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RECENT ADVANCES IN THE DEVELOPMENT OF AN IMPROVED, HUMAN ANTHRAX VACCINE

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Human anthrax vaccines currently licensed in the United States and Western Europe consist of alum-precipitated or aluminum hydroxide-adsorbed supernatant material from fermentor cultures of toxigenic, nonencapsulated strains of *Bacillus anthracis*. These vaccines have several drawbacks, including the need for frequent boosters, the apparent inability to protect adequately against certain strains of *B. anthracis*, and occasional local reactogenicity.

Studies are being undertaken to develop an improved human anthrax vaccine which is safe and efficacious, and which provides long-lasting immunity. Aspects being studied include the identification of antigens and epitopes responsible for eliciting protective immunity, the mechanisms of resistance to anthrax infection, the role of specific antibody in resistance, the differences in immunity elicited by living and chemical vaccines, the potential of new adjuvants to augment immunity, and the feasibility of developing safe vaccine strains having mutationally altered toxin genes. Both living and non-living (chemical) prototype vaccines are being developed and tested.

Anthrax is an ancient disease of animals and humans. Domestic livestock such as sheep, goats, and cattle are frequent victims of the disease, but human cases of anthrax can occur as a result of exposure to infected animals or animal products (32). There is also evidence that anthrax can be transmitted through the bite of insects (55). The etiological agent of anthrax is *Bacillus anthracis*, a large, facultative, gram-positive rod. *Bacillus anthracis* possesses two plasmid-encoded virulence factors: a poly-D-glutamic acid capsule (encoded by pXO2) (16, 58), and a toxic mixture of three proteins, commonly referred to as « anthrax toxin »: protective antigen (PA), lethal factor (LF), and edema factor (EF), all encoded by pXO1 (21, 22, 44). The capsule inhibits phagocytosis (27). Individually, the three proteins possess no demonstrable toxic activity by themselves; however, intravenous injection of PA plus LF, « lethal toxin », kills rats, mice, and guinea pigs (10). Intradermal injection of PA plus EF, « edema-producing toxin », elicits edematous lesions in the skin of guinea pigs and rabbits (33). All three toxin components have molecular masses of between 80 and 90 kilodaltons (kDa) (32). PA is believed to bind to host cells and facilitate the entry of LF and EF (32). Edema factor has been identified as a calmodulin-dependent, adenylate cyclase, which raises the level of cyclic AMP in eukaryotic cells (30, 31). The molecular basis for the toxicity of PA + LF combinations remains unknown.

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Although vaccines against anthrax have been available for over a century, the mechanisms of immunity to the disease and the relevant *B. anthracis* antigens which elicit a protective immune response have not been completely defined. Louis Pasteur is generally credited with development of the first anthrax vaccines for livestock (39). These vaccines consisted of cultures which had been attenuated by growth at elevated temperature; unfortunately, their efficacy was often unpredictable due to varying degrees of attenuation. An explanation for the molecular mechanism of this attenuation was provided by Mikesell et al., who demonstrated that growing *B. anthracis* at 42°C cures the bacillus of the toxin plasmid pX01 (38). For immunization of livestock today, a suspension of viable spores from a toxigenic, nonencapsulated strain similar to the one originally isolated by Sterne (50, 51), is commonly used. The main drawback to the veterinary vaccine is safety; its use occasionally results in necrosis at the site of inoculation, and rarely, death of the animal. Anthrax vaccines for humans in the United States and Western Europe consist of alum-precipitated or aluminum hydroxide-adsorbed supernatant material from fermentor cultures of toxigenic, nonencapsulated strains of *B. anthracis* (4, 42). Brachman et al. (8) conducted a field test of the United States human vaccine and concluded that the vaccine was significantly effective. There are several drawbacks to the human vaccine, however, including the need for numerous boosters, the apparent inability to protect guinea pigs against certain virulent strains of anthrax (2, 36, 57, 60), and occasional local reactogenicity. An obvious need exists for an improved human anthrax vaccine, one which is safe, efficacious against all known virulent strains of *B. anthracis*, and which requires a minimal number of injections to achieve and maintain long-term immunity. Several excellent reviews on the subjects of anthrax, anthrax toxin, and anthrax vaccination exist (7, 18, 33, 56, 65). The central purpose of this article is to describe advances in anthrax vaccine research and development which have occurred within the past few years.

Before an anthrax vaccine can be evaluated, an appropriate experimental animal model must be selected. Anthrax vaccination studies, beginning with the first vaccine trials of Pasteur to present-day investigations, have relied on data collected from animals (2, 9, 10, 13, 14, 18, 21, 22, 24, 28, 33, 35-37, 40, 48-53, 57, 60). The major criterion used to evaluate vaccine efficacy is survival after administration of toxin or virulent spores to a vaccinated animal; other criteria include serum antibody or antitoxin levels, quantity of toxin or numbers of bacilli in the blood at death, and time-to-death (TTD) (11, 17, 18, 33, 37).

Guinea pigs frequently have been used to test the immunogenicity of anthrax vaccines, and, in addition to rabbits, to assay toxicity by edema production in the skin (2, 9, 13, 18, 28, 33, 40, 47, 48, 49, 50, 51, 52, 60). Both animal species can be protected against spore challenge by toxin component and spore vaccines (2, 9, 13, 21, 28, 36, 40, 49, 50, 51, 52, 57, 60).

Certain rodents were reported by several, previous investigators to be immunized poorly with culture filtrates (mice) or attenuated strain spores (mice and rats) against spore challenge (9, 13, 18, 20, 51). However, rats can be protected against a lethal *toxin* challenge after vaccination with toxin component preparations or recombinant PA-producing *B. subtilis* strains (22, 33, 37). In addition, Fischer 344 rats have been used to assay toxin potency because of the exquisite sensitivity of these animals to the lethal effects of toxin (3, 10, 17). Recently, the rat assay was supplemented by a sensitive macrophage cytoxicity assay for lethal toxin (12). Inbred strains of mice are used in several areas of anthrax research and, recently, some strains were immunized successfully against lethal spore challenge (S.L. Welkos, manuscript submitted; 1, 62, 63). In addition to small animals, protection studies have been conducted with primates as well as domestic animals, including cattle, sheep, goats, and horses (13, 14, 18, 24, 40, 50, 53).

Despite extensive data, it is not clear which animal model is appropriate for extrapolation of results to humans. There are several reasons for this. First, anthrax is a complex, poorly understood disease, and the cause of death remains unknown. Development of a lethal infection by *B. anthracis* requires germination of the spore inoculum, systemic invasion, multiplication, and toxin production, leading to death (18, 25, 33, 34, 45, 66). Animal species differ in their natural resistance to disease (1, 10, 25, 29, 33, 35, 37, 48, 62, 66). For example, guinea pigs are susceptible to a lethal spore infection but are resistant to killing by intravenous doses of the toxin (26, 29, 33, 35). In contrast, rats are resistant to establishment of infection but are very sensitive to toxin (29, 33, 35). The data suggest that resistance to infection and to the lethal effects of toxin involve separate host mechanisms (29, 33, 35).

Genetically determined host factors probably also modulate development of acquired immunity to anthrax, as observed for many microbial diseases (6). Indeed, immunizing the naturally resistant rat results in only a slight increase in resistance to spore challenge (as evaluated by increased TTD), compared to immunizing the susceptible guinea pig (25, 37). This response probably explains the relatively poorer degree of immunity observed in the rat as compared to the guinea pig (9). Also, host factors may play a role in the

ability of a *B. anthracis* culture filtrate to protect against heterologous challenge. As discussed below, Auerbach and Wright, and Ward et al. showed that certain strains of *B. anthracis* can overcome immunity induced in guinea pigs by a filtrate preparation of the Vollum strain, whereas rabbits are protected against challenge with all strains tested (2, 60). Finally, whereas the capsule of *B. anthracis* is not an immunizing antigen in most animals studied, a protective effect for mice has been reported (20, 60).

Recently, a mouse model was developed to study the basis of host differences in immunity and the pathogenesis of infection (62, 63). Strains differed significantly in innate susceptibility to lethal infection by both a fully virulent strain of *B. anthracis* and the nonencapsulated Sterne vaccine strain. Genetic studies suggested that resistance to Sterne is controlled in large part by a single, major gene (62, 63). In contrast to previous reports, some strains of mice could be protected against anthrax by immunization with sublethal doses of Sterne spores (S.W. Welkos, manuscript submitted; 62).

There are advantages in using both the guinea pig and inbred mouse system as models for immunization studies. First, there is a large precedent for using guinea pigs. Immunization and protection data with these animals have accumulated from Pasteur's initial studies to the present (2, 9, 13, 18, 28, 33, 40, 47, 48, 49, 50, 51, 52, 60). Also numerous studies with guinea pigs have characterized the pathogenicity of anthrax and the central role played by anthrax toxin in disease (3, 34, 45, 47). In contrast, advantages in using strains of inbred mice include extensively characterized genetic polymorphism, differences in resistance to diseases such as anthrax, and convenient size and ease of maintenance, facilitating use of statistically adequate numbers. Due to the large differences in susceptibility among animal species and the paucity of data on the disease in man, it is difficult to assess which animal responds to vaccination in a manner analogous to humans. Today, most cases of human anthrax occur in places where adequate data cannot be collected (18). Thus, we should probably continue to collect data on vaccine efficacy using several animal hosts and bioassays.

A primary goal in anthrax vaccine studies has been to elucidate those antigens which play a role in eliciting a specific, protective, immune response. Of the antigens studied, only the three toxin components have demonstrated « protective immunogenicity » (5, 21, 37). Somatic components such as capsule, surface polysaccharide, and surface proteins are not protective in most experimental animals (J.W. Ezzell, personal communication; 20, 46). These findings were confirmed in a recent series of experiments (21), summarized in Table 1, in which strains of *B. anthracis* that lacked either the capsule plasmid pX02, the toxin plasmid pX01, or both, were tested for their efficacy as live vaccines against intravenous challenge with anthrax toxin in Fischer 344 rats and against aerosol or intramuscular challenge with virulent anthrax spores in Hartley guinea pigs. Immunization with nontoxigenic, encapsulated ($pX01^-$, $pX02^+$) or nontoxigenic, nonencapsulated ($pX01^-$, $pX02^-$) strains provided no protection from toxin or spore challenge and did not elicit titers to any of the toxin components. In contrast, immunization with either of two toxigenic, nonencapsulated ($pX01^+$, $pX02^-$) strains, Sterne or Texas, provided complete protection against spore or toxin challenge under a variety of immunizing regimens, i.e., several large doses, a few small doses, or a series of graded doses. Thus, live *B. anthracis* strains that produced toxin components were protective, whereas strains that produced capsule, polysaccharide, and other somatic components, but not toxin components, were not protective. During these (21) and other experiments (5) immunization with the human vaccine (prepared by the Michigan Department of Public Health and referred to as MDPH-PA) elicited a small but significant serological response to EF and LF as well as PA. In some studies (36, 57), however, significant antibody titers to LF and EF have not been detected in sera after vaccination with MDPH-PA. The differences between these two sets of data suggest that there may be variations between lots of MDPH-PA, and that at least some of these lots contain LF and EF as well as PA.

In agreement with the guinea pig data, Sterne spores elicited serum anti-PA antibody and protected relatively Sterne-resistant mouse strains against virulent spore challenge (S.L. Welkos, manuscript submitted). However, the vaccine was only effective at doses approaching the Sterne LD_{50}, illustrating the hazards associated with the live vaccine. Sterne-susceptible A/J mice could not be protected by sublethal immunization with Sterne spores.

TABLE 1.
Immunization with *B. anthracis* Strains *.

Plasmid Content of Immunizing Strain	Protection Against Spore Challenge	Protection Against Toxin Challenge	Titers To PA, LF, EF**
pX01 (toxin plasmid)	+	+	+
pX02 (capsule plasmid)	—	—	—
None	—	—	—

* Summary of several experiments (21).
** Determined by enzyme-linked immunosorbent assay (ELISA).

The specific roles of the three toxin components in immunity to anthrax infection have been a source of controversy for over two decades. Protective antigen has been asserted to be the immunogen of importance in the human vaccine (8, 18, 42), yet some lots of MDPH-PA contain all three components (5, 21), and mixtures of PA plus EF were reported by Mahlandt et al. (37) and by Stanley and Smith (49) to be more efficacious than PA alone (37, 49). Furthermore, Mahlandt et al. (37) reported that LF was as protective an immunogen as PA; however, the serological method (agar diffusion) they used to determine the purity of the LF and PA was rather insensitive. In 1985, Berendt et al. (5) reported the results of a series of experiments which shed further light on the problem (Table 2). Guinea pigs were given three biweekly immunizations with either the Sterne spore vaccine, MDPH-PA, or purified toxin components adsorbed onto aluminum hydroxide. Three weeks after the final immunization, the animals received an aerosol challenge of virulent Vollum 1B strain spores. The Sterne spore vaccine was significantly more protective than MDPH-PA ($P<0.05$). The least protective were EF and LF alone. The veterinary and human vaccines were then compared with respect to duration of immunity to an aerosol spore challenge. Sixteen weeks after immunization, 80% of the Sterne spore-vaccinated animals were protected, compared to only 40% of the MDPH-PA vaccinated animals. When guinea pigs were boosted 12 weeks after immunization and then challenged at 27 weeks, 100% of the Sterne-vaccinated animals were protected, as compared to 40% of the MDPH-PA-immunized animals. Surprisingly, in these studies, and also in studies with immunized mice (S. Welkos, unpublished observations), antibody titers to PA were substantially higher in the animals which received MDPH-PA than in the Sterne spore-immunized animals.

TABLE 2.
Immunizing Efficacy of Various Vaccine Preparations*.

Vaccine	Survival		ELISA Antibody Titers to		
	Survivors/ Total	(%)	PA	LF	EF
Veterinary					
(Sterne Spore)	39/45	(87)	14,404	11,286	182
PA + EF	25/30	(83)	14,400	107	4,660
PA + LF	13/16	(81)	6,979	3,200	83
PA	25/36	(69)	7,759	7	5
Human					
(MDPH-PA)	20/30	(67)	58,310	2,190	34
PA + EF + LF	8/14	(57)	19,740	2,934	9,870
LF + EF	8/15	(53)	362	3,490	18,102
LF	3/15	(20)	35	6,979	117
EF	4/29	(14)	1,210	88	3,530
None	0/31	(0)	<4	<4	<4

* Groups of 14 to 35 female, Hartley guinea pigs, 250 to 350 g, received three biweekly injections of the indicated vaccine preparation. Three weeks after the final injection the animals received an aerosol challenge of 10 LD_{50} doses of *B. anthracis* Vollum 1B spores. Animal were bled for determination of ELISA antibody titers 1 week before challenge.

The superiority of the veterinary vaccine over the human vaccine was also recently demonstrated by Little and Knudson (36) and by Turnbull et al. (57). These workers confirmed the observation reported previously (2, 60) that certain strains of *B. anthracis* can overcome immunity induced in guinea pigs by alum-precipitated or aluminum hydroxide-adsorbed culture filtrate. They also compared the protective efficacies of live spore and PA vaccines. Although one of the $pX01^+$, $pX02^-$ vaccine strains used (STI strain) caused some lethality, the live vaccines protected guinea pigs against all challenge strains, including the ones that overcame immunity induced by the human PA vaccines (36, 57). Again, the spore vaccines often induced lower antibody titers to PA than did the cell-free preparations (36, 57).

The data from these and previous studies demonstrated that i) all efficacious, anthrax vaccines contain or produce PA; ii) neither EF nor LF alone is a good protective immunogen; iii) the live Sterne spore veterinary vaccine is more protective than the human chemical vaccine; and iv) there is no absolute, direct correlation between anti-PA antibody titers and levels of protection. The reason for the last observation is unclear. Polyclonal anti-PA titers reflect the antibody response to the entire PA molecule, not individual, antigenic domains. Although MDPH-PA elicits a higher polyclonal anti-PA titer, the Sterne spore vaccine may elicit higher titers to specific, protectively immunogenic domains, including those domains presumed responsible for PA binding to LF, EF, and the host cell. Perhaps the PA that is produced and partially purified in vitro differs in its three-dimensional, antigenic structure from PA which is produced and secreted directly in the host. This putative structural difference may lead to differences in the host protective immune response. Alternatively, elaborating a sustained, critical concentration of PA in vivo, similar to that produced by a live spore vaccine, may be necessary for optimal immune protection. Another possibility is that cellular immunity, which may be stimulated better by Sterne spores than by an aluminum-containing PA vaccine (61), plays a role in the immune response to anthrax infection. Finally, *B. anthracis* antigens other than PA may augment protection.

Further studies were conducted which demonstrated that PA produced in the complete absence

of EF and LF is a protective immunogen against an anthrax spore challenge (B.E. Ivins, unpublished observations; 22). Vodkin and Leppla (59) cloned a 6-kilobase-pair *Bam* HI digestion fragment of the *B. anthracis* pX01 toxin plasmid. This fragment, which contained the PA gene, was then subcloned into *B. subtilis* by using the vector pUB110. Two clones, designated PA1 and PA2, produced PA in liquid cultures at levels equal to or greater than those produced by *B. anthracis*. The PA produced by the *B. subtilis* recombinants was indistinguishable from *B. anthracis* PA with respect to molecular weight and antigenicity, as determined by migration on sodium dodecyl sulfate-polyacrylamide gels and serological reactivity in Western blot assays. The PA1 and PA2 culture supernatants were not toxic by themselves, but addition of EF or LF to the supernatants generated fully active, anthrax edema-producing toxin or lethal toxin, respectively. Although immunization of guinea pigs with 10^7 or more Sterne spores sometimes results in death (36), injection of up to 10^9 cfu of either of the PA-producing *B. subtilis* recombinants elicited neither apparent morbidity nor mortality in guinea pigs. Furthermore, immunization with two doses, 4 weeks apart, of 10^8 to 10^9 cfu of PA1 or PA2, protected against an intramuscular challenge of 20 LD_{50} of anthrax spores. In efficacy comparison tests, PA-producing *B. subtilis* recombinants proved equal to MDPH-PA and Sterne spores in protecting against anthrax infection. In contrast, the *B. subtilis* parent strain of PA1 and PA2, which carried pUB110 but not the PA gene, afforded no protection. In addition, *B. subtilis strain* PA2 was nonlethal for mice, including those of the Sterne-susceptible A/J strain. PA2 induced high titers of anti-PA antibody in all mice, and protected Sterne-resistant CBA/J mice against virulent spore challenge. Since the only *B. anthracis* antigen produced by the recombinant strains is PA, it is obvious that PA alone is a necessary and sufficient immunogen to protect aaginst anthrax.

Immunization with PA-containing chemical vaccines or PA-producing live vaccines elicits partial or complete protection against anthrax infection. Yet, although PA is unquestionably a protective immunogen, the specific antigenic epitopes on the PA molecule which are responsible for eliciting immunity remain unknown. Furthermore, the molecular interactions of the toxin molecules with each other and with host cells also remain to be elucidated. Leppla et al. (S.H. Leppla, personal communication) have been investigating the functional regions of PA, and they recently reported the results of some molecular studies of the molecule. They proteolyzed the 85-kDa PA molecule with trypsin, generating an N terminus, 20-kDa peptide, and a C terminus, 65-kDa peptide. The peptides were then separated by high pressure liquid chromatography. In vitro experiments indicated that removal of the 20-kDa fragment from PA uncovered a specific binding site for EF and LF on the 65-kDa fragment.

To further delineate the protective, immunogenic regions of the PA molecule, the 65-kDa and 20-kDa peptides, as well as unproteolyzed (intact) PA, and PA which had been trypsinized but not separated into the two peptide fragments, were used to immunize guinea pigs. Each immunogen was either suspended in Freund's incomplete adjuvant or adsorbed onto aluminum hydroxide (Table 3). The guinea pigs were protected from an intramuscular virulent spore challenge by intact PA, trypsin-treated PA, and the 65-kDa fragment. The 20-kDa fragment afforded no protection. Animals that had received incomplete Freund's adjuvant had significantly higher anti-PA titers ($P<0.05$ via analysis of variance) than animals that had been given aluminum hydroxide as an adjuvant. As repeatedly demonstrated however, titers to PA did not correlate strictly with the level of immunity to anthrax.

The goal of current research is to develop an improved human anthrax vaccine. Live vaccines appear to stimulate more effective and longer-lasting immunity than do chemical vaccines. (Potential explanations for this observation were given above). Several approaches are being studied

TABLE 3.
Immunization with PA Fragments *.

Immunization	Survivors/Total	Anti-PA Titers
Saline	1/10	<10
Sterne Spores	9/10	2,512
MDHP-PA	6/9	31,623
PA (20 kDa) - with Aluminum hydroxide	1/10	159
PA (65 kDa) - with Aluminum hydroxide	9/10	17,783
PA (65 kDa) - with Incomplete Freund's adjuvant	10/10	79,433
PA (intact) - with Aluminum hydroxide	10/10	11,220
PA (intact) - with Incomplete Freund's adjuvant	10/10	35,482
PA (trypsin-treated) - with Aluminum hydroxide	7/9	15,849
PA (trypsin-treated) - with Incomplete Freund's adjuvant	9/10	44,669

* Guinea pigs received three biweekly injections of: i) 70 μg PA or PA fragment in aluminum hydroxide or Freund's incomplete adjuvant; ii) 0.5 ml of MDPH-PA, iii) Sterne spores (2 X 10^6 to 5 X 10^6 CFU); or iv) saline. Six weeks after the final immunization, all animals were challenged with 2000 (approximately 20 LD_{50s}) virulent strain spores.

to develop effective, nonreactogenic alternates to the current vaccines. These approaches take advantage of the recently elucidated molecular biology of toxin and capsule production and mode of action, the improved methods developed for purification and assay of toxin, and the techniques developed for genetic analysis and transfer of the toxin-encoding and capsule-encoding plasmids of *B. anthracis* (12, 16, 22, 32, 38, 44, 54, 58, 59).

Salmonella typhimurium aro mutants require aromatic metabolites not available in host tissues, and certain of these mutants are effective, prototype vaccines (19). Recently, we used transposon Tn*916* mutagenesis of a nonencapsulated, toxigenic, Sterne strain of *B. anthracis* to generate two, live, *aro* vaccine candidates (23). Immunization of guinea pigs with two biweekly doses of 10^7 or 10^8 colony-forming units of these *aro* strains afforded complete protection against a challenge 4 weeks later with 2,240 virulent strain spores (G.B. Knudson and S.F. Little, personal communication). Further tests will determine the safety of the two strains as well as their efficacy in comparison to the human and chemical vaccines.

Another approach, based on knowledge of the plasmid locations and DNA sequences of the anthrax toxin genes, is to replace the toxin genes of the nonencapsulated vaccine strains with mutationally altered derivatives. The PA gene and the EF gene are being altered by site-specific mutagenesis in attempts to produce nontoxic, immunologically active proteins (D. Robertson, S. Leppla, unpublished data). If achieved, a safer vaccine could be constructed by replacing the wild-type genes of the Sterne strain with the nontoxic DNA sequences.

Similar techniques could also be used to investigate a role for nontoxin antigens, such as capsule or the surface carbohydrate, in augmenting immune protection against anthrax. Finally, combination vaccines (live, attenuated organisms plus a purified antigen) or conjugate vaccines (e.g., PA conjugated to a *B. anthracis* surface antigen) are considerations for future vaccines.

Adjuvants are also being evaluated to determine their safety and usefulness in a human anthrax vaccine. Most adjuvants known to potentiate the host immune response to a vaccine can provoke toxic, allergic, ulcerative, or lethal reactions. For example, Freund's water-in-oil emulsions cause ulceration and thus are unsuitable for human use (61). The present human vaccines contain alum or aluminum hydroxide. They must be refrigerated, because aluminum-containing vaccines cannot be lyophilized (61). The immunity elicited by these vaccines appears to be suboptimal (36, 57), and aluminum is potentially neurotoxic (41, 64). Thus other adjuvants should be tested for their safety and efficacy in potentiating immunity to anthrax.

Substances which have been developed recently as potential immunomodulators include bacterial products (such as the detoxified lipid A component of gram-negative endotoxin, trehalose dimycolate, and muramyl dipeptide) and dimethylglycine (DMG) (15, 43, 61). Antigens, either alone or combined with an adjuvant of bacterial origin, are sometimes administered in microcapsules, liposomes, or nontoxic oil emulsions to enhance their localization and delivery (61).

The development and testing of both live and chemical vaccine candidates, the examination of new adjuvants to increase immunogenicity and reduce time to immunity, and the elucidation of the molecular basis for specific host immunity to anthrax will require substantial research effort. With the aid of modern biotechnological tools, however, we will hopefully achieve our goal of a safe and more efficacious human vaccine against anthrax.

The views of the authors do not purport to reflect the positions of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the « Guide for the Care and Use of Laboratory Animals », as promulgated by he Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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