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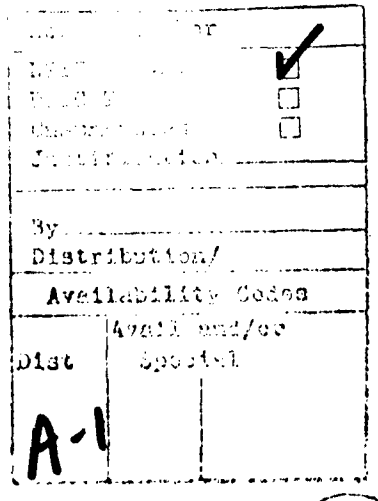

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Differences in Susceptibility of Inbred Mice to *Bacillus anthracis*

Running Title: Susceptibility of Mice to *B. anthracis*

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In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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ABSTRACT

Animal species differ in their resistance both to infection by B. anthracis and to anthrax toxin. A mouse model was developed to study the basis of these host differences and the pathogenesis of infection. When infected with the virulent B. anthracis strain Vollum 1B, all eight strains of inbred mice tested had low LD_{50} values (5 to 30 spores). However, analysis of time-to-death (TTD) data revealed significant differences between the strains, which could be divided into three groups: most susceptible (A/J, DBA/2J, and C3H/HeN); intermediate (C57BL/6J, C57L/J, and C58/J); and least susceptible (CBA/J and C57BR/cdJ). In contrast, the mice were distinctly susceptible or resistant to lethal infection by Sterne vaccine strain. The LD_{50} of the susceptible A/J and DBA/2J mice was approximately 10^3 Sterne spores; whereas the remaining six relatively resistant strains were killed only by 0.2 to 2 x 10^7 spores. Mice lethally infected with B. anthracis had an acute course, characterized by extensive gelatinous edema and large concentrations of bacilli in the blood and organs (e.g. 10^9 CFU/g spleen). To study susceptibility to anthrax toxin, the protective antigen (PA) and lethal factor (LF) components of the toxin were injected i.v. into A/J and CBA/J mice. As reported earlier for various animal species, susceptibilities of the mice to anthrax toxin appeared to be independent of that to infection. The toxin LD_{50} values for both strains were about 12 µg PA combined with 2.4 µg LF. However, CBA/J mice died sooner that A/J mice, with mean TTD values of 0.9 and 3.7 days, respectively, in mice given four LD_{50} of toxin. The mouse model thus appears to be useful in studies on host resistance to anthrax and on pathogenesis of the infection.

INTRODUCTION

Bacillus anthracis, the agent of anthrax, causes disease primarily in domestic and wild animals. However, it can produce either cutaneous anthrax or an often fatal systemic disease (inhalation or gastrointestinal anthrax) in people exposed to infected animals or their products (4, 6, 13, 22).

The two major virulence factors of B. anthracis are a poly-D-glutamic acid capsule and an exotoxin, which are encoded by two plasmids (26; B.D. Green, L. Battisti, and C.B. Thorne, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H99, p. 139). It has been hypothesized that the capsule allows the bacilli to evade early phagocytic defenses(8), and in vitro studies have demonstrated the anti-phagocytic effect of the capsular material (16). Anthrax toxin is composed of three proteins: protective antigen (PA), lethal factor (LF), and edema factor (EF). In guinea pigs and rabbits, PA combined with EF produces edema in the skin, and PA plus LF injected intravenously causes death in guinea pigs, rats, and mice. None of the proteins by itself possesses toxic activity (3, 9, 22, 35, 36, 38). Protective antigen is immunogenic and is the major component in the current human anthrax vaccine used in this country (13, 22).

The pathogenesis of infection by B. anthracis, the role of toxin in the disease, and mechanisms of host defense are poorly understood. Previous studies indicated that animal species vary in their resistance to lethal infection and to killing by the toxin (9, 12, 15, 17, 19, 24, 39). Outbred mice and guinea pigs are killed by low parenteral doses of B. anthracis (LD_{50} values of 5 and 50 spores, respectively), whereas the corresponding LD_{50} dose for rats is approximately 10^6 spores (17, 19, 24, 39; J. Jemski, unpublished

data). Surprisingly, animals such as the rat, that are resistant to lethal infection, appear to be generally more susceptible to killing by injected toxin than are those that succumb to a small infectious dose. Also, the terminal concentrations of toxin and bacilli in the blood of infected animals were found to vary directly with that species' resistance to toxin. For example, just prior to death, infected guinea pigs had 50 units of toxin per ml of blood and 2×10^8 CFU of B. anthracis per ml. In contrast, blood of moribund rats yielded less than eight toxic units per ml and only 1×10^4 CFU/ml (22, 24). Thus, resistance to the organisms and susceptibility to the toxin appear to be separate, aspects of pathogenesis (19, 24).

In order to study pathogenesis and mechanisms of host resistance, an animal model which simulates the range of host responses to anthrax is needed. Abalakin et al. provided evidence for differences in resistance among inbred mice (1). Among six strains tested, five were killed by 400 spores of a fully virulent encapsulated strain of B. anthracis. However, one strain (CC57BR) survived challenge with both the low (4×10^2 CFU) and high (4×10^4 CFU) doses of spores. When challenged with a nonencapsulated, toxin-producing vaccine strain (STI), two mouse strains, A/Sn and DBA/2, died, whereas the other mouse lines tested were resistant. The purpose of our work is to determine the suitability of inbred mice as a model for studying infection by B. anthracis. We have identified and initially characterized eight strains of mice that differ significantly in their susceptibility to infection by B. anthracis and to lethal intoxication.

MATERIALS AND METHODS

Mice. C3H/HeN mice were purchased from Harlan Industries, Inc., Indianapolis, IN. A/J, DBA/2J, CBA/J, C57L/J, C58/J, C57BL/6J, and C57BR/cdJ mice were purchased from Jackson Laboratories, Bar Harbor, MN. Female mice that were 6-weeks old and approximately 18 - 22 g were used in all experiments.

Bacterial strains and media. A toxigenic encapsulated strain (Vollum 1B), toxigenic nonencapsulated strain (Sterne), and nontoxigenic encapsulated strain (Pasteur 6602) were obtained from the culture collection of the U.S. Army Medical Research Institute of Infectious Diseases, USAMRIID, Fort Detrick, MD. Strain VNR-1, obtained from B. Ivins, is a rough derivative of Vollum 1B that was cured of a plasmid required for capsule production by growth in the presence of novobiocin (B. E. Ivins and C. B. Thorne, Abst. 85th Ann. Meet. Amer. Soc. Microbiol. 1985, H100, p. 124). Spore preparations of each strain were made by using the medium and growth conditions described by Leighton and Doi (20). Cultures containing at least 90% spores and 10^9 CFU/ml were collected by centrifugation at 4000 x g for 20 min, washed (twice) in sterile water, and suspended in a volume of water equal to approximately 1/50 the original culture volume. Dilutions of the spore stock were plated for viable counts on trypticase soy agar (TSA) plates, before and after heating the stock at 68°C for 30 min to destroy vegetative bacilli. Plates were incubated at 37°C in an atmosphere of 5% CO_2 in air. Glycerol was added to 10% (v/v), and the spore stock was frozen in aliquots at -70°C. Prior to each infection experiment, a frozen aliquot was thawed, diluted in Hanks balanced salt solution, and the dilutions spread on TSA plates for viable count determinations.

Virulence testing of spores. Mice were inoculated with 0.2-ml volumes of spores. Except where otherwise noted, inoculations were s.c.; some experiments employed i.p., intranasal, and aerosol routes. R. Berendt (Aerobiology Division, USAMRIID), kindly performed the intranasal and aerosol inoculations. Ten mice were infected with each dose of organisms. They were observed for 14 d and the 50% lethal dose (LD_{50}) was calculated by Probit analysis, using the Computerized Biostatistical Analysis Library (CBAL), USAMRIID, Frederick, MD, or by the graphic method of Litchfield and Wilcoxon (25). The time-to-death (TTD) for each mouse was also recorded and the geometric and harmonic mean TTD calculated for each dose of B. anthracis (10, 31). The harmonic mean TTD is determined by the following:

$$TTD = \frac{N}{\Sigma(1/TTD)}$$

where N is the total number of animals infected per dose and TTD is given in days, with TTD equal to ∞ for survivors. Two different methods were used to perform regression analysis of the dose-dependent TTD for each mouse strain. In the first, reciprocal harmonic mean TTD values were determined for each dose and strain and then compared by using computer programs (Statistical Analysis System, SAS Institute, Cary, N.C. 1982). For the second analysis, Cox's proportional hazards model was used to estimate the probability of survival of the infected mice with time after inoculation (SAS program BMDP2L, BMDP Statistical Software, University of California, Berkeley, CA. 1983). The Cox method models the survival instead of TTD of infected mice and is based on instantaneous rates of death ("hazard" rates) relative to the given

covariates of mouse strain and dose of infecting organism. Mouse susceptib ,ity groups were determined in both regression analyses by multiple pairwise comparisons by the Bonferroni method (27). The susceptibility rankings of the mouse strains derived from both methods were the same.

Virulence testing of toxin components. Purified LF and PA components of anthrax toxin, and affinity-purified goat anti-PA antibody, were gifts of S. Leppla (21). PA and LF were combined in a ratio of 5:1 (w/w), and serial twofold dilutions of the mixture were prepared and injected into A/J and CBA/J mice via the tail vein, as described by Ezzell et al. (9). Five mice per strain were inoculated with each dilution of the mixture or with PA, LF, or diluent alone; the experiments were done twice. Heart blood collected from necropsied mice was spread on sheep blood agar medium and the plates incubated at 37°C in air with 5% CO_2. The TTD values were recorded and the LD_{50} was determined as given above for the infection experiments. The TTDs of A/J compared to CBA/J mice were analyzed statistically with CBAL programs for analysis of variance and Fisher's least significant difference test.

The biological activity of the PA and LF preparation used was confirmed by the sensitive rat lethality assay for anthrax toxin (3, 9). Rats injected i.v. with doses containing 12 μg PA and 2.4 μg LF (approx. four LD_{50}s) died within 106 min. When these doses were preincubated with 120 μg of affinity-purified goat anti-PA antibody, the rats were protected from lethal toxicity.

Necropsy and specimen collection. Mice were killed by i.m. injection of 5 mg ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, NY) and 1 mg xylazine (Rompun, Miles Laboratories, Shawnee, Kansas) in 50 μl, and were dissected immediately. Gross pathological changes were noted, heart blood and

subcutaneous edema fluid were collected by needle aspiration, and organs were removed. The latter were weighed for quantitative culture or were processed for staining with hematoxylin and eosin. Edema fluid and blood specimens were smeared on slides for staining with Giemsa and Gram stains. Specimens to be cultured were homogenized and diluted in 0.4% Na_2HPO_4, pH 7.0, with 0.2% gelatin (15), and spread on TSA plates.

RESULTS

Eight strains of inbred mice were screened for differences in susceptibility to lethal infection by B. anthracis. Table 1 shows the response of mice to inoculation with the fully virulent encapsulated and toxin-producing Vollum 1B strain of B. anthracis. Following s.c. challenge with the virulent organism, all of the mice were killed by relatively low doses; the lethal doses (LD_{50}) ranged between 5 to 30 spores. The route of inoculation influenced the general susceptibility of inbred mice to anthrax. Table 1 summarizes the dose of Vollum 1B required for lethality when given by different routes (s.c., i.p., intranasal, and aerosol). In general, mice were most susceptible to infection by the s.c. route and least susceptible to aerosol challenge.

Despite the similarity in the lethal doses of Vollum 1B for the different mouse strains, analysis of the TTD and probability of survival of animals revealed significant strain-related differences. Table 2 summarizes the results in the three groups of mice found to differ significantly in their susceptibility to Vollum 1B. Strains CBA/J and C57BR/cdJ (group III) were the most resistant, surviving for 5.4 and 6.5 d, respectively, after s.c. inoculation with the mean dose (60 spores). The A/J, DBA/2J, and C3H/HeN strains (group I) were the least resistant to killing, dying within 3.5 d after infection. The mice in group II had TTD values intermediate between those of the two significantly different groups. Figure 1 illustrates the kinetics of survival for each strain infected with the mean dose of spores. The group III mice clearly succumbed to infection at a slower rate than group I animals.

The Sterne strain of B. anthracis is a non-encapsulated toxin-producing organism used as a live vaccine in domestic animals. It is considered to be relatively avirulent (2, 13, 37). Inbred mice were found to differ greatly in susceptibility to subcutaneous infection with Sterne. There was a 10^3 to 10^4 difference between the LD_{50}s of two susceptible strains (A/J and DBA/2J) and the LD_{50}s of the remaining six relatively resistant strains (Table 3). VNR-1, a toxigenic non-encapsulated derivative of Vollum 1B, demonstrated similar lethality for mice. The LD_{50} of A/J mice infected with VNR-1 was 1.3×10^3 spores, while that of CBA/J mice was 1.8×10^7 spores. Time-to-death values were also determined for mice infected with Sterne. The TTDs of mice infected with 5×10^5 spores of Sterne were 2.1 d for A/J, 3.0 d for DBA/2J, and 11.4 to more than 14 d for the remaining strains of mice. These results corroborated the division of mouse strains by LD_{50} into susceptible groups (Group I - A/J and Group II - DBA/2J) and a resistant group (III-remaining strains) as shown in Fig. 2. In addition, these data indicate that the A/J and DBA/J mice are not equally susceptible, in that A/J mice succumbed to infection more rapidly than did DBA/2J mice (Fig. 2).

The pathologic and bacteriologic findings were similar to those previously observed in anthrax-infected animals (13, 15, 17, 19, 22, 23). After inoculation with the Vollum 1B strain and just prior to death, the mice became wasted in appearance and very lethargic. They developed paralysis of the left leg (i.e. close to the site of s.c. inoculation) and an often extreme swelling of the abdominal and pelvic region. Typical findings upon autopsy of infected mice included an enlarged, soft, and darkened spleen, a gelatinous and often hemorrhagic s.c. exudate,and dark thickened blood. Occasionally the

lungs and liver appeared to be hyperemic; other major organs were grossly normal. Histologic sections of lung, liver, kidney, and heart from most of the mice appeared to be relatively normal, except for frequent congestion and the presence of numerous bacilli. The latter were seen most notably in the glomeruli of the kidneys, within the alveoli and blood vessels of the lungs, and in vascular spaces and sinusoids of the liver. The most impressive and common changes were noted in the spleens from lethally infected mice, which were often very congested and had large masses of organisms. The normal follicular architecture of the spleen was frequently destroyed and replaced by RBCs, bacilli, histiocytes, and cellular debris. In addition, samples of the extraperitoneal exudate fluid from terminally infected mice usually showed blood cells and many gram- positive bacilli. Stained heart-blood specimens revealed erythrocytes which were often distorted or shrunken, and numerous encapsulated gram positive bacilli. The latter were usually present as single or duplex rods; longer chains of bacilli were often observed in the edema fluid specimens.

Septicemia was confirmed by growth of the organisms in culture. Quantitative cultures of all organs sampled from the mice (spleen, liver, lungs, and blood) were positive for B. anthracis during the period in the infection of rapidly increasing mortality. High concentrations of bacteria were observed particularly in the spleen, where bacilli often exceeded 10^9 CFU/g.

Infection of mice with lethal doses of the Sterne strain of B. anthracis caused similar pathologic and bacteriologic signs in the final stages of disease.

The susceptibility of the mouse strains to anthrax toxin was studied in order to verify a role for toxin in this mouse model and to determine the relative susceptibilities of the strains to toxin. Strains A/J and CBA/J were injected i.v. with preparations of PA antigen and LF mixed in a 5:1 (w/w) ratio. It was recently shown that this ratio of purified toxin components yields maximal toxicity in rats and is lethal to mice and guinea pigs (9). The toxin LD_{50} values for the two strains were similar, 11.0 μg PA/2.2 μg LF for A/J and 12.4 μg PA/2.5 μg LF for CBA/J. However, the CBA/J animals died more rapidly than did the A/J mice, as shown in Fig. 3, which depicts the cumulative mortality in toxin-treated groups having 80 to 100% mortality. The mean TTD of A/J and CBA/J mice given 8 LD_{50} doses, 100 μg PA/20 μg LF, were 3.1 d and 1.1 d, respectively; corresponding values for mice given 4 LD_{50} doses were 3.7 d and 0.9 d, respectively. Mice injected with PA, LF, or diluent alone survived. Heart blood collected from four mice dying between 6 h and 5 d after injection yielded no growth on blood agar.

In vivo production of toxin by B. anthracis presumably has a major role in lethality of the mice, as it does for other animals (22, 36). Encapsulated but nontoxigenic B. anthracis were avirulent for our animals. All of the A/J mice inoculated with doses up to 10^6 spores (highest dose tested) of Pasteur strain 6602 (capsule +, toxin -) survived the infection (data not shown).

DISCUSSION

Systemic anthrax is a rapidly progressive and lethal disease caused by toxigenic and encapsulated strains of Bacillus anthracis (4, 6, 13, 22, 26). Animal species differ considerably in their natural resistance to this disease. Herbivores are the most frequently infected animals, and empirical data have suggested that cattle and sheep are especially susceptible. Studies with laboratory animals have confirmed the large variation in host resistance (12, 15, 19, 24, 39). However, the mechanisms of both host resistance and pathogenesis of disease remain obscure.

Development of a lethal infection by B. anthracis requires germination of the spores at the challenge site, invasion of the bloodstream with systemic multiplication by the bacilli, and toxin production leading to death. Germination and invasion by the organism appear to be dependent on the presence of specific conditions at the site of inoculation. For instance, Hachisuka found that spores inoculated i.p. into rats were engulfed and destroyed by phagocytic cells (12). In contrast, spores rapidly germinated and began vegetative replication in the peritoneal cavities of mice, and thus escaped phagocytosis. Rats survived infection whereas mice succumbed to lethal anthrax. Suspension of anthrax spores in substances such as specific phosphatides or a combination of nutrients was shown to decrease the LD_{50} for rats and hasten the onset of bacteremia (12, 15, 19, 34). It appears that differences in the local milieu might be involved in the species variation in resistance to anthrax.

The inoculation site also influences the pathogenesis and outcome of infection. Although inhalation is presumed to be one of the natural routes of exposure to B. anthracis, animals generally are much more refractory to aerosol than to parenteral challenge (23, 24, 39). This is probably due in part to the mucociliary barrier to infection presented by the respiratory tract. In contrast to the fate of spores inoculated i.p., spores reaching the alveoli appear to be engulfed by alveolar macrophages and are transported by these cells to local lymph nodes (12, 23, 33, 39). Here they germinate and are released into the lymphatics (33). The role of the cellular response in the pathogenesis of anthrax at different sites and in different hosts requires further investigation. In sum, these studies suggest that host- and site-specific responses influence the establishment of infection by B. anthracis and possibly the innate resistance of the animal to lethal disease.

In the present study, we have examined inbred mice as a model for studying host variation in susceptibility to infection by B. anthracis. The course of disease and pathology in lethally infected mice resembled that reported for other animals with anthrax (23). Major findings in the mice included invasive infection and bacteremia, dark thickened blood, an enlarged dark, and soft spleen, and copious amounts of gelatinous edema.

The susceptibilities of eight mouse strains to infection with B. anthracis were characterized. In contrast to previous findings by Abalakin and coworkers (1), none of the mouse strains examined was resistant to lethal infection with capsule- and toxin-producing B. anthracis (Vollum 1B). Nevertheless, one group of mice (Group III - Table 2) had clearly prolonged survival times in comparison to the more rapidly killed group (I) of mice.

The intermediate response of the third group (II) of animals suggests that the variation in susceptibility to Vollum 1B is continuous, and that host response to this toxigenic and invasive microorganism is complex. In contrast, infection with the attenuated Sterne and VNR-1 strains provided a striking division of the mice into resistant and susceptible groups. Both the LD_{50} and survival time data support the separation. The C3H/HeN mouse strain was the only one showing a "crossover" response, being resistant to Sterne, but relatively susceptible to Vollum 1B. These results indicate that susceptibility to the nonencapsulated, toxigenic strains may be under genetic control of one or a few major loci (32). Mice which are resistant to the nonencapsulated strains (CBA/J) and those which are susceptible (A/J) are being bred so that F1 and backcross progeny can be analyzed for single-gene control of resistance.

The Sterne and STI strains of B. anthracis are considered to be avirulent and are currently used as live vaccines for domestic animals and man (2, 13, 37). Consequently, most animals must have a mechanism for controlling infection by nonencapsulated strains of B. anthracis. Our data indicate that this function is reduced or absent in A/J and DBA/2J mice, and we are investigating a genetic basis for this finding. These mice have several abnormalities in host defense mechanisms, yet exhibit organism-specific and not generalized responses to infections (5, 30, 32). For instance, macrophages of A/J mice are defective in their responses to LPS and in cytotoxicity induction; however, these mice are resistant to certain organisms for which the macrophage is presumed to play a major role (11, 14, 28). Both A/J and DBA/2J mice have a genetically-determined deficiency in the C5 protein

of complement, and this defect may have a role in susceptibility to some organisms (7). In vitro data have suggested a role for complement in resistance to B. anthracis. O'Brien et al. (29) showed that normal rabbit serum, but not heat-treated serum, can opsonize the capsule-negative Sterne strain and allow phagocytosis by human PMNs.

Identification of the genetic basis of susceptibility in mice to nonencapsulated B. anthracis would be of practical value in vaccine studies. The efficacy of live attenuated strains in immunization of animals suggests that they might be superior to the widely used PA vaccine in man (13, 18, 37). The latter induces antibody levels that are low and short-lived (13, 22). Inbred mice could be used to screen newly developed live vaccine strains for one which protects mice, such as the A/J animals, against challenge with virulent organisms, without itself causing disease.

Evidence for the toxigenic nature of anthrax was provided by Smith and Keppie more that 30 y ago (35). In recent studies, Leppla showed that the edema factor of anthrax toxin is an adenylate cyclase in tissue culture cells (21). However the primary cause of death in anthrax and the molecular action of anthrax toxin in vivo are still unknown (13, 22, 36).

In past studies, animals injected i.v. with anthrax toxin exhibited a pattern of susceptibility distinct from the pattern following infection. Generally data showed that species which are relatively resistant to lethal infection, e.g. the rat and dog, are killed by much smaller doses of toxin than are the species which are susceptible to infection, e.g. guinea pigs (9, 19,22, 24, 36). These independent responses to toxin and to infection in an animal suggest that the mechanisms responsible for host defense against spore challenge and lethal toxicity are separate entities. The susceptibility of

inbred mice to anthrax toxin was studied using the A/J and CBA/J strains, representing mice that are relatively susceptible or resistant, respectively, to infection. Lethal doses of PA and LF for these mice were similar and agreed well with the value for outbred mice, 12.5 μg PA combined with 2.5 μg LF (9). However, CBA/J mice were killed more rapidly by toxin than were the spore-susceptible A/J animals. In order to determine whether the CBA/J mice are more sensitive to toxin than the A/J, we intend to assay concentrations of toxin and of B. anthracis in lethally infected mice and to study the effect of toxin on mouse cells in vitro. A macrophage cytotoxicity test for LF has been developed which demonstrates that macrophages derived from resistant mice, such as CBA/J, are killed at lower doses of toxin than those from susceptible mice such as A/J (A. M. Friedlander, personal communication).

In summary, the susceptibility of several strains of inbred mice to anthrax was characterized. Mice differed significantly in their susceptibility to lethal infection by B. anthracis and to lethal toxicity. The graded response of the strains to a strain of B. anthracis which is encapsulated and toxigenic implies that the pathogenesis of, and host response to, anthrax is multifactorial. However, the mice were distinctly resistant or susceptible to the nonencapsulated bacilli, making feasible genetic studies on the mechanisms of host response to these organisms. The host response to toxin challenge appeared to be independent of that to infection. This mouse model has potential value in studies on the pathogenesis and prevention of anthrax.

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TABLE 1. Susceptibility of mice to virulent *B. anthracis* strain Vollum 1B

Mouse Strain	LD_{50} by Inoculation Route (# spores)			
	s.c.	i.p.	aerosol	intranasal
A/J	5.5 (2.6)[a]	41	4.2×10^5	5.4×10^3
DBA/2J	<6		$>1.3 \times 10^6$[b]	5.5×10^3 (1.5)[a]
C3H/HeN	<6		$>2 \times 10^5$[b]	7.3×10^4
C57BL/6J	14.5			
CBA/J	25 (3.4)[a]	151	$>2 \times 10^5$[b]	
C58/J	9			
C57L/J	22			
C57BR/cdJ	30[a]			

[a] Geometric mean (geometric standard deviation) of two to three experiments

[b] Highest dose tested

TABLE 2. Differences in susceptibility of mice to lethal infection with virulent B. anthracis (Vollum 1B)

Susceptibility Group[a]	Mouse Strain	Time-To-Death, Days[b]
I	DBA/2J	3.1 (2.8-4.8)
	A/J	3.2 (2.6-3.7)
	C3H/HeN	3.5 (2.8-3.6)
	C57BL/6J	4.1 (3.1-6.0)
II	C57L/J	4.3 (3.3-6.5)
	C58/J	5.2 (3.7-8.5)
	CBA/J	5.4 (4.5-6.8)
III	C57BR/cdJ	6.5 (5.0-9.3)

[a] Mice were divided into three significantly different groups ($p = .002$) by multiple pairwise comparisons (Bonferroni method) of the regression coefficients for each mouse strain (data not shown). Comparisons were made at the $\log_{10}$ geometric mean dose (60 spores) of Vollum 1B and were made relative to C57BL/6J. Analysis of covariance had confirmed that the slopes of the regression curves for each strain were not statistically different.

[b] Harmonic mean TTD of mice infected with the geometric mean dose. Values in parentheses correspond to the 95% confidence interval over the TTD.

TABLE 3. Susceptibility of mice to nonencapsulated strains of *B. anthracis*

Mouse Strain	Susceptibility Group[a]	LD_{50} (# Spores) of Challenge Strain	
		Sterne	VNR-1
A/J	S	1.05×10^3 (1.1)[b]	1.6×10^3
DBA/2J	S	2.0×10^3	
C57BL/6J	R	2.4×10^6	
C3H/HeN	R	8.3×10^6	
CBA/J	R	2.1×10^7(4.7)[b]	1.8×10^7
C58/J	R	$>4.0 \times 10^7$[c]	
C57BR/cdJ	R	N.D.[d]	
C57L/J	R	N.D.[d]	

[a] - Relatively susceptible (S) or relatively resistant (R) to killing after subcutaneous inoculation with *B. anthracis* strains Sterne or VNR-1

[b] - Geometric mean (geometric standard deviation) of two experiments

[c] - All C58/J mice survived a dose of 4×10^7 spores and all succumbed to a dose of 4×10^8 spores

[d] - N.D. - not done. Mice were challenged with 2 doses of spores. All mice survived doses of $1 - 2 \times 10^5$ spores and all were killed by $1 - 2 \times 10^8$ spores

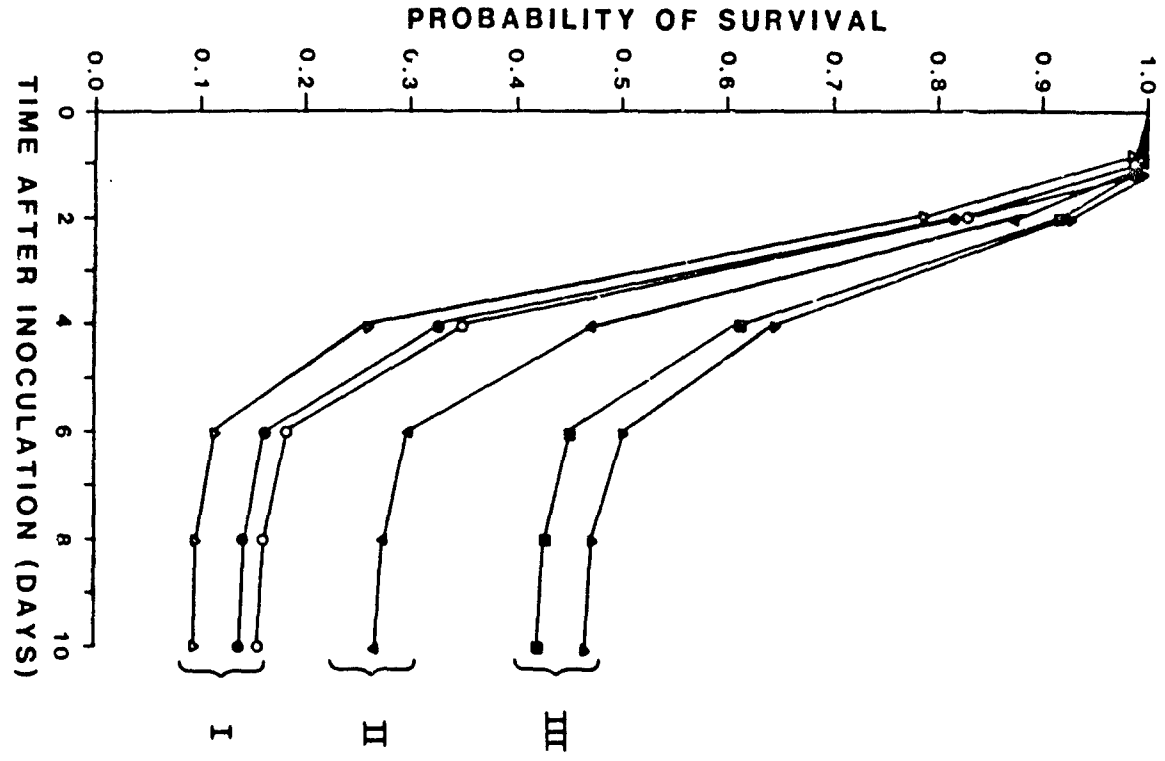
PROBABILITY OF SURVIVAL
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
TIME AFTER INOCULATION (DAYS)
0
2
4
6
8
10
I
II
III

Fig 1

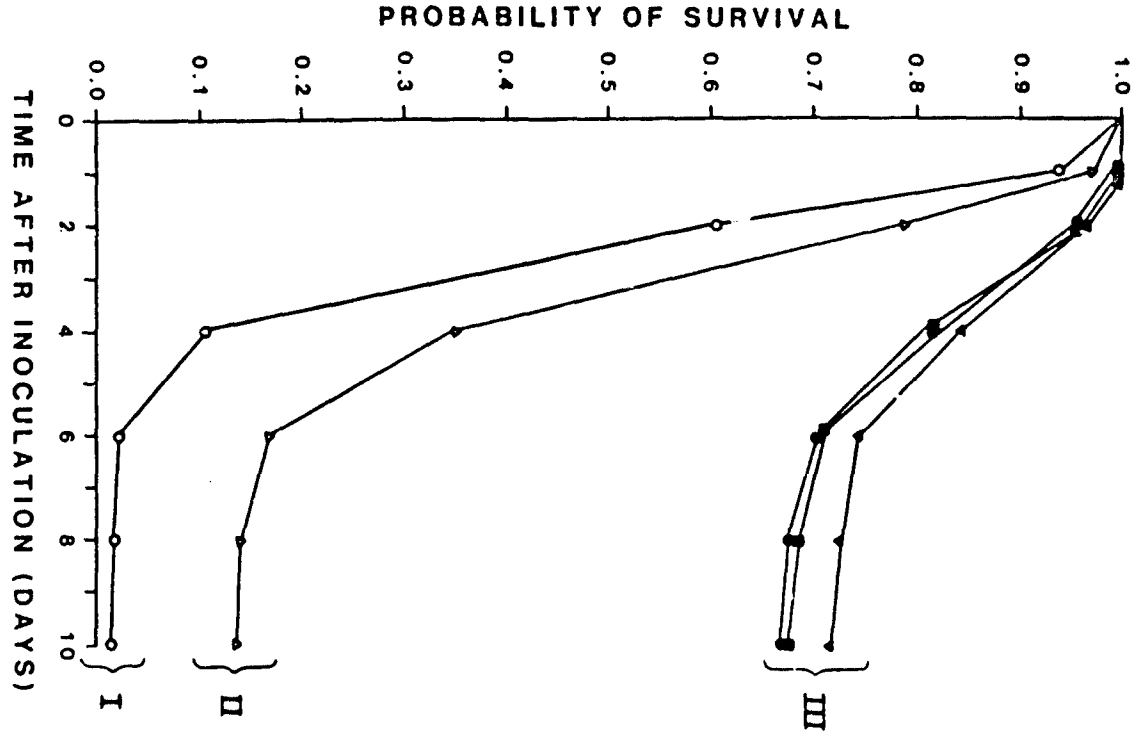
PROBABILITY OF SURVIVAL
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
TIME AFTER INOCULATION (DAYS)
0
2
4
6
8
10
I
II
III

Fig. 2

Fig. 3

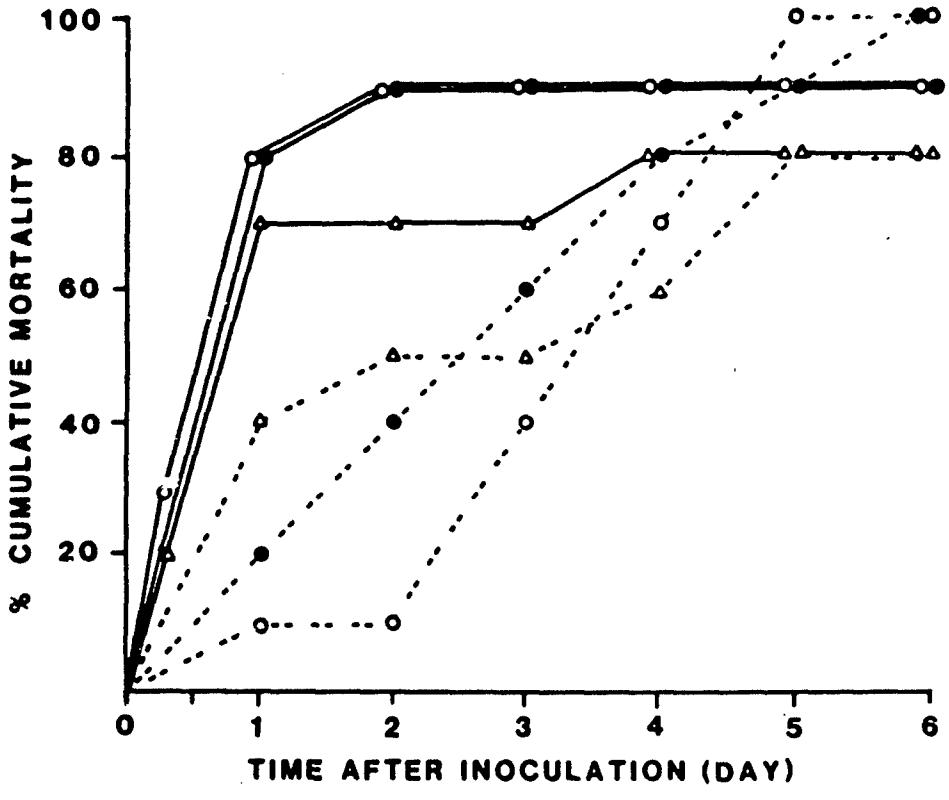
100
80
60
40
20
% CUMULATIVE MORTALITY
0
1
2
3
4
5
6
TIME AFTER INOCULATION (DAY)

FIGURE LEGENDS

FIG. 1. The probability of survival with time of mice infected with the geometric mean dose (60 spores) of Vollum 1B. The probability is shown from 1.0 (100% survival) to 0 (0% survival). Cox's proportional hazards method was used to model survival of the mice, as described in Materials and Methods. The three susceptibility classes are shown, consisting of A/J(○), C3H/HeN (●), and DBA/2J (△) in the most susceptible group I; C57BL/6J (▼), representative of the intermediate group II; and CBA/J (■), and C57BR/cdJ (▲) in the least susceptible group III.

FIG. 2. The probability of survival with time of mice infected with the geometric mean dose (5×10^5 spores) of Sterne. The mouse strains were divided into three significantly different groups ($p=.0005$), two susceptible groups (I and II) and one resistant group (III). The symbols correspond to those in Fig. 1.

FIG. 3. Cumulative mortality of mice injected intravenously with doses of PA and LF. CBA/J mice (——) and A/J mice (---) were inoculated with the following mixtures, given in μg PA/μg LF: 100/20 (●), 50/10 (○), or 25/5 (△). The mean TTD of A/J compared to CBA/J for the two highest doses of PA/LF were significantly different, at $p<.01$ and $p<.001$, respectively.