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Correlation between anthrax lethal toxin neutralizing antibody levels and survival in guinea pigs and nonhuman primates vaccinated with the AV7909 anthrax vaccine candidate

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ABSTRACT

The anthrax vaccine candidate AV7909 is being developed as a next generation vaccine for a post-exposure prophylaxis (PEP) indication against anthrax. AV7909 consists of the Anthrax Vaccine Adsorbed (AVA, BioThrax®) bulk drug substance adjuvanted with the immunostimulatory oligodeoxynucleotide (ODN) compound, CPG 7909. The addition of CPG 7909 to AVA enhances both the magnitude and the kinetics of antibody responses in animals and human subjects, making AV7909 a suitable next-generation vaccine for use in a PEP setting. The studies described here provide initial information on AV7909-induced toxin-neutralizing antibody (TNA) levels associated with the protection of animals from lethal *Bacillus anthracis* challenge. Guinea pigs or nonhuman primates (NHPs) were immunized on Days 0 and 28 with various dilutions of AV7909, AVA or a saline or Alhydrogel + CPG 7909 control. Animals were challenged via the inhalational route with a lethal dose of aerosolized *B. anthracis* (Ames strain) spores and observed for clinical signs of disease and mortality. The relationship between pre-challenge serum TNA levels and survival following challenge was determined in order to calculate a threshold TNA level associated with protection. Immunisation with AV7909 induced a rapid, highly protective TNA response in guinea pigs and NHPs. Surprisingly, the TNA threshold associated with a 70% probability of survival for AV7909 immunized animals was substantially lower than the threshold which has been established for the licensed AVA vaccine. The results of this study suggest that the TNA threshold of protection against anthrax could be modified by the addition of an immune stimulant such as CPG 7909 and that the TNA levels associated with protection may be vaccine-specific.

1. Introduction

Anthrax is considered a serious biological threat due to the highly lethal effects of exposure via the inhalational route and the relative ease of weaponizing *Bacillus anthracis* spores. While antimicrobials administered post-exposure can reduce the incidence or progression of anthrax disease, they do not protect against subsequent disease resulting from germination of residual spores that may remain in the body after the cessation of the recommended 60-day antibiotic regimen [1,2]. Such additional protection may be achieved by post-exposure vaccination. AVA, the only FDA-approved anthrax vaccine licensed for pre-exposure prophylaxis, was recently approved under the FDA Animal Rule for post-exposure prophylaxis (PEP) of disease following suspected or confirmed *B. anthracis* exposure [3,4]. The anthrax vaccine candidate AV7909, is composed of the AVA drug substance and the adjuvant CPG 7909, an immunostimulatory Toll-like receptor 9 (TLR9) agonist, and is being developed as a next generation anthrax vaccine candidate for PEP that is expected to confer protection earlier and to require fewer immunisations. CPG 7909 is an immunostimulatory oligonucleotide (short deoxyribonucleic acid [DNA] sequence) shown to be a potent vaccine adjuvant [5–11]. CPG 7909 has been shown to induce both an enhanced antigen-specific antibody response and a natural killer T-cell response when used in combination with prophylactic or therapeutic vaccines [12–14].

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The current pre-exposure regimen for AVA is a series of 3 intramuscular (IM) doses at 0, 1, and 6 months with subsequent booster at 12 and 18 months and annual boosters thereafter. The AVA PEP regimen of three subcutaneous doses (SC) at 0, 2, and 4 weeks, combined with the recommended 60-day course of antibiotics is considered one of the most effective medical countermeasures to prevent inhalational anthrax following widespread exposure in a biological attack [15]. The AV7909 vaccine candidate administered as two IM immunisations two weeks apart may offer an improvement over the current licensed PEP vaccination regimen [7]. To provide the highest level of additional protection following inhalation of anthrax spores, immunity to anthrax should be gained as rapidly as possible. To decrease logistical complexity in a mass immunisation scenario, a post-exposure vaccine regimen should include as few injections as possible.

Because it is not ethical to evaluate efficacy of anthrax vaccines in humans, and field trials are no longer feasible due to the rarity of naturally occurring anthrax in humans in the United States, the licensure of an anthrax vaccine for PEP must be achieved using the U.S. Food and Drug Administration (FDA) "Animal Rule". Using this FDA guidance a vaccine may be licensed based on adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans [16,17]. Rabbits and NHPs are preferred animal models for inhalational anthrax and have been widely used to study disease pathogenesis, examine bacterial characteristics such as virulence, and assess efficacy of vaccines and therapeutics [18,19]. A rabbit model of inhalational anthrax was used to set a TNA threshold for the AVA PEP indication. However, the rabbit model was found to be inadequate for AV7909 development because rabbits do not respond strongly to CPG adjuvants such as CPG 7909 that act via the TLR9 receptor [20]. Therefore, a guinea pig model was developed as the small animal model for evaluation of AV7909 vaccine immunogenicity and efficacy. The guinea pig and cynomolgus macaque models of inhalational anthrax have been characterized extensively to support their use for licensure of AV7909, and the clinical signs of disease observed in these models are similar to those of inhalational anthrax in humans [21–23]. Furthermore, studies in these animal models have demonstrated that serum TNA and anti-PA IgG titres are reliable predictors of survival following lethal *B. anthracis* challenge [24–27]. The TNA assay can selectively quantitate functional antibodies by measuring the ability of serum from immunized animals or human subjects to neutralize lethal toxin (LT). For protective antigen (PA)-based anthrax vaccines, pre-challenge TNA titres correlate with animal survival post-challenge and provide the means for deriving an antibody titre associated with a specific probability of survival in animals [3,28]. Since the TNA assay is species-independent [29], it can be used to directly compare functional immune responses across species, thereby providing a mechanism for bridging animal and human immunogenicity data to support licensure of the vaccine under the Animal Rule.

A series of studies was performed to evaluate the immunogenicity and protective efficacy of AV7909 in guinea pig and NHP models of inhalational anthrax. The primary goal of these studies was to establish the relationship of TNA antibodies circulating in serum at the time of challenge with survival following lethal inhalation exposure to anthrax spores.

2. Material and methods

2.1. Experimental animals

Animal studies were performed at Battelle Biomedical Research Center (West Jefferson, OH), and all animal procedures were approved by Battelle's Institutional Animal Care and Use Committee (IACUC). The studies were conducted in compliance with the Animal Welfare Act and followed the principles of the Guide for the Care and Use of Laboratory Animals from the National Research Council. Animal room temperatures (64–84 °F) and relative humidity (30–70%) were maintained and recorded a minimum of twice daily. The light/dark cycle was approximately 12 h each per day using fluorescent lighting.

Male and female Hartley guinea pigs (*Cavia porcellus*) weighing approximately 350–400 g, were purchased from Charles River Laboratories (Saint Constant, Quebec Canada). Guinea pigs were single-housed in polycarbonate cages on stainless steel racks equipped with a watering system. Male and female Asian-origin cynomolgus macaques (*Macaca fascicularis*) weighing 2.29–4.11 kg (2.9–11.1 years of age) were procured from Covance Research Products (Alice, TX). All NHPs were tested and verified negative for tuberculosis, Simian Immunodeficiency Virus, Simian T-Lymphotrophic Virus-1, Macacine herpesvirus 1 (Herpes B virus), Simian Retroviruses 1 and 2 and *Trypanosoma cruzi*. NHPs were pair housed during quarantine and the pre-challenge period in stainless steel cages on racks equipped with automatic watering systems. Animals were individually housed when moved into the biosafety level 3 (BSL-3) laboratory seven days prior to challenge and while housed in the BSL-3 after challenge.

2.2. Test and control material

AVA (Emergent BioSolutions, Lansing, MI)] is prepared from cell-free culture filtrates of an avirulent, nonencapsulated strain of *B. anthracis* adjuvanted with Alhydrogel®. AV7909 final drug product was made by combining AVA bulk drug substance with CPG 7909 to achieve a concentration of 0.5 mg CPG 7909 per mL. AVA and AV7909 were serially-diluted from the human dose (0.5 mL) in sterile saline for use in animal studies. Adjuvant control groups were injected with 0.5 mL of a sterile saline containing 0.650–0.730 mg of Alhydrogel and 0.25 mg of CPG 7909. Saline control groups were injected with 0.5 mL of a sterile saline.

2.3. Study design

Four independent studies were performed: two in guinea pigs and two in NHPs. In each study, groups of animals were immunized by intramuscular (IM) injection on Day 0 and 28 with 0.5 mL of various dilutions of AV7909 or AVA. The vaccine dilutions used and the number of animals used in each study are shown in Table 1. Guinea Pig Study 1 and NHP Study 1 controls received 0.5 mL of adjuvant control. Guinea Pig Study 2 and NHP Study 2 controls received 0.5 mL of saline. Equal numbers of male and female animals were used in all study groups. Animals were challenged via the inhalational route with aerosolized *B. anthracis* (Ames strain) spores on Day 70, and observed for mortality and clinical signs of disease for up to 21 (guinea pigs) or 28 (NHPs) days post-challenge.

2.4. Aerosol challenge

B. anthracis Ames strain spores were prepared and characterized as described previously [2]. Aqueous suspensions of *B. anthracis* spores were aerosolized by a 3-jet Collison nebulizer and delivered to the animals via a nose-only (guinea pigs) or head-only (NHPs) inhalational exposure system. The anthrax target challenge dose was 200 LD_{50} of *B. anthracis* Ames spores, using the LD_{50} values of 5.01×10^4 spores/animal for guinea pigs [21] and 6.18×10^4 spores/animal for cynomolgus macaques [23]. Actual challenge doses achieved in the studies are shown in Table 1. The atmospheric concentration of spores in the exposure system was con-

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