Characterization of a Therapeutic Model of Inhalational Anthrax Using an Increase in Body Temperature in New Zealand White Rabbits as a Trigger for Treatment

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The development of an appropriate animal therapeutic model is essential to assess the potential efficacy of therapeutics for use in the event of a *Bacillus anthracis* exposure. We conducted a natural history study that showed New Zealand White rabbits exhibited a significant increase in body temperature (SIBT), changes in hematologic parameters, and increases in C-reactive protein and succumbed to disease with an average time to death of approximately 73 h following aerosol challenge with *B. anthracis* Ames spores. The SIBT was used as a trigger to treat with a fully human monoclonal antibody directed at protective antigen (PA). Ninety percent (9/10) of the treated rabbits survived the lethal inhalational challenge of *B. anthracis*. Further characterization investigated the protective window of opportunity for anti-PA antibody administration up to 12 h post-onset of SIBT. Eighty-three percent (5/6) of the rabbits treated at SIBT and 100% (6/6) of those treated at 6 h after SIBT survived challenge. Only 67% (4/6) of the rabbits treated at 12 h after SIBT survived. The increase in body temperature corresponded with both bacteremia and antigenemia (PA in the blood), indicating that SIBT is a suitable trigger to initiate treatment in a therapeutic model of inhalational anthrax.

*Bacillus anthracis*, the etiologic agent of anthrax, is a Gram-positive, spore-forming bacterium that can cause human disease via the gastrointestinal, cutaneous, or inhalation (pulmonary) routes, each exhibiting different clinical manifestations of disease, with pulmonary being the most lethal (3). The virulence of *B. anthracis* is predicated on the bacterial poly-D-glutamate capsule and a tripartite toxin consisting of three polypeptides, protective antigen (PA), lethal factor (LF), and edema factor (EF), which interact to form two interlinked toxins (17). PA and LF combine to produce anthrax lethal toxin (LT), and the PA and EF combine to produce edema toxin (ET). Upon binding to the host cell, the N-terminal region of PA (PA83) is cleaved, and the resulting 63-kDa protein (PA63) heptamerizes, forming a ring structure with competitive binding sites for three molecules of LF and/or EF (18). Once the enzymatic moiety (EF and/or LF) binds to the oligomerized PA63, the complex enters the cell via receptor-mediated endocytosis. Conformational changes to PA63 in the acidic endosome facilitate the translocation of EF and LF into the cytosol (1). EF is a calmodulin-dependent adenyl cyclase that forms 3',5'-AMP from ATP in many different types of cells (14). LF is a zinc metalloprotease with mitogen-activated kinase kinases 1, 2, 3, 4, 6, and 7 as the only known substrates (5, 21, 29). The enzymatic actions of these toxin components inhibit signaling cascades required for proper immune cell function and contribute to the pathology associated with disease, such as edema and hemorrhage of infected tissues.

A humoral response to PA confers protection against anthrax, and PA is the dominant antigen in the current licensed anthrax vaccine adsorbed (AVA). Protection of rabbits vaccinated with recombinant PA (rPA) correlates directly with anti-PA titer (15). Passive immunization with anti-PA antibodies has also been shown to provide protection in animal models (11, 16, 20, 22, 23).

The ability to generate high titers of *B. anthracis* spores by using basic microbiological techniques, combined with the ability of this agent to be disseminated by aerosolization, has made anthrax a bioterrorist and military threat. It is current practice to vaccinate at-risk individuals, such as military personal, first responders, and laboratory workers with AVA, but due to the rare occurrence of anthrax in the human population it may not be feasible to vaccinate the general population. However, there is the need to protect an unvaccinated population exposed to an intentional release, as evidenced in 2001 with the anthrax letter attacks. Such protection would come in the form of antibiotics, postexposure vaccination, and passive immunization. To develop these medical countermeasures, animal models are required to assess the efficacies of vaccines and therapeutics. The three general indications of medical countermeasures against anthrax are the following: (i) general-use prophylaxes, given prior to exposure (e.g., vaccines); (ii) postexposure prophylaxes, given after exposure, prior to onset of symptoms (e.g., vaccines and antibiotics); and (iii) therapeutics, given once the subject has presented with symptoms (e.g., antibiotics and passive immunization).

The rabbit and nonhuman primate have been used extensively in medical countermeasure development and are considered appropriate animal models of human inhalational anthrax (6, 27, 28, 32). Development of a true therapeutic treatment model requires that the animal demonstrate clinical signs of disease (e.g., bacteremia) prior to treatment. However, by the time bacteremia can be confirmed by culture results during the conduct of an efficacy study, the disease may have progressed to a state where therapeutic intervention is no longer effective. Therefore, we explored the use...
of clinical and physiological changes observed following a lethal exposure to *B. anthracis* as potential “triggers for treatment.” We utilized a significant increase in body temperature (SIBT) as the trigger to treat with a fully human monoclonal antibody to PA. Our hypothesis was that treatment following exhibition of SIBT would result in increased protection of animals exhibiting signs of inhalational anthrax. In the current body of work, the antibody was administered therapeutically (animals confirmed as bacteremic at the time of treatment) and showed significant protection in inhalational anthrax. In the current body of work, the antibody would result in increased protection of animals exhibiting signs of inhalational anthrax. In the current body of work, the antibody was administered therapeutically (animals confirmed as bacteremic at the time of treatment) and showed significant protection in the New Zealand White (NZW) rabbit model of inhalational anthrax.

**MATERIALS AND METHODS**

**New Zealand White rabbits.** NZW rabbits (specific pathogen free) were obtained from Covance Research Products (Denver, PA). The animal procedures were approved by Battelle’s Institutional Animal Care and Use Committee. All work was done in a biosafety level 3 (BSL-3)/animal BSL-3 laboratory registered with the Centers for Disease Control and Prevention and inspected by the Department of Defense and the U.S. Department of Agriculture.

**Aerosol challenge.** A modified Microbiological Research Establishment-type three-jet collision nebulizer (BGI, Waltham, MA) with a precious fluid jar was used to generate a controlled delivery of aerosolized *B. anthracis* Ames spores from a liquid suspension into a muzzle-only exposure chamber. Atmospheric samples from within the exposure chamber were collected in an impinger (model 7541; Ace Glass Inc.) filled with approximately 20 ml of sterile water. The liquid in the impinger was diluted and enumerated by the spread plate technique to quantify viable spore counts per ml. The viable counts per ml of liquid, exposure time, and impinger sampling rate were used to determine the total number of spores per liter of atmospheric air (aerosol concentration) in the exposure chamber. During each exposure, the aerosol particle size in the exposure chamber atmosphere was determined using an APS spectrometer (model 3321; TSI Inc., Shoreview, MN).

Whole-body plethysmography was performed in real time on each animal during agent challenge to measure important respiratory parameters. These parameters (tidal volume, total accumulated tidal volume, and minute volume) were calculated from the measured volumetric displacement of air caused by the movement of the thoracic cavity of an animal while in a sealed plethysmograph. The total accumulated tidal volume, along with the aerosol concentration, was used in calculating the inhaled dose. The inhaled dose was converted to median lethal dose (LD$_{50}$) equivalents by using the LD$_{50}$ cited by Zaucha et al. of 1.05 × 10$^6$ *B. anthracis* Ames spores (32).

**Temperature monitoring.** Each rabbit was sedated with acepromazine (1 to 5 mg/kg of body weight), and a programmable temperature transponder (IPTT-300; BMDS, Seaford, DE) was implanted subcutaneously (at the shoulder blade level) prior to exposure to *B. anthracis* Ames spores. Temperature readings were taken twice daily prior to challenge to establish a normal baseline for each animal. Body temperatures were monitored hourly from 12 to 72 h postchallenge. The threshold for an elevated body temperature was set as the average baseline temperature plus two times the standard deviation of the rabbit’s baseline temperature. The SIBT was dependent upon the inherent variation in baseline body temperature and defined as three consecutive readings that were greater than the threshold for an elevated temperature. Following the hourly temperature monitoring period, surviving rabbits were monitored twice daily.

**Bacteremia.** Whole blood was collected into EDTA tubes, streaked over blood agar plates, and incubated at 37°C for 24 to 48 h. Plates containing colonies with a morphology consistent with *B. anthracis* were reported as positive.

**Circulating protective antigen ELISA.** Serum was isolated from whole blood collected in serum separator tubes (SST). Serum samples were then evaluated for quantitative circulating PA levels in an enzyme-linked immunosorbent assay (ELISA). A double-affinity-purified polyclonal, monospecific rabbit anti-PA IgG capture antibody (produced by Battelle, Columbus, OH; purified from rPA-vaccinated rabbit serum and first passed over a protein A column and then over a PA column) was used to coat the wells of a 96-well plate at a concentration of 2.0 µg/ml. The plates were blocked with 5% skim milk and then incubated with rabbit serum samples containing native PA (lot number NR-164; BEI Resources), reference standard, or quality control samples consisting of rPA spiked differentially into naïve rabbit serum. The PA was detected by first incubating with diluted goat anti-PA serum, followed by incubation with a bovine anti-goat secondary antibody conjugated to horseradish perox-
idase (Santa Cruz Biologicals, Santa Cruz, CA) and developed with a 2,2'azinoisobenzene-3-ethylbenzothiazoline-6-sulfonic acid-diiammonium salt substrate for 30 min, and the reaction was terminated with the supplied stop solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The plates were read at 405 nm, and the data were analyzed using a four-parameter logistic log (4PL) model to fit the eight-point calibration curve.

The concentrations of PA in unknown samples were determined by computer interpolation from the plot of the reference standard curve data (Softmax Pro Molecular Devices). The limit of detection (LOD) for this assay was 2.0 ng/mL.

**Complete blood cell counts and C-reactive protein levels.** Complete blood cell counts (CBCs) were performed on blood samples collected into EDTA blood collection tubes by using the Advia 120 chemistry analyzer (Siemens). Plasma was then harvested from the whole blood by centrifugation, and C-reactive protein (CRP) levels were determined with the Advia 1200 chemistry analyzer (Siemens). The LOD for CRP was 0.5 mg/dl.

**Characterization of inhalational anthrax in New Zealand White rabbits.** Twelve NZW rabbits weighing between 2.5 and 3.5 kg (50% male and 50% female) were aerosol challenged with a mean dose of 225 ± 35 LD₅₀ of *B. anthracis* Ames spores; two rabbits (one male and one female) were utilized as unchallenged controls. The unchallenged control animals were handled in the same manner as the challenged group, with the exception of not being placed into the challenge chamber or subjected to plethysmography. Blood samples were taken every 6 h between 24 and 48 h postchallenge and at 60 and 72 h postchallenge. Blood samples were assayed for bacteremia, hematology parameters, CRP, and PA.

**Efficacy of passive immunization.** Nineteen vascular access port (VAP)-implanted NZW rabbits weighing between 2.5 and 3.5 kg (Covance) were challenged with a mean dose of 325 ± 61 LD₅₀ of *B. anthracis* Ames strain. Two rabbits (one male and one female) received 8 mg/kg (intravenously via the VAP) of a fully human monoclonal anti-PA antibody (23, 25, 30) on an individual basis immediately following exhibition of SIBT. Nine rabbits (6 male and 3 female) were included in an untreated control group.

**Efficacy of delayed passive immunization.** Twenty-four VAP-implanted NZW rabbits (50% male and 50% female) weighing approximately 2.5 to 3.5 kg were challenged with 325 ± 71 LD₅₀. The rabbits were treated on an individual basis with 10 mg/kg of fully human monoclonal antibody (six rabbits per group) immediately following an SIBT, 6 h following an SIBT, or 12 h following an SIBT. Six rabbits served as untreated controls.

**Statistical analysis.** The time to death was calculated from the end of the aerosol exposure to the time the animal was found dead or euthanized due to morbidity. One-sided Fisher’s exact tests were utilized to compare the survival rates between groups. Kaplan-Meier curves were plotted, and the log-rank test was computed to determine if differences were statistically significant. If the log-rank test was significant, pairwise log-rank tests were computed to determine group differences. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 level of significance.

For the purposes of determining the exact time from challenge to positive bacteremia, circulating PA, CRP, or an abnormal hematology result, the time from the end of aerosol exposure until the scheduled blood draw was used. A result from the PA ELISA or measured CRP was considered positive if the value was greater than the LOD for each assay. For each respective hematology parameter, the threshold for an abnormal parameter was defined as each individual animal’s baseline parameter value plus or minus two times the root mean squared error from an analysis of variance (ANOVA) model.

Significant differences in group geometric mean PA and CRP levels and cell counts were determined using ANOVA models with any group effect fitted separately for each time point. If there was a significant group effect in the data, Tukey’s multiple comparisons analysis was performed to determine which pairs of groups were significantly different. Data were analyzed using SAS software, version 8 (SAS Institute Inc., Cary, NC).

**RESULTS**

**Characterization of disease progression.** To characterize the disease progression in the NZW rabbit model of inhalational anthrax, 12 rabbits were challenged with *B. anthracis* Ames strain and monitored for bacteremia, circulating PA, changes in body temperature, hematology parameters, and CRP levels. All 12 challenged rabbits died, with a mean time to death of 73.37 ± 24.07 h (Fig. 1). A febrile response (defined by SIBT) was observed for challenged rabbits, while the body temperature for naïve rabbits remained relatively unchanged during the pre- and postchallenge phases (Fig. 2). The mean time from challenge to SIBT was 27.1 ± 7.2 h for challenged animals (Table 1), in which the average time to death from SIBT was 46.3 ± 26.2 h. There was no correlation between the onset of fever and the postchallenge survival time.

Bacteremia, circulating PA levels, hematology parameters, and CRP were examined in addition to monitoring body temperature. The times from challenge to bacteremia, detection of circulating PA, or changes in hematology parameters or CRP levels for each animal are listed in Table 1. The mean time to the first positive blood culture was 25.5 ± 3.6 h; all terminal blood samples from challenged rabbits were positive for *B. anthracis* (data not shown). In addition, PA detection in the serum corresponded with positive bacteremia, circulating PA, hematology parameters, and CRP levels. All 12 challenged rabbits died, with a mean time to death of 73.37 ± 24.07 h (Fig. 1). A febrile response (defined by SIBT) was observed for challenged rabbits, while the body temperature for naïve rabbits remained relatively unchanged during the pre- and postchallenge phases (Fig. 2). The mean time from challenge to SIBT was 27.1 ± 7.2 h for challenged animals (Table 1), in which the average time to death from SIBT was 46.3 ± 26.2 h. There was no correlation between the onset of fever and the postchallenge survival time.

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blood cultures for 68% of the rabbits. The mean time to the detection of circulating PA was 28.0 ± 4.3 h, with the mean levels peaking at 60 h postchallenge. While the times from challenge to detectable levels of PA were relatively consistent, a wide range (e.g., 15.6 to 4,365.5 ng/ml) in the levels of circulating PA was observed (Fig. 3).

White blood cells (WBCs), in particular lymphocytes, decreased in all challenged rabbits from 30 to 42 h postchallenge and increased toward baseline levels at subsequent collection time points (Fig. 4a and b). There was a significant decrease in lymphocyte counts in challenged animals at 36 h postchallenge versus controls (P < 0.0008). Heterophil levels increased 6 h after challenge and remained elevated until 30 h postchallenge (Fig. 4c). While empirical changes to total WBC and heterophil counts occurred after challenge, there was no statistical difference between challenged and control levels. Changes from baseline occurred at 31.5 ± 3.7, 28.51 ± 8.0, and 21.8 ± 20.1 h postchallenge for WBCs, lymphocytes, and heterophils, respectively (Table 1). The challenged rabbits exhibited an increase in CRP levels at a mean time of 23.0 ± 14.9 h postchallenge (Table 1). The CRP levels were significantly higher in the challenged animals than controls from 36 to 72 h postchallenge (P < 0.05) (Fig. 4d).

**Efficacy of passive immunization at SIBT.** Consistent with the
above findings, a febrile response was observed following challenge, with a mean time from challenge to SIBT of 26.11 ± 2.96 h in the treatment group (Fig. 5; Table 2). The threshold for an elevated body temperature was set as the average baseline temperature plus two times the standard deviation of that rabbit’s baseline temperature. Treatment was initiated after three consecutive readings above threshold; therefore, the rabbits exhibited the first febrile responses 3 h prior to passive immunization, approximately 16 h after treatment administration. All untreated, challenged controls died, with an average time to death of 77.46 ± 35.44 h following challenge (Fig. 6). A significant increase in survival was observed in the treated animals compared to the untreated controls (P < 0.001, Fisher’s exact test). The passive immunization dose was based on the previously published results using this antibody in B. anthracis-challenged Dutch belted rabbits (23). In that report, a dose of 10 mg/kg was used in the delayed treatment experiment. In order to conserve the antibody for additional studies, we decided to use a dose slightly lower (8 mg/kg) when treating at SIBT.

In the current study, the mean time from challenge to a positive blood culture was 26.15 ± 8.61 h in the treated animals, and 90% of the rabbits were bacteremic prior to treatment (Table 2). The one rabbit not bacteremic prior to treatment was culture positive using SIBT as a noninvasive surrogate for bacteremia enables the treatment of a majority of the animals in a therapeutic manner.

The mean time from challenge to PA detection was 26.41 ± 2.96 and 37.65 h in the treated and untreated rabbits, respectively. Most of the untreated animals became toxemic within the same time frame as the treated rabbits. However, PA was not detected in two untreated animals until the 60- and 72-h blood collection time points, resulting in a greater mean time to toxemia. Thirty percent of rabbits were positive for circulating PA prior to treatment (Table 2). In the untreated rabbits, the mean PA level was 592.5 ± 583.0 ng/ml at the last blood sample collected prior to death. The one treated animal that succumbed to disease had a PA level of 53.9 ng/ml prior to treatment at 24 h postchallenge. The PA levels reached 3,433.3 ng/ml at the last blood draw time point.

### TABLE 2 Time from challenge to event in animals treated at SIBT

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Group mean time (h) from challenge to event (± SD)</th>
<th>% with detectable PA prior to treatment</th>
<th>% bacteremic prior to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>SIBT</td>
<td>Treatment Bacteremia PA</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>30.46 ± 14.93</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Treated</td>
<td>26.11 ± 2.96</td>
<td>34.40 ± 19.35</td>
<td>37.65 ± 21.13</td>
</tr>
</tbody>
</table>

*NA, not applicable.*
prior to death. After treatment, the PA levels in all surviving animals began to decline and were below the limit of detection by 96 h postchallenge. Conversely, PA levels in untreated rabbits continued to increase until death (Fig. 7).

**Efficacy of delayed passive immunization following SIBT.** To determine the window of therapeutic opportunity, treatment with the monoclonal antibody (10 mg/kg) was administered individually to rabbits either immediately following SIBT or at 6 or 12 h after SIBT. The antibody dose was increased from 8 mg/kg to 10 mg/kg to maximize the effect in the delayed treatment groups. All groups exhibited a febrile response, with the mean time to SIBT of $28.20 \pm 6.22$ h across groups. Table 3 shows the mean time from challenge to SIBT and treatment for each group. Eighty-three percent (5/6) of the rabbits treated immediately following SIBT survived challenge, with the one death occurring at 2.6 days after challenge. All (6/6) of the rabbits treated 6 h after exhibition of SIBT survived, while only 67% (4/6) of those treated 12 h after exhibition of SIBT survived (Fig. 8). It is important to note that one of the animals in the 12-h group died before receiving treatment. All of the untreated control rabbits (6/6) succumbed to infection following challenge, with a mean time from challenge to death of $90.5 \pm 22.1$ h. Fisher’s exact test demonstrated that all treatment groups had a significantly greater survival rate than the untreated control group ($P < 0.05$).

**DISCUSSION**

Prior to 2001, the last case of inhalational anthrax in the United States was reported in 1978 (26). In the 2001 anthrax attack, the median incubation time from exposure to symptom onset was 4 days. Patients sought care a median of 3.5 days (range, 1 to 7 days) after the onset of symptoms. The most common symptoms were fever, fatigue, malaise, lethargy, cough, nausea or vomiting, and dyspnea. Upon examination by a health care professional, physical findings were fever, tachycardia, elevated WBCs, neutrophilia, and abnormal chest X-ray findings (10). Blood cultures from

### Table 3

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Group mean time (h) from challenge to event (± SD)</th>
<th>% with detectable PA prior to treatment</th>
<th>% bacteremic prior to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$26.16 \pm 5.91$</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>SIBT</td>
<td>$25.05 \pm 4.14$</td>
<td>$25.27 \pm 5.92$</td>
<td>$23.55 \pm 5.61$</td>
</tr>
<tr>
<td>SIBT + 6 h(^a)</td>
<td>$31.98 \pm 7.05$</td>
<td>$38.11 \pm 6.65$</td>
<td>$27.27 \pm 12.85$</td>
</tr>
<tr>
<td>SIBT + 12 h(^b)</td>
<td>$29.59 \pm 6.59$</td>
<td>$39.97 \pm 4.97$</td>
<td>$24.14 \pm 8.08$</td>
</tr>
</tbody>
</table>

\(^a\) NA, not applicable.

\(^b\) Time between SIBT and treatment.
patients prior to treatment showed all were positive for *B. anthracis*. With multiple antibiotic treatment regimens and supportive care, the mortality rate was 45% (9, 10). This intentional release of *B. anthracis* spores in 2001 highlights the need for animal models for testing the safety and efficacy of medical countermeasures against intentional release of biological agents.

Rabbits are considered an acceptable animal model to evaluate potential anthrax vaccine candidates, because the pathophysiological response to disease closely resembles human inhalational anthrax (8, 32). The work presented here suggests that the NZW rabbit follows a similar clinical course to humans, although disease progression is more rapid.

These results confirmed the findings of Yee et al., who demonstrated that bacteremia was closely followed by detection of PA in the circulation by electrochemiluminescence (ECL), with fever occurring shortly following the detection of the toxin component (31). The correlation between detection of PA and bacteremia was also demonstrated by Koliber et al. by using the ECL assay (12). In the current study, PA levels were variable over time and this may have been due to differences in disease progression for each individual animal. A similar variation was observed in guinea pigs after intranasal inoculation of 20 to 50 LD$_{50}$ of Vollum strain spores (12). The confounding factors of asynchronous germination of the *B. anthracis* spores and animal-to-animal variability make it difficult to compare PA levels at set time points postchallenge. More work is needed to correlate PA levels at the time of onset of bacteremia and just prior to death as a reliable maker or as a surrogate at these stages of disease.

The febrile response after being challenged with *B. anthracis* spores is not limited to the NZW rabbit model. Through constant telemetric monitoring, Lawrence et al. showed that Dutch belted dwarf rabbits exhibit increases in body temperature just prior to death (13). While the results of the series of studies presented here show that NZW rabbits consistently produce fevers following challenge, results from primate studies have been more variable. Studies with African green monkeys determined that a febrile response was inconsistent after challenge with *B. anthracis* spores and did not always correlate with bacteria and toxemia (24). This finding suggests that the use of body temperature as a trigger for treatment may be limited to rabbit models of inhalation anthrax.

To determine the efficacy of a compound in a truly therapeutic manner, the test subject must be treated after clinical symptoms. In animal models, a positive bacteremic culture, prior to treatment, has been considered a requirement for signaling administration of therapeutics (8). In this study, the correlation of SIBT with bacteremia and PA levels in blood following inhalational exposure to *B. anthracis* Ames spores was used as a trigger for treatment with an anti-PA monoclonal antibody. Ninety percent of these monoclonal antibody–treated animals survived challenge, which was similar to rabbits treated with raxibacumab (a human
antibody to PA) after SIBT or PA detection, where a 40-μg/kg dose protected 44% of the rabbits from a lethal inhalational anthrax exposure (11).

In addition to treatment efficacy studies, there are published studies with human monoclonal antibodies to PA that were designed to determine a window of opportunity for antibody treatment at a defined time postchallenge. The antibody ETI-204 (20 mg/rabbit) administered at 24 or 36 h after aerosol challenge with \textit{B. anthracis} Ames spores showed significant protection; however, no difference in survival was observed when the antibody was administered 48 h postchallenge (19). Similarly, the human monoclonal antibody AVP-21D9 demonstrated significant protection when given 6, 12, or 24 h post-nasal instillation challenge with \textit{B. anthracis} spores in the Dutch Belted rabbit model (23).

While not statistically significant, 60% of rabbits were protected when treatment was delayed until 48 h postchallenge. Using SIBT as the primary indicator for delayed treatment, the present study showed significant protection above controls when treatment was delayed up to 12 h following SIBT. This protection afforded by the monoclonal was consistent with observations in previous studies (14, 18).

While passive immunization against anthrax is promising as a prophylactic/therapeutic treatment, it is likely that future recommendations for individuals at risk of exposure will include active immunization and antibiotic treatment, as well as neutralizing monoclonal anti-PA antibodies. In 2006, a 44-year-old Pennsylvania man was diagnosed with naturally acquired inhalational anthrax and was treated with antibiotics, supportive care, and human anthrax immunoglobulin; the patient survived the infection (23). However, the definitive effect of passive immunization is difficult to determine from this single instance, and more studies on the passive immune therapy during anthrax infections are needed.

The work presented here indicates that use of SIBT as a trigger for treatment may be a valuable tool in evaluating therapeutics in the rabbit model of inhalational anthrax. There have been recent advances in sensitive methods to detect circulating anthrax toxins and capsular material (2, 4, 7), which may also be a suitable trigger for initiation of treatment in a therapeutic model. However, laboratories that do not have access to these assays may find the febrile response in the rabbit model as a reliable, cost-effective trigger for treatment when testing therapeutics against anthrax. The model may also be acceptable for determining if passive immunization adds benefit over antibiotic treatment alone. An aggressive antibiotic regimen along with supportive care is the current standard of care for anthrax patients. Any adjunct therapy will most likely have to show an added benefit above the current standard. The logical next phase in the characterization of the NZW rabbit as a therapeutic model will be to determine if it can be used in these added benefit studies.

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