis* strain 7702 as described in the text. Challenged mice received a retained dose of between 1×10^6 and 5×10^6 spores per mouse (which equals the total lung count). Mice were sacrificed on day 5 postchallenge. The number of CFU present in the lungs and in the spleens is presented. The lower limit of detection for *B. anthracis* in spleen or lung was 250 CFU in each case. Negative spleen cultures were not graphed.

to determine the number of CFU as described above. Although spore numbers in the lungs decayed between day 0 and day 5, large numbers of CFU were observed in the lungs of all of the mice from all three groups, and no difference was observed in the number of CFU present in the lungs between the three groups (Fig. 2). In A/J mice, large numbers of *B. anthracis* were recovered from the spleens of 6 of 10 mice, indicating that the death of these animals is associated with systemic spread of the pathogen and septicemia. In contrast, no bacteria were recovered from the spleens of C57BL/6 mice, indicating that bacteria are contained within the respiratory tract in these mice which are highly resistant to the Sterne strain. However, when C57BL/6 mice were treated with CVF, they lost their ability to contain *B. anthracis* within the respiratory tract. Bacteria were observed in the spleens of 9 of 11 CVF-treated C57BL/6 mice, indicating that in normally resistant strains of mice depleted of complement, the Sterne strain is able to cause fulminant bacteremia and death, similar to that of the virulent strain in wild-type animals.

Together these data support the use of the acapsular Sterne strain in complement deficient mice as a safe and efficient means to study the biology of anthrax. Because capsule is an important virulence determinant in natural *B. anthracis* infections, experiments using an acapsular strain must be designed and interpreted with care, and important conclusions should eventually be confirmed in additional animal models. However, because *B. anthracis* capsule is unusually toxic in mice, the pathology observed upon infection of mice with a capsule-positive *B. anthracis* strain is unlikely to accurately reflect the pathology observed in other animal models. Except when examining the role of capsule, the use of the acapsular Sterne strain in complement-deficient mice is arguably a better model for the initial studies of *B. anthracis* pathogenesis. There are a number of substantial advantages to this experimental system. (i) Use of an acapsular strain of *B. anthracis* eliminates the problems of interpretation associated with the high toxicity of capsule in mice, which is not reflective of the effect of capsule in other animal models of anthrax, including humans. (ii) Since CVF treatment is broadly applicable, specific immunodeficient mouse strains could be used to dissect the relevant immune parameters involved in control and/or clearance of *B. anthracis*. Other research-specific issues may make it desirable to use specific strains of mice that are resistant to infection with the Sterne strain. CVF treatment greatly increases the number of mouse strains that are available for use in this model. (iii) If a high-containment laboratory is not required for anthrax animal studies, the cost associated with these experiments will be greatly decreased and, more importantly, the number of researchers in the field who will be capable of independently pursuing their ideas will be expanded. This should relieve pressures on those limited numbers of high-containment labs, allowing them to focus on those high-priority projects for which use of the fully virulent strain is required. In principle, the approach that we have taken, to pair reciprocal defects in pathogen and host, could also be applied to the study of other organisms, thereby broadening the scope of research for other important pathogens.

We thank Gopa Raychaudhuri, Manual Osorio, and Karen Meysick for their critical reading of the manuscript.

REFERENCES

1. **Collier, R. J., and J. A. T. Young.** 2003. Anthrax toxin. Annu. Rev. Cell Dev. Biol. **19:**45–70.
2. **Koch, R.** 1876. Die etiologie der milzbrand krankheit hegrundet auf die entwickelungsgeschichte des Bacillus anthracis. Beit. Biol. Pflanz. **2:**277–283.
3. **Lacy, D. B., and R. J. Collier.** 2002. Structure and function of anthrax toxin. Curr. Top. Microbiol. Immunol. **271:**61–85.
4. **Li, S., V. M. Holers, S. A. Boackle, and C. M. Blatteis.** 2002. Modulation of mouse endotoxic fever by complement. Infect. Immun. **70:**2519–2525.
5. **Moayeri, M., and S. H. Leppla.** 2004. The roles of anthrax toxin in pathogenesis. Curr. Opin. Microbiol. **7:**19–24.
6. **Mock, M., and A. Fouet.**2001. Anthrax. Annu. Rev. Microbiol. **55:**647–671.
7. **Pickering, A. K., M. Osorio, V. K. Grippe, G. M. Lee, M. Bray, and T. Merkel.** 2004. The cytokine response to infection with *Bacillus anthracis* spores. Infect. Immun. **72:**6382–6389.
8. **Shapiro, S., D. O. Beenhouwer, M. Feldmesser, C. Taborda, M. C. Carroll, A. Casadevall, and M. D. Scharff.** 2002. Immunoglobulin G monoclonal antibodies to *Cryptococcus neoformans* protect mice deficient in complement component C3. Infect. Immun. **70:**2598–2604.
9. **Vogel, C.-W.** 1991. Cobra venom factor: the complement-activating protein of cobra venom, p. 147–188. *In* A. T. Tu (ed.), Handbook of natural toxins: reptile venoms and toxins, vol. 5. Dekker, New York, N.Y.
10. **Welkos, S. L., and A. M. Friedlander.** 1988. Pathogenesis and genetic control of resistance to the Sterne strain of *Bacillus anthracis*. Microb. Pathog. **4:**53–69.
11. **Welkos, S. L., T. J. Keener, and P. H. Gibbs.** 1986. Differences in susceptibility of inbred mice to *Bacillus anthracis*. Infect. Immun. **51:**795–800.
12. **Welkos, S. L., N. J. Vietri, and P. H. Gibbs.** 1993. Nontoxigenic derivatives of the Ames strain of *Bacillus anthracis* are fully virulent for mice - role of plasmid pX02 and chromosome in strain dependent virulence. Microb. Pathog. **14:**381–388.

Editor: A. D. O'Brien