species. AVA does not protect hamsters at all [36]. In mice, the PGA capsule appears to be the primary virulence factor, and PA-based vaccines confer only limited protection [37]. There is no direct correlation between anti-PA titers and protection in mice and hamsters [36,37]. In guinea pigs, AVA provides partial protection [35,38,39], but AVA appears to be more effective in rabbit and macaque models [35,40-42]. It is noteworthy that "efficacy", as defined in these studies, is relative. When macaques were exposed experimentally to doses of up to 900 times the LD_{50}, 88–100% of the animals were protected [31]. However, simulation studies suggest that a person opening a letter filled with anthrax spores and standing over it for 10 min could inhale up to 3,000 times and perhaps as much as 9,000 times the LD_{50} for humans [2].

A controlled human trial was conducted in the 1950s with a vaccine similar to AVA but derived from a different attenuated, non-encapsulated strain of *B. anthracis* grown aerobically. In a susceptible population of textile mill workers in the northeastern states of the US who processed occasionally contaminated goat hair, vaccination provided 92.5% protection against cutaneous anthrax [43,44]. However, no assessment of inhalational anthrax could be made, because cases were too few.

There are several concerns regarding AVA: (i) It does not protect all animal hosts against different strains of *B. anthracis*. AVA or PA-based vaccines in general induce toxin-neutralizing antibodies. The mechanism underlying the protective action of PA-based vaccines is unclear. It is thought that anti-PA vaccines protect the host from intoxication and thus allow the immune system to deal with the organism. However, evidence that primates vaccinated with AVA or PA vaccine develop transient episodes of bacteremia suggests that vaccination does not prevent the growth of bacilli. (ii) The administration of AVA is burdensome, requiring subcutaneous injections at 0, 2, and 4 weeks and 6, 12, and 18 months with subsequent yearly boosters [31,32]. In a field trial of a vaccine similar to AVA, one case of cutaneous anthrax occurred 5 months after the initial 3-dose series and just before the scheduled 6-month booster [9]. This case suggests that immunity is not long-lasting and that frequent boosters may be necessary. (iii) The preparative processing of AVA is crude and lacks consistency. Furthermore, there are relatively high rates of local and systemic adverse reactions, likely due to residual toxicity in AVA or other contaminants.

Improvement: highly purified recombinant PA

The limitations of AVA have raised widespread interest in developing improved anthrax vaccines consisting of well-characterized components. A new generation of vaccines based on highly purified recombinant PA is currently being developed and evaluated. There have been numerous attempts to establish high-level PA expression systems based on a variety of organisms, including attenuated strains of *B. anthracis*, *B. subtilis*, *B. brevis*, *Salmonella typhimurium*, *E. coli*, viruses, insect cells, and plants [45-50]. In addition, genetic immunization with DNA encoding for PA is being explored [51].

The highly purified PA vaccines are expected to induce essentially the same immunity as AVA. While some disadvantages of AVA due to its "dirty" preparation may be overcome, limitations in protection and lack of an immune memory response may be intrinsic to PA itself.

New strategies are needed for further improvement. One possibility is that other antigens or cellular immunity in addition to PA-specific antibodies are required for full protection in different animal species. This is supported by studies showing that the live veterinary vaccine provides significantly greater protection against anthrax in experimental animals than does AVA, despite the fact that it frequently induces lower levels of antibodies to PA [33,39,42,52,53]. After a naturally acquired infection, and depending on when samples are taken, 68–93% of cases develop antibodies to PA, 42–55% of cases develop antibodies to LF, and antibodies to EF are less frequently detected [34,54-56]. Interestingly, antibodies to the capsule are detected in 67–94% cases [55,56], whereas no response to the capsule is expected in the vaccinees who have been vaccinated with AVA or non-encapsulated live vaccines.

Further improvement: two-in-one postexposure antitoxic therapy/vaccine

Post-exposure vaccination is the most likely scenario, given the rarity of natural inhalational anthrax infection. However, the use of PA as a postexposure vaccine may be limited. Since PA is a natural component of anthrax toxin and may contribute to toxin formation, it may not be safe to administer a PA-based vaccine to persons who have been or are suspected of having been exposed to anthrax. We recently proposed the replacement of PA in vaccines with a dominant-negative inhibitor (DNI) of anthrax toxin [57]. DNI is a translocation-deficient mutant of PA carrying double mutations of K397D and D425K and has been demonstrated to interfere with the intoxication process, providing immediate therapeutic protection against anthrax toxin in vivo [58,59]. Furthermore, when used as a vaccine, DNI is more immunogenic than PA [57].

The symptoms and incubation period of human anthrax vary depending on the route of transmission. The reported incubation period of inhalational anthrax, the most lethal form, ranges from 1 to 43 days [60]. Data from animal studies suggest that anthrax spores persist in the host for several weeks after infection and that antibiotics can