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Review

Advances in the development of next-generation anthrax vaccines

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ABSTRACT

Anthrax, a disease of herbivores, only rarely infects humans. However, the threat of using *Bacillus anthracis*, the causative agent, to intentionally produce disease has been the impetus for development of next-generation vaccines. Two licensed vaccines have been available for human use for several decades. These are composed of acellular culture supernatants containing the protective antigen (PA) component of the anthrax toxins. In this review we summarize the various approaches used to develop improved vaccines. These efforts have included the use of PA with newer adjuvants and delivery systems, including bacterial and viral vectors and DNA vaccines. Attempts to broaden the protection afforded by PA-based vaccines have focused on adding other *B. anthracis* components, including spore and capsule antigens.

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Contents

1. Introduction

Anthrax, a disease of herbivores caused by *Bacillus anthracis*, only occasionally infects humans and usually in an agricultural setting in less-developed countries. The major virulence factors of *B. anthracis* are two exotoxins, lethal toxin and edema toxin, and a poly-γ-D-glutamic acid (γDPGA) capsule. The two exotoxins share a common cell-receptor-binding protein, protective antigen (PA), originally identified as a protein providing protection against infection in experimental animals [1]. PA is proteolytically activated either after binding to the cell surface receptor(s) by furin or a furin-like protease or by a protease present in plasma. The resulting 63 kDa fragment (PA63), which remains bound to the receptor, forms oligomers that competitively bind a zinc-dependent metalloprotease (lethal factor; LF) that cleaves MAPKK or a calmodulin-dependent adenylate cyclase (edema factor; EF) generating lethal toxin or edema toxin, respectively. The toxin complexes are endocytosed and within acidic endosomes, the PA oligomer forms a pore, facilitating transport of LF and EF into the cytosol [2]. The toxins contribute to virulence by suppressing major signaling pathways of the innate and adaptive immune systems [3] while the γDPGA capsule is responsible for resistance of the bacillus to phagocytosis.

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The current human anthrax vaccines, anthrax vaccine adsorbed (AVA; BioThrax) and anthrax vaccine precipitated (AVP) licensed in the US and UK, respectively, contain PA as the major protective component. Vaccination, originally intended for individuals in high-risk occupations such as veterinarians, farmers, and laboratory personnel working with *B. anthracis*, was expanded for military use in the Persian Gulf War (1990). Although AVA and AVP are effective, their undefined composition, lot-to-lot variation, and extensive dosing regimen were the impetus for the development of a second-generation vaccine containing recombinant PA (rPA). Development of a third-generation vaccine to broaden the efficacy of the rPA vaccine has been propelled by the possible intentional use of *B. anthracis* to cause disease, as unfortunately occurred in 2001 when spore containing letters were sent through the US postal system, and the creation of genetically engineered strains [4] that may resist current vaccines.

The rarity of anthrax and inability to perform human volunteer trials requires using surrogate animal models to develop correlates between survival and an immunological response(s). Mice, in contrast to other animals, are uniquely susceptible to non-toxinogenic encapsulated strains and very difficult to protect with PA vaccines although one study reported modest protection against intranasal challenge with a fully virulent strain and a correlation between toxin neutralizing antibody titers and survival [5]. Although a correlation between survival and neutralizing antibody titer was reported for PA vaccinated guinea pigs [6], AVA vaccinated guinea pigs in contrast to rabbits show variable protection against challenge with different strains [7] and are poorly protected against aerosol challenge [8]. While all animal models represent less than optimal substitutes for human efficacy trials, the rabbit and nonhuman primate have been accepted as the best alternatives even though inhalational anthrax in both models shows significant differences from the disease in humans. Anti-rPA IgG ELISA titer and toxin neutralizing antibody titer were identified as serological correlates of immunity in a rabbit aerosol challenge model [9,10] and neutralizing antibody was a better correlate in an intranasal (IN) challenge model [11]. Initial studies in small numbers of rhesus monkeys, considered the best human surrogate model, suggest that anti-PA IgG and toxin neutralizing antibody titers might correlate with immunity and a study using AVA in larger numbers of animals demonstrates a correlation between the magnitude of the antibody responses to a 3 dose intramuscular (IM) schedule and survival (C.P. Quinn, personal communication).

This review summarizes the scientific literature describing the different approaches taken to make a better anthrax vaccine, focusing on those evaluating efficacy against infection.

2. Licensed acellular culture supernatant vaccines

AVA [12] is prepared by adsorbing filtered microaerophilic culture supernatant fluids from the *B. anthracis* V770-NP1-R strain onto aluminum hydroxide gel and AVP [13] by precipitating aerobic culture filtrates of *B. anthracis* Sterne with alum. Benzethonium chloride and formaldehyde are added to AVA and thiomersal to AVP. AVA, licensed in 1970 to be administered subcutaneously (SC) (0.5 ml) as three biweekly injections followed by injections at 6, 12, and 18 months and yearly boosters thereafter was recently relicensed to be given IM at 0, 1, 6, 12 and 18 months followed by yearly boosters [14]. While this caused fewer local reactions it resulted in an inferior anti-PA antibody response from week 8 to 6 months after vaccination [15] during which protection may be reduced. AVP (0.5 ml) is administered IM at 0, 3, 6, and 32 weeks with yearly boosters thereafter. AVA and AVP contain LF and EF and other proteins whose contribution toward protective immunity, while unlikely, is unknown [16–19]. An earlier less potent cell-free vaccine is the only one to be evaluated in a human field trial in mill workers in which it showed efficacy, although most of the cases were cutaneous [20]. An extensive review by the National Academy of Sciences found AVA to be safe and effective for protection against anthrax, including inhalational anthrax [12].

Various compounds have been added to AVA and AVP to improve the immune response in experimental animals [16,21], and whole cell pertussis vaccine was used clinically in association with AVP [22]. CpG oligodeoxynucleotides (ODN) possessing adjuvant properties, added to AVA increased antibody titers to PA in mice, guinea pigs and nonhuman primates and protection against infection in mice and guinea pigs compared to AVA alone [23–25]. In a Phase I study in humans, CpG similarly enhanced the immune response to AVA but was associated with increased adverse effects [26].

3. PA-based vaccines

PA given with aluminum adjuvants confers a high degree of protection against aerosol challenge in rabbits [10] and nonhuman primates [27]. These results have been the basis for the advanced development of rPA vaccines for human use. Several Phase I trials of PA vaccines produced in either *B. anthracis* [28,29] or *E. coli* [30] and formulated with aluminum adjuvants have been reported. The vaccines appear to be safe and immunogenic but the optimal formulation, dose schedule and demonstration of non-inferiority with AVA remain to be established. Ongoing efforts to improve the immunogenicity and efficacy of PA vaccines involve the use of other adjuvants, *B. anthracis* antigens and alternative delivery systems. Further efforts are examining domains of the PA molecule possessing protective epitopes [31,32] and have shown that PA mutants impaired in cell binding, protease cleavage, oligomerization, or pore formation retain immunogenicity and may be less reactogenic, although native PA has not proved to be very reactogenic (see below).

3.1. PA combined with adjuvants

In addition to aluminum-based adjuvants licensed for human use, several newer adjuvants are reported to increase the immune response to PA vaccines as well as to AVA (see above) and protect against experimental infection. PA combined with CpG ODN, CpG ODN with Pluronic F127, a non-ionic block copolymer [33], bacterial DNA fragments and modified *Escherichia coli* lipopolysaccharide [34], or the mast cell activator C48/90 [35] increased the antibody response of mice. Other approaches combining PA with complement receptor 2 monoclonal antibody to target antigen-presenting cells increased antibody levels [36] and conjugating PA to Complement C3d also slightly increased efficacy in a mouse model [37].

3.2. Augmenting PA with B. anthracis components

While LF and EF can induce toxin neutralizing antibodies and EF is reported to have adjuvant properties and increase the anti-PA response [38], their role in protective immunity remains unclear [39–41]. Some effect of LF alone expressed on a plasmid vaccine was observed with an increased the time to death in rabbits after aerosol challenge but no increase in survival [42]. Similarly, the N-terminal fragment of EF expressed on an adenoviral vector partially protected mice against challenge with an unencapsulated strain [43]. Other studies have proposed using mutants of PA, LF, and EF that are incapable of forming lethal or edema toxin as a safer vaccine and possible therapeutic as they can also inhibit the activity of native toxin [44–48]. Similarly live attenuated spore vaccines with inactivating mutations in LF and EF have been proposed to decrease reactogenicity (see below).