SHORT COMMUNICATION

Protein- and DNA-based anthrax toxin vaccines confer protection in guinea pigs against inhalational challenge with Bacillus cereus G9241

John Palmer¹, Matt Bell¹, Christian Darko¹, Roy Barnewall² & Andrea Keane-Myers¹

1 Vaccines and Medical Countermeasures, Biological Defense Research Directorate, Navy Medical Research Center, NMRC-Frederick, Fort Detrick, MD, USA
2 Battelle, Columbus, OH, USA

This is a straight-forward approach to examine the ability to protect guinea pigs from a spore challenge with the ‘anthrax like’ Bacillus cereus strain G9241 with DNA and protein-based anthrax toxin vaccines.

Keywords
inhalation anthrax; DNA vaccine; recombinant protein vaccine; immunization.

Abstract

In the past decade, several Bacillus cereus strains have been isolated from otherwise healthy individuals who succumbed to bacterial pneumonia presenting symptoms resembling inhalational anthrax. One strain was indistinguishable from B. cereus G9241, previously cultured from an individual who survived a similar pneumonia-like illness and which was shown to possess a complete set of plasmid-borne anthrax toxin-encoding homologs. The finding that B. cereus G9241 pathogenesis in mice is dependent on pagA1-derived protective antigen (PA) synthesis suggests that an anthrax toxin-based vaccine may be effective against this toxin-encoding B. cereus strain. Dunkin Hartley guinea pigs were immunized with protein- and DNA-based anthrax toxin-based vaccines, immune responses were evaluated and survival rates were calculated after lethal aerosol exposure with B. cereus G9241 spores. Each vaccine induced seroconversion with the protein immunization regimen eliciting significantly higher serum levels of antigen-specific antibodies at the prechallenge time-point compared with the DNA–protein prime-boost immunization schedule. Complete protection against lethal challenge was observed in all groups with a detectable prechallenge serum titer of toxin neutralizing antibodies. For the first time, we demonstrated that the efficacy of fully defined anthrax toxin-based vaccines was protective against lethal B. cereus G9241 aerosol challenge in the guinea pig animal model.
capsule (PDGA) expression (Hammerstrom et al., 2011) and possesses, unlike B. anthracis pXO1, a functional hasACB operon that directs synthesis of a hyaluronic acid (HA) capsule (Oh et al., 2011). pBC218 lacks synergy to any known B. cereus plasmid, and although harboring genes for additional copies of PA as well as a truncated LF and AbxA, the pBC218-encoding proteins exhibit only 60%, 36%, and 78% amino acid identities, respectively, compared with the B. anthracis homologs (Hoffmaster et al., 2004). pBC218 also possesses the bpsX-H operon that directs synthesis of BPS, a second polysaccharide-based capsule (Oh et al., 2011).

To date, the relative virulence of B. cereus G9241 spores compared to B. anthracis strains has been evaluated in rabbit and mouse models. In New Zealand rabbits, B. cereus G9241 is essentially avirulent for exposure via the subcutaneous route or attenuated 100-fold for inhalation-mediated infection compared with the corresponding routes and doses of fully virulent B. anthracis Ames (Wilson et al., 2011). In the mouse model, B. cereus G9241 infection in both immune-competent C57BL/6 mice and complement-deficient A/J mice is lethal and elicits an anthrax-like disease progression. Challenge studies in both mouse strains indicate B. cereus G9241 is less virulent than B. anthracis Ames (Wilson et al., 2011). In contrast, compared to B. anthracis Sterne, an attenuated strain that lacks the PDGA capsule-encoding virulence plasmid pXO2, B. cereus G9241 LD50 values were either similar for subcutaneous challenge or c. fivefold greater for intranasal and subcutaneous delivery, respectively (Wilson et al., 2011). Mortality rates in mice infected with G9241 strains that were cured for one or both of the virulence plasmids, deleted for the pBCXO1-borne PA encoding gene (ΔpagA1) or HA-encoding operon (ΔhasACB) or the pBC218-borne BPS encoding operon (ΔbpsX-H), indicate expression of toxin and both capsules is necessary for full virulence (Oh et al., 2011; Wilson et al., 2011). In subcutaneous challenge studies with plasmid-cured strains using A/J mice, only a B. cereus G9241 pBCXO1ΔpagA1ΔbpsX-H derivative caused mortality at a dose >3 logs higher than the wild-type LD50 value (Wilson et al., 2011). C57BL/6 mice challenged intraperitoneally with 30× LD50 wild-type equivalents of B. cereus G9241 variants either deleted for the toxin subunit PA or one or both of the HA and BPS capsules exhibited attenuated phenotypes with mortality rates of 50% (ΔbpsX-H) or 60% (ΔpagA1 or ΔhasACB) compared with the wild-type parent strain (Oh et al., 2011). Deletion of both capsules (ΔhasACB and ΔbpsX-H) rendered B. cereus G9241 spores avirulent (Oh et al., 2011).

Given the recent fatalities and wide geographical distribution of B. cereus G9241-mediated anthrax-like illness, the identification of effective medical countermeasures against future similar cases is urgent. The finding that B. cereus G9241 pathogenesis in mice is dependent on pagA1-derived PA synthesis suggests an anthrax vaccine formulated using the PA subunit may be efficacious against exposure to a toxin-encoding B. cereus strain (Oh et al., 2011). Currently, most anthrax vaccines are toxin-based. The FDA-licensed anthrax vaccine BioThrax®, also known as AVA (Anthrax Vaccine Adsorbed, Emergent Biosolutions, Rockville, MD), contains a PA-aluminum hydroxide-adsorbed precipitate derived from a B. anthracis pXO1 pXO2 culture supernatant, and rPA-based next-generation anthrax vaccines are currently undergoing clinical trials (Brown et al., 2010; Bellanti et al., 2012). In addition, vaccine-engineered plasmids expressing PA and LF can protect A/J mice against lethal challenge with aerosolized B. anthracis spores (Albrecht et al., 2012a, b). Recent work has also demonstrated that immunization with formalin-inactivated spores derived from nontoxicigenic, nonencapsulated B. cereus G9241 strains (pBCXO1Δ-, pBC210Δ) containing the immunodominant spore protein BclA (dcG9241) or a deletion mutant (dcG9241ΔbclA) conferred significant protection in mice against spore challenge with B. anthracis Ames or Ames ΔbclA (Vergis et al., 2013).

The viability of using an anthrax PA-based vaccine as a prophylactic against B. cereus G9241 infection was recently demonstrated (Oh et al., 2013). AVA-immunized C57BL/6 mice exhibited robust protection against exposure to B. cereus G9241 spores via both the intraperitoneal (100% survival at 37×LD50) and aerosol (90% survival at 30×LD50) routes of infection. Consequently, we decided to extend the above observations by evaluating the protective efficacy of PA and LF recombinant protein- and DNA-based anthrax toxin subunit vaccines in a second aerosol-based larger animal model. In this study, we utilized Dunkin Hartley guinea pigs as our animal model against challenge with B. cereus G9241 spores.

DNA and recombinant protein PA and LFn (first 254 amino acids of LF) subunit vaccines were prepared as described elsewhere (Albrecht et al., 2012a) with the following modifications. DNA sequences encoding PA and LFn were codon-optimized for human expression using NMRC-patented methodology (Goldman & Albrecht, 2011). The optimized genes were then synthesized by Gene Oracle Inc. (Mountain View, CA) and transferred to the DNA vaccine plasmid pDNAVACCultra2 (Nature Technology Corporation, Lincoln, NE) to produce pPAho and pLFnho. The DNA vaccines were administered intramuscularly at 100 μg plasmid per vaccination event (100 μL of vaccine plus 100 μL of adjuvant were combined, divided in half, then injected into each quadriceps). The lipid-based adjuvant was formulated by combining PE (1,2-Diphytanoyl-sn-Glycero-3-Phosphoethanolamine) and DDAB (Dimethyldioctadecylammonium bromide) at a PE-to-DDAB ratio of 4:1 (Avanti J, Alabaster, AL). rPA and rLFn were administered subcutaneously at 50 μg per vaccination event with Imject Alum adjuvant (200 μL protein plus 200 μL adjuvant). Groups of guinea pigs (n = 11 for groups treated with rPA, rLFn or rPAho + rPA; n = 10 for group treated with rLFnho + rLFn) were vaccinated on days 0, 14, and 28; for each DNA vaccination regimen, a protein boost of 50 μg of the corresponding recombinant protein was administered subcutaneously on Day 14 in addition to the DNA vaccine. Control groups (n = 6) were given either empty pDNAVaccultra2 vector or saline. On Day 42, a six-jet Collison nebulizer (BGI, Waltham, MA) in conjunction with a CH Technologies
nose-only inhalation exposure system (Westwood, NJ) was used to aerosol challenge each group of guinea pigs with a target dose of \(2.5 \times 10^7\) \(B.\) cereus G9241 spores [500 \(\times\) LD\(_{50}\) \(B.\) anthrax Ames equivalents (Savransky et al., 2013)]. Each total-inhaled dose was determined from impinger sample concentration, sampling parameters, and exposure duration. Mean-calculated inhaled doses as colony-forming units (CFU) for each group were as follows: rPA, 2.35 \(\times\) \(10^7\) CFU; rLFn, 2.54 \(\times\) \(10^7\) CFU; pPAho-rPA, 2.55 \(\times\) \(10^7\) CFU; pLFnho-rLFn, 2.52 \(\times\) \(10^7\) CFU; saline, 2.49 \(\times\) \(10^7\) CFU and empty vector, 2.59 \(\times\) \(10^7\) CFU.

Serum from blood samples collected via the cranial vena cava was used in ELISAs and toxin neutralization assays to determine antigen-specific IgG titers (for sera obtained on days 0, 41, 44, 49, and 56) and the ability to neutralize anthrax toxin (for sera obtained on Days 41 and 56), respectively, as previously described (Albrecht et al., 2007).

Consistent with previous work in guinea pigs and other animal models (Price et al., 2001; Reuveny et al., 2001; Galloway et al., 2004; DuBois et al., 2007; Albrecht et al., 2012b; Cote et al., 2012), immunization with each of the PA

<table>
<thead>
<tr>
<th>Vaccine treatment</th>
<th>TNA as percentage of total antigen-specific antibody [Ratio of TNA to antigen-specific antibody ((\mu\text{g mL}^{-1})]]</th>
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<tbody>
<tr>
<td></td>
<td>Day 41</td>
</tr>
<tr>
<td>rPA</td>
<td>51 (684 : 1329)</td>
</tr>
<tr>
<td>rLFn</td>
<td>9 (79 : 874)</td>
</tr>
<tr>
<td>pPAho-rPA</td>
<td>6 (4 : 72)</td>
</tr>
<tr>
<td>pLFnho-rLFn</td>
<td>ND</td>
</tr>
</tbody>
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ND. Not detected.
and LF vaccines induced seroconversion (Fig. 1a and b). However, the antigen-specific IgG synthesis profiles for the protein and DNA–protein prime-boost vaccination regimens were different. On Day 41, the prechallenge time-point, anti-PA IgG, and anti-LF IgG levels were 18- and 43-fold greater for the recombinant protein vaccines, respectively, compared with each corresponding DNA vaccine. For the postchallenge protein vaccination time-points, with the exception of a modest rise in the anti-PA IgG level observed at the study’s conclusion on Day 56, anti-PA and anti-LF IgG levels did not significantly change ($P = 0.05$). In contrast, while antigen-specific IgG responses elicited from the DNA–protein prime-boost vaccinations at Day 44 remained low, by Day 49 and Day 56, anti-PA and anti-LF IgG serum titers had increased significantly to levels similar or exceeding those found for the corresponding protein vaccines, suggesting that the DNA–protein prime-boost vaccination schedule had induced a memory response in those guinea pigs that survived.

Previous findings in rabbits, guinea pigs, and mice have demonstrated that the toxin neutralizing antibody (TNA) fractions of anti-PA and anti-LF serum titers constitute a major component of protective immunity against anthrax and can function as a surrogate marker for protection (Pitt et al., 1999, 2001; Reuveny et al., 2001; Staats et al., 2007; Chun et al., 2012). In parallel with the results observed for total antigen-specific IgG levels, the TNA serum fraction elicited by each vaccine was also significantly different and ranged immediately prior to challenge at Day 41 from 51% and 9% for rPA and rLF, respectively, to 6% for the pPAho-rPA prime-boost vaccination schedule (Fig. 1c and Table 1). At the prechallenge time-point, there was no detectable TNA response from the pLFho-rLF prime-boost series of vaccinations. Guinea pig survival rate subsequent to aerosol challenge on Day 42 with $B. $ cereus G9241 spores was associated with the presence of detectable prechallenge levels of serum TNA titers (Fig. 1c and d). After pathogen challenge, all guinea pigs in the control groups that received either saline or empty DNA vector succumbed within 3–4 days, a time to death interval of 1 day more than that determined for guinea pigs aerosol challenged with an equivalent dose of $B. anthracis$ Ames spores (Savransky et al., 2013). Protein and DNA–protein vaccinated animals conversely showed either partial (i.e. 30% for the animals administered pLFho-rLF, $P < 0.05$) or complete protection (in the groups administered rPA, rLF, or pPAho-rPA, $P < 0.0001$).

End of study TNA serum titers were also elevated, compared with prechallenge levels, with a detectable TNA response now also present in the group vaccinated with pLFho-rLF. Final percentage of TNA serum titers, compared with total antigen-specific IgG levels, were 81% and 20% for rPA and rLF, respectively, and 42% and 3% for pPAho-rPA and pLFho-rLF, respectively (Fig. 1c and Table 1). The data from this study suggest that even though the TNA serum titers generated by the rLFn and pPAho-rPA vaccination regimens were ninefold (79 µg mL$^{-1}$) and 171-fold (4 µg mL$^{-1}$) lower, respectively, than that for the rPA (684 µg mL$^{-1}$) vaccination schedule, they were still sufficient to confer complete protection from challenge and suggest a threshold serum TNA level to achieve robust protection against $B. cereus$ G9241 spore challenge for this study’s experimental conditions. Recently, it has been demonstrated that mice immunized with the FDA-approved AVA vaccine are protected against challenge with $B. cereus$ G9241 spores (Oh et al., 2013). Here, we demonstrate for the first time that fully defined DNA- and protein-based anthrax toxin vaccines can also confer protection against lethal aerosol challenge with $B. cereus$ G9241 spores in Dunkin Hartley guinea pigs.

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**References**


