Published in final edited form as:
Mol Aspects Med. 2009 December ; 30(6): 490–502. doi:10.1016/j.mam.2009.08.006.

Anthrax vaccination strategies

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Abstract

The biological attack conducted through the U.S. postal system in 2001 broadened the threat posed by anthrax from one pertinent mainly to soldiers on the battlefield to one understood to exist throughout our society. The expansion of the threatened population placed greater emphasis on the reexamination of how we vaccinate against *Bacillus anthracis*. The currently-licensed Anthrax Vaccine, Adsorbed (AVA) and Anthrax Vaccine, Precipitated (AVP) are capable of generating a protective immune response but are hampered by shortcomings that make their widespread use undesirable or infeasible. Efforts to gain U.S. Food and Drug Administration (FDA) approval for licensure of a second generation recombinant protective antigen (rPA)-based anthrax vaccine are ongoing. However, this vaccine's reliance on the generation of a humoral immune response against a single virulence factor has led a number of scientists to conclude that the vaccine is likely not the final solution to optimal anthrax vaccine design. Other vaccine approaches, which seek a more comprehensive immune response targeted at multiple components of the *B. anthracis* organism, are under active investigation. This review seeks to summarize work that has been done to build on the current PA-based vaccine methodology and to evaluate the search for future anthrax prophylaxis strategies.

Keywords

Bacillus anthracis; anthrax; vaccine

Introduction

Bacillus anthracis is a highly virulent organism of intense interest within the biodefense field. The natural characteristics of *B. anthracis*, particularly the persistence and transmissibility of anthrax spores and the high rates of morbidity and mortality that result from spore exposure, make the bacterium an ideal candidate for weaponization. During the Cold War, the principal perceived threat of an anthrax-based weapon was its use as part of a military campaign. In the post-Cold War world this remains a concern, but following the attacks conducted through the U.S. postal system in 2001 (Jernigan et al., 2001; Jernigan et al., 2002) this scenario has been eclipsed by the worry that anthrax spores might be used against the general population. These and other developments have led to a renewed interest in anthrax vaccines.

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The vaccine currently used in the U.S. has its origins in research dating back more than 50 years (Wright et al., 1951; Wright and Slein, 1951; Belton and Strange, 1954; Puziss and Wright, 1954; Wright et al., 1954; Auerbach and Wright, 1955; Henderson et al., 1956). Human field trials conducted with an earlier version of the vaccine demonstrated effectiveness in reducing rates of cutaneous, and perhaps inhalational, anthrax among those exposed to *B. anthracis* (Brachman et al., 1962). However, despite the tremendous step forward that the United States' anthrax vaccine, adsorbed (AVA) and the United Kingdom's anthrax vaccine, precipitated (AVP) represent, numerous reports have questioned the safety, practicality and long-term efficacy of the vaccine regimens (Turnbull, 2000; Baillie, 2001; Sever et al., 2004; Brey, 2005; Grabenstein, 2008). These concerns have resulted in the search for new and improved anthrax vaccines, including the possible development of a vaccine that incorporates multiple antigens presented by *B. anthracis* and targets different aspects of the organism's pathogenicity (Tournier et al., 2009). This review will attempt to summarize and assess research into new approaches to vaccinate against *B. anthracis*.

Toxin-Based Anthrax Vaccines

AVA

The currently-licensed AVA vaccine used in the United States and the closely-related AVP vaccine used in the United Kingdom strive to protect vaccinees from the lethal effects of toxins elaborated by *B. anthracis* following spore germination (Mock and Fouet, 2001). The key component of the vaccine is protective antigen (PA), the element common to lethal toxin (LT) and edema toxin (ET). These toxins play critical roles in *B. anthracis* pathogenicity (Keppie et al., 1955; Smith et al., 1955a; Smith et al., 1955b; Molnar and Altenburn, 1963; Pezard et al., 1991). Early work demonstrating the protective efficacy of an antibody-based immune response to PA, along with the subsequent development of PA-based anthrax vaccines, is the subject of numerous reviews (Hambleton et al., 1984; Nass, 1999; Baillie, 2001; Little, 2005; Brey, 2005; Wang and Roehrl, 2005; Grabenstein, 2008). Studies performed by Brachman and colleagues (Brachman et al., 1962) demonstrated the capacity of a PA-based vaccine to lower the incidence of anthrax among exposed human populations and provided a strong rationale for administration to certain at-risk populations within the military and health-care communities. It is worth noting that differences exist between the AVA and AVP vaccines even though both are supernatant-based preparations designed to generate a protective response to PA. Compared to AVA, the British AVP contains lower levels of PA and higher concentrations of additional *B. anthracis* antigens, such as lethal factor (LF), edema factor (EF), and certain bacillus surface proteins (Turnbull, 1991; Baillie et al., 2003; Whiting et al., 2004). These additional or enriched components in the British AVP anthrax vaccines, which reflect the production strain used and/or the vaccine preparation techniques employed, may impart a slight enhancement in protection (Baillie et al., 2004), and may also be the source of the increased transient reactogenicity seen in comparison to AVA (Turnbull, 2000).

In recent times, events such as the Persian Gulf War of 1991 and the anthrax attacks of 2001 caused the perceived “at-risk” population to grow. As the target population grew, so too did concerns over the efficacy, cumbersome regimen (6 shots over 18 months and an annual booster), and possible side effects of the AVA vaccine (Pittman et al., 2001; Swanson-Biearman and Krenzelok, 2001; Geier and Geier, 2002; Greidanus and Honl, 2002; Hoffman et al., 2003; Lange et al., 2003; Wasserman et al., 2003; Geier and Geier, 2004; Hunter et al., 2004; Pittman et al., 2004; Grabenstein et al., 2006; Vasudev and Zacharisen, 2006; McNeil et al., 2007; Payne et al., 2007; Smith et al., 2007). Much energy has been devoted to investigating and addressing these concerns (Friedlander et al., 1999; Joellenbeck et al., 2002; Niu et al., 2009). Recent studies demonstrated efficacious vaccination schedules that require fewer shots to achieve necessary antibody titers (Pittman et al., 2000; Pittman et al., 2002; Pittman et al., 2006; Marano et al., 2008; Singer et al., 2008). However, questions persist

regarding variability of AVA production batches and reactogenicity, a common yet variable side effect of repeated vaccination (Brachman et al., 1962) that may be attributable to the less well-defined elements of the vaccine (Turnbull, 2000). Moreover, the AVA vaccine fails to fully protect against certain geographic isolates of *B. anthracis* (Little and Knudson, 1986; Fellows et al., 2001). Taken together, these questions about the tolerability and efficacy of AVA as administered in the current regimen have provided the impetus for the development of a 2nd generation vaccine.

Recombinant PA

In part to address the existing concerns over issues such as the safety and lot-to-lot variability of AVA, any 2nd generation anthrax vaccine needed to contain well-defined and controlled components. Given the essentiality of protection against intoxication, and the knowledge that an immune response to PA blocks the action of both key anthrax toxins, it was clear that this vaccine would be based on a recombinantly expressed PA (rPA) molecule. Studies with guinea pigs (McBride et al., 1998), rabbits (Little et al., 2004) and monkeys (Ivins et al., 1998) have demonstrated the capacity of rPA to protect against aerosol challenge. Like AVA, protection afforded by rPA-based vaccines is primarily antibody-based (Williamson et al., 2005). Titers of toxin-neutralizing antibodies, which are the most reliable predictor of protection against lethal challenge (Weiss et al., 2006), can be induced to similar levels with an rPA-based vaccine as with AVA (Gorse et al., 2006; Campbell et al., 2007). The rPA molecule can also be truncated (Hepler et al., 2006) or modified (Ramirez et al., 2002; Ribot et al., 2006; Rhie et al., 2005) while still maintaining the capacity to induce a protective humoral response. As with AVA, results with rPA-based vaccines consistently indicate the requirement for annual boosters to maintain levels of toxin-neutralizing antibodies sufficient to ensure protection (Ivins et al., 1998; Pittman et al., 2002; Little et al., 2006). Human clinical trials meant to investigate the safety and immunogenicity of the rPA-based vaccine PA102 are currently underway (Gorse et al., 2006; Keitel, 2006).

Alternative Approaches to PA-based Vaccines

Modified Recombinant PA

Despite the efficacy of the rPA-based anthrax vaccine currently under development, many researchers strive to develop improved rPA vaccines that demonstrate more rapid humoral induction, more durable efficacy and/or broader utility. One approach with potentially broad application involves the use of Dominant Negative Inhibitors (DNIs), mutated forms of PA that can undergo heptamerization at the cell surface but not the conformational changes necessary for membrane translocation. In the absence of that PA translocation step, anthrax toxin trafficking and function cease (Sellman et al., 2001a; Sellman et al., 2001b; Singh et al., 2001). These DNIs are protective against intoxication when administered with active toxin (Sellman et al., 2001b; Singh et al., 2001). They are also capable of inducing antibodies that successfully neutralize the activity of normal rPA (Aulinger et al., 2005; Yan et al., 2008). Unlike fully-functional rPA, DNIs might be given to a patient post-exposure without risk of enhancing intoxication during an infection. Hence, DNIs could potentially serve as both a pre-exposure prophylactic vaccine and a post-exposure therapeutic.

Alternate Adjuvants

Much research has been devoted to improving anti-PA humoral responses through modified delivery of rPA, rather than modification of rPA itself. One strategy involves the investigation of new adjuvants that enhance the host response when compared with the alum adjuvants currently approved for human use (Edelman, 2000). Adjuvants capable of evoking a more rapid or more durable response offer hope of a reduced number of shots during initial vaccination, or extension of the period between sustaining boosts. Early studies that used certain microbial

products and/or oil chemical compounds, such as saponin, achieved antibody levels and protection equivalent to that achieved with approved alum adjuvants in a variety of animal models (Ivins et al., 1992; Ivins et al., 1995; Ivins et al., 1998). Experiments with the Ribi adjuvant (a mixture of squalene, Tween-80, mycobacterial products and the bacterial compound monophosphoryl lipid A) demonstrated greater protection of guinea pigs against inhalational anthrax despite lower doses of immunogen (McBride et al., 1998).

CpG oligodeoxynucleotide molecules (ODMs) have also been evaluated as possible adjuvants for anthrax vaccines. CpG ODMs work as an adjuvant by stimulating the immune response through the induction of Toll-Like Receptor 9 (TLR9) (Takeshita et al., 2004). These molecules induced a humoral response that was more rapid, more avid, and longer lasting than when rPA (or AVA) was administered with alum (Klinman et al., 2004). This stronger, broader response led to protection that was more rapidly generated and longer in duration for animal models ranging from mice (Coeshott et al., 2004) to macaques (Klinman et al., 2004). Given some of the afore-mentioned limitations of the AVA regimen, an additive capable of increasing both the speed and the endurance of a protective humoral response warrants further investigation.

Needle-free Delivery

Aside from attempts to augment the response generated to rPA when administered through the traditional intramuscular route, numerous investigators have sought to use alternate delivery routes to achieve more relevant or robust immune responses. Intradermal injection shows evidence of inducing toxin-neutralizing antibodies whose levels correlate well with protection in rabbits (Mikszta et al., 2005). Some results (Mikszta et al., 2006) suggest that these intradermal injectors produce a more rapid humoral induction than that achieved through classic intramuscular injection. Skin patches that contained rPA adjuvanted with *E. coli* heat-labile toxin conferred protection onto A/J mice subsequently challenged with a toxigenic, unencapsulated anthrax strain (Kenney et al., 2004; Matyas et al., 2004). This model system (Welkos and Friedlander, 1988), which involves a complement-deficient mouse (The Jackson Laboratory, 2008) and an attenuated *B. anthracis* strain, is commonly used for preliminary research but does not always accurately reflect an infection with fully-virulent anthrax. Indeed, further mouse experimentation demonstrated that upon challenge with fully virulent *B. anthracis* the protection was only partial (Peachman et al., 2006). The patches nonetheless do induce significant levels of IgA in the lungs of vaccinated animals (Kenney et al., 2004). New variations in the intradermal delivery of PA (Knockenhauer et al., 2008) offer the continued possibility of successful anthrax vaccination through this approach.

Mucosal Vaccination

Perhaps the most likely scenario for a biological attack involving anthrax entails the inhalation of aerosolized spores. Because of the known persistence of spores in the lung following such exposure (Barnes, 1947; Henderson et al., 1956), the presence of anti-PA IgA molecules in the lung may be valuable in the prevention of disease. A reasonable approach for stimulating anti-PA IgA in the lung would be to intranasally administer a vaccine, installing the immunogen into the same sites likely to be exposed during inhalational infection. Intranasal administration of rPA to mice not only yielded higher anti-PA IgA titers than did subcutaneous administration but also generated similar levels of anti-PA IgG and toxin-neutralizing antibodies (Gaur et al., 2002). However, later studies that examined the immunogenicity and protection afforded to mice (Sloat and Cui, 2006a; Sloat and Cui, 2006b; Zeng et al., 2007; Baillie et al., 2008), guinea pigs (Bielinska et al., 2007) and rabbits (Huang et al., 2007) yielded mixed results. The technique appears to be most effective when rPA is provided in powder form, rather than as a liquid (Wimer-Mackin et al., 2006; Klas et al., 2008). Intramuscular priming of rPA following by an intranasal boost may prove to be the most effective strategy (Flick-Smith et al., 2002).

An alternate approach to inducing mucosal immunity involves oral administration of PA in the form of PA-expressing bacterial vectors. Efforts thus far have mostly focused on either nontoxigenic, unencapsulated *B. anthracis* strains expressing PA or attenuated, PA-producing *Salmonella enterica*. Oral administration of live, unencapsulated, nontoxigenic *B. anthracis* spores expressing rPA yielded significant anti-PA IgA and toxin-neutralizing levels but afforded protection to guinea pigs that was less than that provided by subcutaneous administration of the same vector (Aloni-Grinstein et al., 2005). Meanwhile, after less than encouraging early results (Garmory et al., 2003), *S. enterica*-based vaccines show greater promise. Efforts to improve PA expression led to improved immune responses (Galen et al., 2004; Osorio et al., 2009). As a consequence, enhanced induction of toxin-neutralizing antibodies has resulted in substantial protection against aerosol exposure in experiments involving A/J mice (Stokes et al., 2007; Osorio et al., 2009). Recent results (Mohamadzadeh et al., 2008; Mohamadzadeh et al., 2009) with PA-encoding *Lactobacillus* vehicles offer to further expand the possibilities of this approach.

Live, attenuated bacilli injection

Another approach to generating a protective anti-toxin response is through the direct injection of attenuated *B. anthracis*. The injection of rats and guinea pigs with a toxigenic, nonencapsulated strain generated a robust antibody response to PA, LF and EF and resulted in protection against subsequent challenge with virulent anthrax (Ivins et al., 1986). A more recent approach afforded protection in mice, guinea pigs and rabbits via the delivery of nonfunctional but immunogenic PA, LF and EF through a killed but metabolically-active (KBMA) strain. This unencapsulated *B. anthracis* construct is capable of secretion despite its photochemically-inactivated and asporogenic state (Skoble et al., 2009). This most recent approach succeeds in achieving immunogenicity and protection while exhibiting less reactogenicity than rPA adjuvanted with alum (Skoble et al., 2009).

DNA Vaccination

By introducing immunogen-encoding genetic material into a host cell, DNA vaccinations offer the prospect of maintaining levels of target antigen within a vaccinated individual for a longer period than with protein injection. The potential utility of this approach is illustrated by available evidence which suggests that while PA-encoding DNA vaccines might not achieve the same intensity in the initial immune response as is seen with a protein-based vaccine, the resulting immunological memory is longer-lasting (Hahn et al., 2006a). Work toward a DNA vaccine for anthrax has included demonstration of the generation of an anti-PA immune response in mice, rats and rabbits (Gu et al., 1999; Luxembourg et al., 2008). However, reports of successful protection of hosts through the administration of PA-encoding DNA alone have been limited (Riemenschneider et al., 2003; Midha and Bhatnagar, 2009). In an attempt to enhance the intensity of the immune response to the antigen targets, several groups have pursued a DNA prime/protein boost strategy (Williamson et al., 1999; Price et al., 2001; Galloway et al., 2004). These researchers achieved higher anti-PA IgG and toxin neutralizing antibody levels through this approach than they did with a series of DNA injections alone. Other investigators found that the co-administration of LF-encoding DNA alongside the PA-encoding DNA yielded higher IgG and toxin-neutralizing antibody responses than when either target was administered alone (Price et al., 2001; Hermanson et al., 2004; Liu et al., 2009). Still other have succeeded in generating anti-PA responses (Lee et al., 2003; Shivachandra et al., 2007; Liu et al., 2009) and protection from challenge (Hashimoto et al., 2005) through application of PA-encoding viral vectors. Thus far, almost all positive findings with viral vectors and other DNA vaccines have been achieved with mouse models. The ability to translate these successes into demonstrated efficacy in more advanced model systems remains to be seen.

New Vaccine Targets

Despite the potential improvements in PA-based vaccine strategies, there is reason to remain uneasy about continued reliance upon antibodies to PA as the sole protector against anthrax. Titers of total anti-PA IgG (Turnbull et al., 1988; Pitt et al., 1999; Pitt et al., 2001; Pittman et al., 2005; Little et al., 2004), or more specifically, toxin-neutralizing antibodies (McBride et al., 1998; Reuveny et al., 2001; Marcus et al., 2004; Pittman et al., 2005; Weiss et al., 2006; Hewetson et al., 2008), are generally considered to be good predictors of a protective immune response. However, certain studies suggest that the anti-PA humoral response generated by PA-based vaccines and the level of protection conferred by antibodies to PA are more variable than was first thought (Welkos and Friedlander, 1988a; Ivins et al., 1992; Ivins et al., 1995; Ivins et al., 1998; Fellows et al., 2001). In situations where patients face infection from anthrax in the midst of immunocompromise, a robust response to PA may be insufficient to confer complete protection (Welkos and Friedlander, 1988b; Peachman et al., 2006). Also, the need to subject a patient to extended vaccination regimens in order to reach acceptable levels of anti-PA antibodies remains a major drawback to the PA-based approach. Furthermore, the potential exists for development of a genetically-engineered *B. anthracis* strain in which PA has been supplanted by an alternative virulence factor. Certain naturally-occurring isolates which are at least partially refractile to AVA, through molecular bases not currently understood (Little and Knudson, 1986; Fellows et al., 2001), raise concerns over the PA-based approach.

Undoubtedly, the generation of a robust response to PA will remain a critical component of any future anthrax vaccine. The absence of PA negates any potential benefits from other vaccine components (Ivins et al., 1986; Pezard et al., 1995; Cohen et al., 2000; Brossier et al., 2002; Brahmbhatt et al., 2007; Cybulski, Jr. et al., 2008). However, it seems altogether wise to consider a comprehensive, multi-targeted approach to anthrax vaccination that mirrors successful approaches taken with other bacterial pathogens such as *Bordetella pertussis*. The existence of distinct life stages and virulence factors, each of which is essential to bacterial virulence and immunogenic during the course of a natural infection (Sirisanthana et al., 1988; Harrison et al., 1989; Zhang et al., 2008), provides vaccine researchers with an array of potential targets.

Capsular Antigens

By virtue of its position as the outermost surface of the bacilli, the antiphagocytic (Ezzell and Welkos, 1999) poly-γ-D-glutamic acid (PGGA) capsule is the face presented to the host immune system upon spore germination. Examination of the sera from survivors of anthrax infection demonstrates an almost universal response to the *B. anthracis* capsule (Sirisanthana et al., 1988; Harrison et al., 1989), though this information alone does not necessarily ensure that such a response has protective benefits. Early investigations (Goodman and Nitecki, 1966) demonstrated the relatively poor immunogenicity of PGGA administered alone. Therefore, a common approach to investigating the possible benefit of PGGA has involved conjugation of capsular polypeptides to protein carriers. Vaccinating with a PA:PGGA conjugate holds the potential for a dual benefit, with the PA protein enhancing the immune response to the capsule while simultaneously immunizing against intoxication. This approach successfully induced IgG responses to both PA and PGGA in mice (Schneerson et al., 2003; Rhie et al., 2003; Wang et al., 2004; Sloat and Cui, 2006a). Simultaneous administration of the conjugated components did not deleteriously affect the response to PA compared to PA administered alone (Sloat and Cui, 2006a). Enhanced protection against spore challenge was demonstrated in mice (Chabot et al., 2004; Joyce et al., 2006) and rabbits (Wimer-Mackin et al., 2006) with PA:PGGA conjugates compared to PA alone or PA plus unconjugated PGGA. Anti-PGGA antibodies show a capacity *in vitro* to induce opsonophagocytosis (Schneerson et al., 2003; Wang et al., 2004) and complement-mediated killing (Rhie et al., 2003).

The spore

When considering possible approaches to the development of more comprehensive protection against anthrax, one must also consider research involving attenuated strains of *B. anthracis* administered in spore form. Pasteur and Greenfield's original vaccine strains (Tigertt, 1980; Sternbach, 2003), as well as the strain isolated by Sterne in the 1930's and still in use today as a veterinary vaccine (Sterne, 1939; Turnbull, 2000), relied on administration of whole spores. An attenuated, live spore anthrax vaccine, developed by scientists in the former Soviet Union, is still in use in Russia and China (Dong, 1990; Shlyakhov and Rubinstein, 1994). Spore vaccines constructed from toxigenic, unencapsulated strains have repeatedly demonstrated the capacity to confer protection to mice and guinea pigs against anthrax challenge (Klein et al., 1962; Ivins et al., 1986; Little and Knudson, 1986; Welkos and Friedlander, 1988a; Ivins et al., 1990; Brossier et al., 2000). Certain studies indicate that these attenuated spore vaccines are more protective than existing PA-based vaccines (Little and Knudson, 1986; Ivins et al., 1986; Welkos and Friedlander, 1988a). However, one must always bear in mind the key role for protection against intoxication in any vaccination scheme. Spores made from unencapsulated, nontoxigenic strains fail to protect against subsequent challenge (Ivins et al., 1986; Pezard et al., 1995; Cohen et al., 2000), whereas the reintroduction of PA into such toxin-minus strains via rPA-encoding plasmids restores protective efficacy (Barnard and Friedlander, 1999; Cohen et al., 2000; Aloni-Grinstein et al., 2005; Mendelson et al., 2005). Effective generation of protective immunity can also be achieved by the administration of spores made from an unencapsulated strain encoding for nonfunctional but immunogenic toxin molecules (Brossier et al., 2000). However, utilization of live spores for vaccination has been hampered in the U.S. and Europe, despite its possible benefit, due in part to concerns over residual virulence, safety and the efficacy of the approach. In order to harness the protective benefit of the spore, an alternative strategy must be identified.

Studies involving the use of formalin-inactivated spores (FIS) administered as a vaccine in conjunction with PA offer an alternate approach to harnessing the spore to generate enhanced protective immunity (Brossier et al., 2002; Gauthier et al., 2009). These investigations demonstrated enhanced protection of both mice and guinea pigs by vaccination with FIS plus PA compared to PA alone. This work provided one potential strategy to achieve a broader immune response in the absence of a live *B. anthracis* strain. A later study demonstrated that immunization with spores induced a cellular immune response that might contribute to protective immunity against anthrax (Glomski et al., 2007). Demonstrations that protective anti-spore responses were not dependent on the presence of a live organism raise the possibility that individual spore parts might, in association with PA, augment a protective immune response.

One enticing target from among the long list of spore components is *Bacillus* Collagen-like antigen (BclA), the immunodominant glycoprotein responsible for the hair-like protrusions present on the outermost surface of *B. anthracis* (Hachisuka et al., 1966; Sylvestre et al., 2002; Steichen et al., 2005). Studies that examined the efficacy of BclA delivered with PA in either DNA (Hahn et al., 2006a; Hahn et al., 2006b) or protein (Brahmbhatt et al., 2007) form showed enhanced protection compared to PA alone. Enhancement correlated with the appearance of an anti-BclA humoral response. More recently, a screen of a list of proteins known to be visible to the host and immunogenic in the context of whole spores broadened the inventory of potentially-protective spore antigens (Cybulski, Jr. et al., 2008). This study identified the structural protein BxpB and the previously-undescribed antigen p5303 as immunogens capable of enhancing the protection afforded by PA when A/J mice are challenged with toxigenic, unencapsulated *B. anthracis*. Each of these investigations also reaffirmed the importance of PA, as they failed to observe any significant protection when the spore antigens were administered alone.

Passive Antibody Therapy

Our growing understanding of protective immune responses to anthrax can be used not only to develop and improve prophylactic vaccines but also to design post-exposure therapies. One approach involves the use of those same vaccines, which have been shown to improve the outcome of exposure in previously-unvaccinated individuals when combined with antibiotic therapy (Friedlander et al., 1993; Fowler et al., 2005; Peterson et al., 2006; Baccam and Boechler, 2007). Another tactic, valuable perhaps in situations where vaccination is precluded by limited supply or host immunocompromise, is the passive administration of protective antibodies. Protective benefits of the passive administration of AVA-induced antisera seem to be derived primarily from the toxin-neutralizing activity of anti-PA antibodies (Little et al., 1997; Beedham et al., 2001; Reuveny et al., 2001; Kobiler et al., 2002; Hewetson et al., 2008), though studies by Welkos and colleagues indicate that antibodies raised against PA also have anti-spore characteristics (Welkos et al., 2001; Welkos et al., 2002).

Much of the recent work in the field of anti-anthrax passive antibody therapeutics has been devoted to the identification and development of human monoclonal antibodies (mAbs). The identification of human mAbs with the capacity to neutralize PA has come primarily through the screening of sera belonging to AVA-vaccinated individuals (Wild et al., 2003; Sawada-Hirai et al., 2004; Zhou et al., 2008). While these reagents carry the potential benefit of better characterization and standardized production compared to polyclonal sera, the challenge lies in the identification of mAbs that match the efficacy of the polyclonal approach (Casadevall, 2002) and do not have the unintended effect of enhancing infection (Mohamed et al., 2004). Anti-PA mAbs have demonstrated the capacity to protect a number of animal models, including mice (Peterson et al., 2006), guinea pigs (Mabry et al., 2005; Peterson et al., 2006) and rabbits (Mohamed et al., 2005; Peterson et al., 2006; Peterson et al., 2007; Wild et al., 2007). Protective efficacy of mAbs against anthrax upon subcutaneous administration of antibodies (Peterson et al., 2007; Wild et al., 2007) offers the possibility of a less cumbersome regimen than the intravenous antibody administration utilized to date. Evidence of a synergistic effect when antibodies are administered alongside antibiotics (Peterson et al., 2006) is also promising. This latter point may prove crucial given that the effectiveness of passive therapy declines as time passes between exposure and mAb administration (Kozel et al., 2004; Mohamed et al., 2005; Peterson et al., 2007; Wild et al., 2007).

PA is an obvious target upon which to focus efforts for development of a therapeutic human mAb. However, as in the case of vaccination, mounting evidence supports the value of pursuing additional or alternate targets for antibody therapy. One such target is the LF molecule, whose interaction with PA is essential for the intoxication that PA-based therapies aim to block (Ascenzi et al., 2002). Several studies (Albrecht et al., 2007; Staats et al., 2007) indicate that anti-LF mAbs are perhaps a superior therapeutic to anti-PA mAbs. *In vitro* evidence indicates that, unlike anti-PA mAbs, anti-LF mAbs are able to protect cell lines from intoxication despite delayed administration after exposure to toxin (Staats et al., 2007). A mouse protection study suggested that anti-LF mAbs are retained in the bloodstream longer, and protect hosts at a lower concentration, than do anti-PA mAbs (Albrecht et al., 2007). An additional advantage of a potential anti-LF mAb is that it might be administered alongside a PA-based vaccine with minimal risk of interference with the development of an anti-PA response (Pelat et al., 2007).

Aside from major virulence factors such as PA and LF, the targeting of other bacterial components may also hold protective potential. For example, monoclonal antibodies directed against the poly-D-γ-glutamic capsule afforded mice protection against anthrax (Kozel et al., 2007). Moreover, a recent study demonstrated that mAbs to anthrolysin O provided partial protection of A/J mice against infection with *B. anthracis* Sterne strain (Nakouzi et al., 2008). Our laboratory found that antibodies to certain spore antigens promoted the

opsonophagocytosis of spores (Brahmbhatt et al., 2007; Cybulski, Jr. et al., 2008), a step which helps confer partial protection by promoting spore clearance and reducing the effective infectious load. As is the case with vaccination, it is hard to conceive of a passive therapeutic approach that does not incorporate protection against intoxication. However, these results suggest that it may be possible to find ways to enhance any protection afforded by a PA- or LF-based approach.

Conclusion

The threat of an intentional anthrax outbreak, and the search for improvements in the available anthrax prophylaxis tools, will likely remain a subject of great interest within the realm of biodefense for the foreseeable future. The AVA and AVP vaccines represented a significant step forward in an era before modern molecular biological techniques and provided a major defense mechanism for key populations at risk for anthrax exposure. However, the 1991 Persian Gulf War and the 2001 anthrax attacks led to the massive expansion of the population of potential vaccinees, which, in turn, led to a focus on the limitations of these vaccines. The development of a defined, recombinant PA-based vaccine promises to address some of concerns, but questions remain. Without a firm understanding of how important the "undefined" components of AVA and AVP are in the levels of protection afforded by that vaccine, it remains unclear whether or not rPA will entirely recapitulate the efficacy of these vaccines in non-human primates and humans. While numerous strategies that involve molecular alteration, improved adjuvants, and novel delivery offer the possibility of enhancing the effectiveness of a PA-based approach, there is ample reason to remain skeptical that a vaccine relying on PA alone can be a final answer. The surge of research interest that resulted from the biological attacks of 2001 has led to tremendous progress in the understanding of the basis molecular processes of the *B. anthracis* pathogenesis. As has recently been discussed elsewhere (Tournier et al., 2009), these new insights have broadened, and will likely continue to broaden, the array of possible vaccine targets that might enhance the current PA-based protection scheme. Consequently, a multi-pronged approach targeting distinct aspects of the *B. anthracis* organism seems like a logical goal for future anthrax vaccines.

Acknowledgments

We thank Ms. Sophia Echelmeyer and Mr. Patrice Bolté for help with illustration production. This work was supported by NIH/NIAID Middle Atlantic Regional Center of Excellence grant U54 AI057168 and research funds from the United States Navy through Uniformed Services University grant # G173HS. Special thanks also to the United States Army Long-Term Health Education and Training Program for support of one of us (R. Cybulski). The opinions and assertions in this paper are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Department of Defense.

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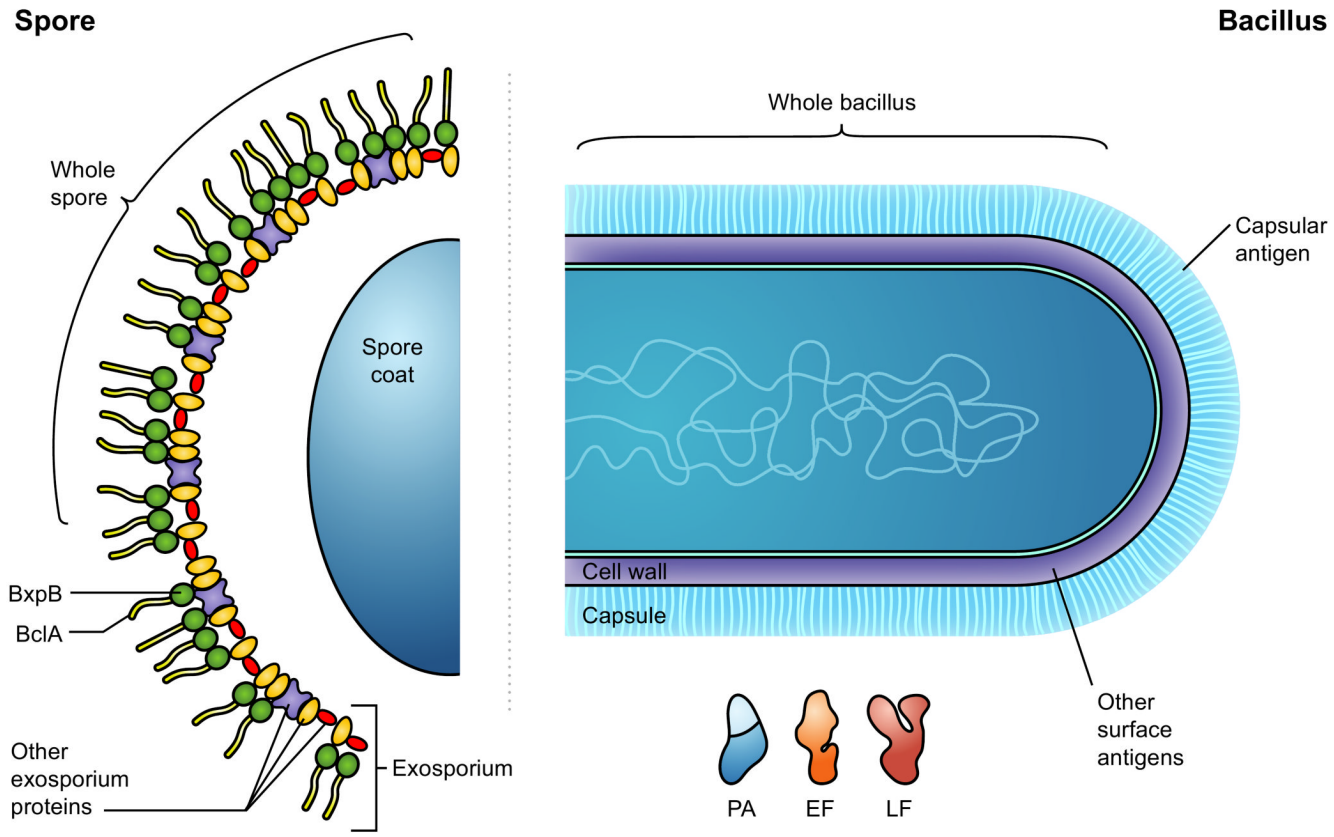

Figure 1.

Potential anthrax vaccination targets. Protective or partially-protective immune responses can be directed at either phase of the *Bacillus anthracis* life cycle: the spore or the vegetative bacilli. Aside from the administration of live, attenuated spores, an anti-spore humoral response can be elicited via the delivery of individual spore components such as BclA or BxpB. Prime targets from the vegetative life cycle include the anthrax toxin components protective antigen (PA), lethal factor (LF) and edema factor (EF) as well as the antigen that comprises the antiphagocytic poly-γ-D-glutamic acid capsule. Direct administration of live, attenuated bacilli, primarily as a vehicle for delivery of PA, has also been explored as a vaccination strategy.

Table 1

Potential Anthrax Vaccine Strategies

- PA-based Vaccination Strategies
  - First Generation (AVA)
  - 2nd Generation (PA102)
  - 3rd Generation (alternative rPA-based)
    - Modified rPA
    - Alternative adjuvants
    - Needle-free delivery
      - Intradermal injection
      - Transcutaneous administration
    - Mucosal administration
      - Intranasal delivery
      - Oral delivery (attenuated bacterial vector)
    - Live, attenuated bacilli injection
    - DNA vaccination
      - Viral vector
      - Plasmid injection
- Alternative vaccination strategies
  - Bacilli capsular antigens
  - Attenuated whole spores
  - Spore antigens
    - BclA
    - BxpB
    - other
- Passive antibody therapy
  - Anti-PA
  - Anti-LF
  - Anti-capsule
  - other