# Prevention and treatment of bacterial diseases caused by bacterial bioterrorism threat agents

Ronald A. Greenfield and Michael S. Bronze

**There is general consensus that the bacterial agents or products most likely to be used as weapons of mass destruction are *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* and the neurotoxin of *Clostridium botulinum*. Modern supportive and antimicrobial therapy for inhalational anthrax is associated with a 45% mortality rate, reinforcing the need for better adjunctive therapy and prevention strategies. Pneumonic plague is highly contagious, difficult to recognize and is frequently fatal. Therefore, the development of vaccines against this agent is crucial. Although tularemia is associated with low mortality, the highly infectious nature of aerosolized *F. tularensis* poses a substantive threat that is best met by vaccine development. Safer antitoxins and a vaccine are required to meet the threat of the use of botulinum toxin as a weapon of mass destruction. In this article, the current status of research in these areas is reviewed.**

**Ronald A. Greenfield***
**Michael S. Bronze**
The Infectious Diseases Section
Department of Medicine
University of Oklahoma Health Sciences Center
& The Oklahoma City Veterans Administration Medical Center
Oklahoma City
OK 73190, USA
*e-mail: Ronald-Greenfield@ouhsc.edu

▼ Multinational organizations and military and public health authorities of many nations have identified anthrax, plague, tularemia and botulism as the most probable consequences of a biological attack employing bacterial agents or the products of bacterial agents as weapons of mass destruction. Although they are responsible for very different illnesses, the causative agents of these diseases share availability, ease of production, an ability to cause widespread disease by aerosol attack, and for the live organisms, the potential for naturally occurring or genetically engineered resistance to currently available antimicrobial therapy. Effective biodefense includes antimicrobial, adjunctive and/or antitoxin therapy and, ideally, universally administered safe and effective pre-exposure prophylaxis, or at least effective post-exposure prophylaxis. This review describes the current therapy and prevention of these illnesses, together with areas of active investigation for the development of more effective treatment agents and effective vaccines for pre-exposure prophylaxis.

## Anthrax

Anthrax has long been considered the most probable bioweapon-induced disease. Bioterrorism was achieved by distribution of *Bacillus anthracis* spores through the postal system in the US in the autumn of 2001, resulting in cases of cutaneous and inhalational anthrax [1]. Anthrax occurs as a result of infection with *B. anthracis* and intoxication with the toxins of *B. anthracis*. The observations that the mortality associated with inhalational anthrax was 45% [1] and that all patients who exhibited toxemia before the initiation of appropriate antimicrobial therapy died despite such therapy [2] make it crucial to review the status of anthrax treatment and prevention.

### *Antimicrobial and adjunctive therapy*

There are three clinical forms of anthrax: cutaneous, inhalational and gastrointestinal. Ciprofloxacin has become a treatment of choice for all clinical forms of anthrax (Table 1) [1,3]. This fluoroquinolone has become the treatment of choice owing to potential resistance to penicillins (the classical therapeutic agents) and tetracyclines. *B. anthracis* possesses a penicillinase gene and inducible, low-level production of its product has been demonstrated [4]. *B. anthracis* also contains a constitutive cephalosporinase [4]. Furthermore, a key phase of *B. anthracis* multiplication is intracellular and β-lactam antibiotics penetrate poorly into macrophages [4]. Additionally, it is reported that the former Soviet Union engineered penicillin and tetracycline resistant *B. anthracis*, or possibly developed antimicrobial resistant

1359-6446/03/$ – see front matter  PII: S1359-6446(03)02847-2 

**Table 1. Current therapy, prophylaxis and investigational approaches for anthrax**

| Type of therapy | Primary | Alternative (s) | Adjunctive therapy | Investigational prospects |
|---|---|---|---|---|
| **Anthrax** | | | | |
| Therapy for cutaneous anthrax | Ciprofloxacin[a] or doxycycline[b] [1,3] | Amoxicillin if susceptibility is established<br>Levofloxacin [7]<br>Gatifloxacin [7,8]<br>Other tetracyclines | Corticosteroids if extensive edema [1] | Polyclonal antibody [14]<br>Synthetic, polymeric, polyvalent inhibitor of reaction of PA with LF [15]<br>Dominant negative PA mutants [16,17]<br>PlyG lysin [18]<br>Yeast β-1,3 glucan immune modulators [19]<br>Recombinant antibody to PA fragments [23] |
| Therapy for inhalational anthrax | Ciprofloxacin[c] or Doxycycline[d] plus 1–2 additional antimicrobial agents[e] with established or expected activity Against *B. anthracis* [1–3] | Other fluoroquinolone or tetracycline class antimicrobial plus 1–2 additional agents[e] | Supportive therapy (intravascular volume repletion, vasopressors, ventilatory support) as needed.<br>Thoracostomy drainage of pleural effusions if present [1–3]<br>Corticosteroids if serious neck or mediastinal edema or meningitis | |
| Therapy for gastrointestinal anthrax | As for inhalational anthrax | As for inhalational anthrax | | |
| Pre-exposure prophylaxis | US and UK cell-free culture filtrate vaccines [1] | Live attenuated spore vaccine has been used in countries of the former USSR | | Polyclonal antibody to PA [14]<br>Recombinant antibody to PA fragments [23]<br>Recombinant PA encapsulated and surface bound to polymeric microspheres [24]<br>Immunization with dominant negative PA mutants [16,17]<br>VEE virus-vectored PA vaccine [25] |
| Post-exposure prophylaxis | Presumptive therapy with ciprofloxacin[c] or doxycycline[d] | | | Presumptive therapy plus PA-based vaccine [28] |

[a]Ciprofloxacin 500 mg (15 mg/kg not to exceed 500 mg if ≤8 yrs or <45 kg) PO q12h for ≥60 days (this and all subsequent doses are those for individuals with normal drug excretory organ function. Consult appropriates sources for dose modification for individuals with abnormal excretory function)
[b]Doxycycline 100 mg (2.2 mg/kg not to exceed 500 mg) if ≤8 yrs or <45 kg) PO q12h. >60 days
[c]Ciprofloxacin 400 mg (10 mg/kg not to exceed 400 mg if ≤8 yrs or <45 kg) IV q12h with switch to oral therapy when clinically appropriate, total duration ≥60 days
[d]Doxycycline dosed as for cutaneous anthrax but administered IV, with switch to oral therapy when clinically appropriate, total duration ≥60
[e]Penicillin G, clindamycin, rifampin or chloramphenicol
Abbreviations: LF, lethal factor; PA, protective antigen; PlyG lysin, a lysin derived from γ phage of *B.anthracis*

*B. cereus* or *B. subtilis* and transformed these organisms to pathogenicity by incorporating the virulence plasmids of *B. anthracis* [5]. There has been no evidence of naturally occurring fluoroquinolone resistance, although laboratory subculturing and multiple cell passage has led to *in vitro* development of ofloxacin resistance [6].

Among the fluoroquinolones, ciprofloxacin is the agent of choice because of demonstrated efficacy in a primate

model [1]. Based on *in vitro* susceptibility and pharmacodynamic modeling, levofloxacin and gatifloxacin are expected to be therapeutically equivalent to ciprofloxacin [7], and trovafloxacin, moxifloxacin and gemifloxacin are probably also effective. Trovafloxacin and gatifloxacin possess *in vivo* activity in a murine model [8]. Doxycycline is the preferred agent among the tetracycline class because of its demonstrated efficacy in primates and ease of administration [1].

Cutaneous anthrax therapy is usually of seven to ten days duration. In the bioweapon setting, however, therapy should be continued for ≥60 days as post-exposure prophylaxis against possible concurrent inhalation exposure [1]. If susceptibility of the epidemic strain is documented, amoxicillin is the preferred therapy for pregnant or lactating women and for children <18 years of age [1].

The recommended therapy for inhalational anthrax (Table 1) is also ciprofloxacin or doxycycline, but administered intravenously [1,3]. Furthermore, it is recommended that one or two additional antimicrobial agents with activity against *B. anthracis* are simultaneously administered [1,3]. Limited data from the recent US outbreak indicate that treatment with two or more active antimicrobial agents improved survival [2]. Reasonable agents for this role are penicillin G, clindamycin, rifampin and chloramphenicol (Table 1). There are no clear data regarding choice of these additional agents. When there is comorbid meningitis, ciprofloxacin is preferred over doxycycline, and penicillin, rifampin and/or chloramphenicol might be preferred because of their established roles in the treatment of meningitis caused by other pathogens [1]. Clindamycin could have a particular role in the treatment of anthrax in that, as an inhibitor of protein synthesis, it might limit toxin production more quickly or effectively than either β-lactam or fluoroquinolone agents, as has been demonstrated for another gram-positive bacterium, *Streptococcus pyogenes* [9].

Adjunctive therapy is essential for the management of a patient with inhalational anthrax. Provision of physiologic supportive therapy is crucial. Thoracostomy tube drainage of pleural effusion and empyema is associated with clinical improvement [2]. Corticosteroid therapy has been recommended for serious neck or mediastinal edema or meningitis [1]. The role of modern anti-inflammatory strategies, such as antibody to tumor necrosis factor or nitric oxide scavengers [10], in patients with overwhelming septicemia is currently unexplored, but is an intriguing prospect.

The duration of parenteral therapy for inhalational anthrax is determined by the clinical response. The total duration of therapy, completed with either ciprofloxacin or oral doxycycline, should be ≥60 days. This is because the disease might be initiated again by persisting, originally inhaled *B. anthracis* spores because these are not killed by antimicrobial therapy [1]. In addition, early antimicrobial therapy might abrogate the establishment of host immunity [11].

### *Novel therapies in development*

The production of toxins by *B. anthracis* is crucial to pathogenesis. In fact, animal studies suggest a 'point of no return' in this intoxication, after which death is inevitable, even if all *B. anthracis* are killed. Therapeutic approaches that are in development focus on interrupting production of the anthrax toxins. To discuss this, the pathogenesis of anthrax intoxication must be first briefly reviewed.

The PX01 plasmid of *B. anthracis* encodes genes for what is classically described as a tripartite exotoxin complex, but is better described as precursors [edema factor (EF) and lethal factor (LF)] of the two principal toxins, and a delivery module [protective antigen (PA)]. PA initially binds the target cell membrane and is then 'trimmed' to PA63 by a cellular furin-like protease. PA63 then drifts in the membrane until contact is made with six additional PA63 monomers. These seven identical protein units then link together into a ring that serves as the delivery module. This heptamer contains a high-affinity binding site that binds either EF or LF. The ring and the factor are then internalized into the cell by receptor-mediated endocytosis. The endocytic vacuole is then fused to an endosome. During normal cellular function, this endosome acidifies, resulting in a conformational change in the PA63 heptamer, which now forms a transmembrane pore. The associated factor is then delivered through this pore into the cell cytoplasm, now as either edema toxin (ET) or lethal toxin (LT). ET is a calcium- and calmodulin-dependant adenylate cyclase, the activity of which leads to edema and compromises neutrophils. LT is principally responsible for the toxemia of anthrax. LT is a zinc metalloprotease that cleaves key cellular protein kinases, causing the death of macrophages, resulting in the release of proinflammatory mediators.

Because of the central role of PA in pathogenesis, treatment directed at interrupting toxin effects has focused on rendering PA non-functional. Furthermore, of the several currently available antibodies to *B. anthracis* and its toxins, investigators have found anti-PA to be the most effective in treatment [12,13]. Several studies have demonstrated the effectiveness of passive immunization with polyclonal antibodies to PA in the treatment of rodent and primate models of anthrax [14]. However, because human anti-PA antibodies are not readily available (although they could perhaps be obtained from vaccinated military personnel), and passive immunization with antibodies developed in some other species would be complicated by potential hypersensitivity reactions, modern techniques have been applied to the search for PA inhibitors. Mourez *et al.* [15] developed a polyvalent inhibitor of LT action in a rat model by developing a peptide that binds to PA

and prevents its interaction with EF and LF, and covalently linking multiple copies of this peptide to a flexible backbone. Two groups have engineered dominant negative mutants of PA [16,17]. When incorporated into the heptameric pre-pore formed by PA, these dominant negative mutants prevent the pre-pore from converting into a functional membrane pore and delivering ET and LT intracellularly to their site of action. These approaches could have therapeutic potential.

Two approaches to anthrax therapy unrelated to PA inhibition have also been advanced. Schuch and colleagues [18] demonstrated the therapeutic effect of a lysin derived from γ phage of *B. anthracis* (PlyG lysin) in infections in closely related *Bacillus* spp. in mice. They were able to purify the lytic enzyme of this naturally occurring, commonly used bacteriophage γ and demonstrated *in vivo* therapeutic efficacy after intravenous administration. Kournikakis *et al.* [19] demonstrated the therapeutic efficacy of yeast β-1,3 glucan immunomodulators in a murine model of anthrax. β-1–3 glucans are believed to act by enhancing macrophage and neutrophil function.

### *Pre-exposure prophylaxis*

Pre-exposure prophylaxis for anthrax currently involves vaccination [20]. As its name implies, the dominant immunogen that affords protection is PA. US and UK cell-free culture (of a non-encapsulated *B. anthracis* strain) filtrate vaccines are currently licensed, but are available only to military personnel. These vaccines are highly effective [21]. Mild local reactions occur in ~30% of vaccinees, but systemic reactions occur in <0.2% of recipients [22]. A major drawback of these vaccines is the need for an initial series of multiple injections and annual booster immunizations. Therefore, work is in progress to develop better vaccines using modern vaccine strategies.

Short-to-intermediate term protection has been achieved in rodent models by passive immunization with polyclonal antibody to PA [14]. Maynard and co-workers [23] developed recombinant PA antibody fragments that were protective in mice.

Active immunization strategies are expected to confer longer-term protection. In this regard, the dominant negative mutants previously described were found to lead to development of anti-PA antibodies in rodents [16,17]. Flick-Smith *et al.* [24] associated recombinant PA with microspheres and found that mucosal or parenteral administration resulted in antibody production and provided protection to mice. Recently, Lee and colleagues [25] engineered a DNA subunit PA vaccine, inserted it into a Venezuelan equine encephalitis virus vector, and demonstrated protection of mice from anthrax infection by administration of this product.

### *Post-exposure prophylaxis*

Post-exposure prophylaxis for anthrax is presumptive therapy for those with a high risk of having sustained exposure. Ciprofloxacin or doxycycline is recommended in full therapeutic doses for 60–100 days [1,3]. Data pertinent to the duration of post-exposure prophylaxis reveal that human anthrax occurred 43 days after spore release in the Sverdlovsk accident [26] and that traces of spores were present in monkeys for up to 100 days after inhalational exposure [27]. Vaccination in combination with antimicrobial therapy has been considered for post-exposure prophylaxis – there is recent experimental support in guinea pigs for this approach [28].

During the 2001 US outbreak, only 44% of potentially exposed postal workers reported full adherence to a 60-day course of antimicrobial prophylaxis [29,30]. Nonetheless, no anthrax occurred in the group given post-exposure prophylaxis and an estimated nine cases of human anthrax were prevented [31]. At least one adverse event was encountered in 77% of ciprofloxacin-treated and 71% of doxycycline-treated individuals ($P<0.01$), and, overall, 16% of individuals sought medical attention for an adverse event [30]. These problems with adherence to and tolerability of post-exposure antimicrobial prophylaxis highlight the need for improved methods for pre-exposure prophylaxis.

## Plague

The causative agent of plague is *Yersinia pestis*. Plague occurs in classical bubonic, pneumonic and septicemic forms. *Y. pestis* is a gram-negative bacterium, and the septicemic manifestations of plague infection are believed to result from gram-negative endotoxemia [32].

Streptomycin or gentamicin is considered the therapy of choice for plague (Table 2) [32]. Because US supplies of streptomycin are limited, gentamicin might be the preferred therapy. *In vitro* studies [33] and a murine trial [34] demonstrate the comparable or superior activity of gentamicin compared with streptomycin, and gentamicin has been successfully used in the treatment of human plague [35]. The well-known nephrotoxicity and ototoxicity of these aminoglycosides are a major concern. Additionally, parenteral therapy for all exposures in a mass-casualty scenario will probably overwhelm attempts to supply and administer such therapy.

Doxycycline is a recommended alternative therapy for patients who cannot be given aminoglycosides [32]. The fluoroquinolones are also a reasonable alternative therapy, based on murine-model trials in which efficacy similar to that of aminoglycosides was demonstrated [34,36]. Ciprofloxacin is preferred because it is the most studied, and at least one case of successful treatment of human plague

**Table 2. Current therapy, prophylaxis and investigational approaches for plague, tularemia and botulism**

| Type of therapy | Primary | Alternative (s) | Adjunctive therapy | Investigational prospects |
|---|---|---|---|---|
| **Plague** | | | | |
| All clinical forms | Streptomycin[a] **or** gentamicin[b] | Doxycycline[c] [32]<br>Ciprofloxacin[d] [34,37]<br>Chloramphenicol for plague meningitis[e] [32] | Supportive therapy for endotoxemia as needed<br>Sympathetic blockade for acral gangrene [37] | Passive immunization with monoclonal antibodies [14] |
| Pre-exposure prophylaxis | None available in US other than strict isolation of patients with primary or secondary pneumonic plague.<br>Killed cell vaccine possibly available in UK [40] | | | Recombinant subunit vaccines containing F1 and V antigens [39,43]<br>DNA vaccine coding for derivatives of the F1 antigen [44] |
| Post-exposure prophylaxis | Presumptive therapy with doxycycline | Ciprofloxacin<br>Chloramphenicol | | |
| **Tularemia** | | | | |
| Therapy for all clinical forms | Streptomycin[a] **or** gentamicin[b] [46] | Doxycycline<br>Chloramphenicol<br>Ciprofloxacin or other fluoroquinolones | | |
| Pre-exposure prophylaxis | US live attenuated vaccine for high-risk military personnel | | | Live attenuated vaccine [45]<br>Outer membrane proteins subunit vaccine [51,53]<br>Lipopolysaccharide subunit vaccine [49,50] |
| Post-exposure prophylaxis | Doxycycline for 14 days, **or** ciprofloxacin for 10 days [46] | | | |
| **Botulism** | | | | |
| Therapy for all clinical forms | US licensed AB equine antitoxin with or without investigational E equine antitoxin[f] **or** US investigational heptavalent equine antitoxin[f] [54] | | | Monoclonal or oligoclonal antitoxins [55] |
| Pre-exposure prophylaxis | US investigational pentavalent botulinum toxoid vaccine for high-risk military personnel and laboratory workers [54] | | | Recombinant toxoid vaccines [56] |
| Post-exposure | Equine antitoxin generally not recommended<br>Careful medical scrutiny | | | Monoclonal or oligoclonal antioxins [55] |

[a]Streptomycin 15 mg/kg IM q12h
[b]Gentamicin 5 mg/kg IV or IM qd
[c]Ciprofloxacin 500 mg (15 mg/kg not to exceed 500 mg if ≤8 yrs or ≤45 kg) PO q12h
[d]Doxycycline 100 mg (2.2 mg/kg not to exceed 500 mg) if ≤8 yrs or ≤45 kg) PO q12h
[e]Chloramphenicol 25 mg/kg q6h
[f]Because specific dosage and administration guidelines for these products can vary, consult instructions for specific product

with ciprofloxacin has been reported [37]. Although there are no substantiating clinical trials, chloramphenicol has been used to treat human plague and has been recommended for the treatment of plague meningitis because of its known activity against meningitis caused by other pathogens [32]. β-lactam antibiotics are not effective in treating plague [32]. Treatment for plague should be continued for ten days [32].

There is concern that a biological attack with plague might employ a natural or engineered antimicrobial-resistant strain. In the former regard, natural resistance of *Y. pestis* to the antimicrobials used for therapy is very rare, but a 1995 isolate from Madagascar contained a transferable multidrug resistance plasmid. This strain was resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, spectinomycin, sulfonamides, tetracycline and minocycline [38]. There are reports that the bioweapons operations of the former Soviet Union engineered multidrug resistant and fluoroquinolone resistant *Y. pestis* [5,32]. Therefore, all isolates of *Y. pestis* should be submitted for antimicrobial susceptibility testing and the results should guide further antimicrobial therapy selection.

Supportive therapy is crucial to the management of severe plague. Modern anti-inflammatory strategies [10] for the treatment of the endotoxemia of plague are untested, but intriguing. Passive immunization with monoclonal antibodies has been demonstrated as an effective treatment for plague in murine models [14,39].

There is no currently available pre-exposure prophylaxis for plague. Hospitalized patients with known or suspected plague pneumonia should be placed in strict isolation for at least 48 h after appropriate antimicrobial therapy is initiated. A disposable surgical mask is adequate for preventing transmission of plague by respiratory droplets and should be used by healthcare workers caring for such patients [32], but N-95 masks for respiratory protection would be prudent for unidentified severe epidemic respiratory illness.

There is no currently available vaccine for plague in the US. A formalin-killed whole-cell vaccine was licensed in the US for military personnel and researchers but has been withdrawn after studies found that protection was limited to bubonic plague (no protection was afforded for pneumonic plague) [32]. A similar vaccine (with a similar deficiency) is available for researchers in the UK, Canada and Australia [40].

Efforts are under way to develop a vaccine that is effective against both bubonic and pneumonic plague. Strategies have focused on providing for the development of immunity to the fraction 1 capsular protein (F1) and the V antigen. Both of these proteins are thought to be involved in protecting *Y. pestis* from macrophage uptake [40]. Immunization with either protein provides protection against pneumonic or bubonic plague in animal models, but greater than additive protection is achieved when F1 and V (LcrV) antigen are combined [41]. Furthermore, F1 negative strains exist and have caused human disease [42]. One vaccine under development at the Defence Science and Technology Laboratory at Porton Down (http://www.mod.uk), the UK military laboratory, is a recombinant protein-based vaccine composed of two parts F1 and one part V. This vaccine has demonstrated efficacy in murine models and safety in human trials [39]. Similarly, investigators at the US Army Medical Research Institute of Infectious Diseases (USAMRIID; http://www.usamriid.army.mil) have developed a recombinant capsular F1-V antigen fusion-protein vaccine and established its protective efficacy against bubonic and pneumonic plague in mice [43]. Finally, investigators at the Israel Institute for Biological Research (http://www.iibr.gov.il) reported the development of a DNA vaccine coding for a derivative of F1 (F1 devoid of its putative signal peptide) and demonstrated its efficacy against bubonic plague [44].

Post-exposure prophylaxis for plague should be administered to individuals with close contact (<2 m) with an infectious case, or who have had a potential respiratory exposure. The recommended regimen is oral doxycycline or ciprofloxacin, administered, as for therapy, for seven days [32]. In a community experiencing a pneumonic plague epidemic, individuals with a temperature ≥38.5°C or a new cough should promptly receive therapy, preferably with parenteral gentamicin [32].

## Tularemia

Tularemia results from infection with *Francisella tularensis*, a small, gram-negative coccobacillus that is an intracellular pathogen [45]. *F. tularensis* lipopolysaccharide (LPS) does not exhibit the properties of a classical endotoxin [45]. Tularemia occurs in six forms: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic and typhoidal. Although tularemia does not produce mortality rates as high as anthrax or plague, it is highly contagious and could be used to produce substantial morbidity and panic [46].

Recommended therapy for all clinical forms of tularemia is a seven-day course of streptomycin or gentamicin (Table 2) [46]. Doxycycline or fluoroquinolones (for ten days) have traditionally been considered an alternative therapy [46]. However, in a non-randomized comparison of streptomycin (1 gm/24 h for seven to ten days) and ciprofloxacin therapy (750 mg orally twice daily for 14–28 days) for treatment of infection with an epidemic strain of *F. tularensis* serovar *palearctica*, ciprofloxacin therapy was more effective [47]. β-lactam antibiotics and macrolides are ineffective in treating tularemia.

There is no currently available pre-exposure prophylaxis for tularemia. A live attenuated [live vaccine strain (LVS)] tularemia vaccine, administered by scarification, is being studied under investigational new drug protocols at USAMRIID. The development of LVS has been recently reviewed [45]. It has been reported to reduce the incidence of and attenuate the illness incurred after exposure to *F. tularensis* by various routes. DynPort Vaccine Company LLC (http://www.dynport.com) has been contracted to develop, manufacture, test and license a LVS vaccine in the US with a target completion date of 2009 [48]. It might, however, still produce side-effects owing to the presence of LPS.

Efforts are also in progress to engineer a stable and safe subunit vaccine for tularemia. One approach is immunization with modified *F. tularensis* LPS, which can be safely administered to humans in an immunogenic dose and has demonstrated efficacy in protection in rodent models [49,50]. Another approach has been recognition and development of outer membrane protein antigens that elicit humoral and T-cell immunity [51–53], both of which seem required for full protective benefit to be achieved by subunit vaccines [45]. It is likely that a subunit vaccine will be composed of several protective antigens [45].

## Botulism

Botulism is a condition resulting from intoxication with the neurotoxin of *Clostridium botulinum* (BoNT). There are seven serological types of BoNT, types A-G, with A, B and E the most common naturally occurring types [54].

Therapy of BoNT intoxication involves supportive care and neutralization of BoNT effects. To date, specific antibodies are the only substances known to neutralize BoNT. Specific antibodies neutralize circulating unbound toxin, but not cell-bound toxin. Passive immunization with antitoxin antibodies is key to treating botulism. Prompt administration of antitoxin does not reverse existing paralysis, but it does minimize subsequent nerve damage and thus limits the severity of the disease [54].

In the US, a licensed bivalent (AB) equine antitoxin and an investigational anti-E equine antitoxin are available; both should be administered if the serological type is unknown [54]. The US government also maintains a limited supply of pentavalent (ABCDE) antitoxin product for use in emergencies [54]. In general, hypersensitivity to horse serum is first assessed by administration of a small dose by scratch test. If positive for substantial wheal and flare response, desensitization is required before administration of therapeutic doses. The antitoxin, mixed in physiologic saline, is administered by slow intravenous infusion, in accordance with manufacturer's recommendations. Epinephrine, diphenhydramine and corticosteroids should be readily available for intravenous administration. Major hypersensitivity reactions (e.g. anaphylaxis, urticaria, serum sickness) and minor hypersensitivity reactions are the major toxicities of these equine antisera, although precise incidence data are not available [54]. A variety of monoclonal antitoxin antibodies are in development [14] with the intention of producing therapies that will obviate hypersensitivity reactions.

As with diphtheria, the logical method for pre-exposure prophylaxis for botulism is vaccination with toxoid. In the US, an investigational heptavalent (ABCDE) toxoid vaccine is available for use by military personnel and laboratory workers at high risk of exposure [54]. Several recombinant toxoid vaccines are in development [55]. Because toxoid induces immunity over a period of weeks to months, this strategy is not suitable for post-exposure prophylaxis [54]. There would be a unique potential consequence of universal vaccination for BoNT, namely rendering therapeutic and cosmetic use of BoNT no longer tenable.

Careful medical scrutiny for initial symptoms and signs of botulism is the recommendation for post-exposure prophylaxis. Antitoxin is generally not recommended for post-exposure prophylaxis because of the toxicity [54]. However, the development of safe and effective antitoxins would lead to agents with a likely role in post-exposure prophylaxis.

## Conclusions

*B. anthracis*, *Y. pestis*, *F. tularensis* and BoNT are capable of producing wide-spread illness and terror if employed as weapons of mass destruction. Although current therapies possess substantial efficacy, there remain issues with availability of resources, toxicity of therapies and the potential for terrorists to employ or develop agents highly resistant to available therapeutic agents. Current research efforts into improved therapy involve the application of information from the sequencing of the genomes of these agents and the molecular biological detailing of the mechanisms by which they produce human illness. New treatment strategies have been defined and agents are in development. These data should enable the development of safe and effective prevention strategies suitable for universal application.

## References

1 Inglesby, T.V. *et al.* (2002) Anthrax as a biological weapon. Updated recommendations for management. In *Bioterrorism. Guidelines for Medical and Public Health Management.* (Henderson, D.A. *et al.*, eds), pp. 63–97, American Medical Association Press

2 Jernigan, J.A. *et al.* (2001) Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States. *Emerg. Infect. Dis.* 7, 933–944

3 Bartlett, J.G. *et al.* (2002) Management of anthrax. *Clin. Infect. Dis.* 35, 851–858

4 Bell, D.M. *et al.* (2002) Conference summary: clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. *Emerg. Infect. Dis.* 8, 222–225

5 Alibek, K. and Handelman, S. (1999) *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World*, Random House
6 Choe, C. *et al.* (2000) *In vitro* development of resistance to ofloxacin and doxycycline in *Bacillus anthracis* Sterne. *Antimicrob. Agents Chemother.* 44, 1766
7 Bryskier, A. (2002) *Bacillus anthracis* and antibacterial agents. *Clin. Microbiol. Infect.* 8, 467–478
8 Elliott, T.B. *et al.* (2002) Antimicrobial therapy for *Bacillus anthracis* - induced polymicrobial infection in $^{60}$Co γ-irradiated mice. *Antimicrob. Agents Chemother.* 46, 3463–3471
9 Steigbigel, N.H. (2000) Macrolides and clindamycin. In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (Mandell, G.L. *et al.*, eds), pp. 366–382, Churchill Livingston
10 Riedemann, N.C. and Ward, P.A. (2003) Anti-inflammatory strategies for the treatment of sepsis. *Expert Opin. Biol. Ther.* 3, 339–350
11 Friedlander, A.M. *et al.* (1993) Postexposure prophylaxis against experimental inhalation anthrax. *J. Infect. Dis.* 167, 1239–1243
12 Little, S.F. *et al.* (1997) Passive protection by polyclonal antibodies against *Bacillus anthracis* infection in guinea pigs. *Infect. Immun.* 65, 5171–5175
13 Kobiler, D. *et al.* (2002) Efficiency of protection of guinea pigs against infection with *Bacillus anthracis* spores by passive protection. *Infect. Immun.* 70, 544–550
14 Casadevall, A. (2002) Passive antibody administration (immediate immunity) as a specific defense against biological weapons. *Emerg. Infect. Dis.* 8, 833–841
15 Mourez, M. *et al.* (2001) Designing a polyvalent inhibitor of anthrax toxin. *Nat. Biotechnol.* 19, 958–961
16 Sellman, B.R. *et al.* (2001) Dominant negative mutants of a toxin subunit: an approach to therapy of anthrax. *Science* 292, 695–697
17 Singh, Y. *et al.* (2001) A dominant negative mutant of *Bacillus anthracis* protective antigen inhibits anthrax toxin action *in vivo*. *J. Biol. Chem.* 276, 22090–22094
18 Schuch, R. *et al.* (2002) A bacteriolytic agent that detects and kills *Bacillus anthracis*. *Nature* 418, 884–889
19 Kournikakis, B. *et al.* (2003) Anthrax - protective effects of yeast Beta 1,3 glucans. *MedGenMed* 5, 1
20 Friedlander, A.M. *et al.* (2002) Anthrax vaccines. *Curr. Top. Microbiol. Immunol.* 271, 33–60
21 Brachman, P.S. *et al.* (1962) Field evaluation of a human anthrax vaccine. *Am. J. Public Health* 52, 632–645
22 Friedlander, A.M. *et al.* (1999) Anthrax vaccine: evidence for safety and efficacy against inhalational anthrax. *J.A.M.A.* 282, 2104–2106
23 Maynard, J.A. *et al.* (2002) Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. *Nat. Biotechnol.* 20, 597–601
24 Flick-Smith, H.C. *et al.* (2001) Mucosal or parenteral administration of microsphere-associated *Bacillus anthracis* protective antigen protects against anthrax infection in mice. *Infect. Immun.* 70, 2022–2028
25 Lee, J.S. *et al.* (2003) Venezuelan equine encephalitis virus-vectored vaccines protect mice against anthrax spore challenge. *Infect. Immun.* 71, 1491–1496
26 Guillemin, J. (1999) *Anthrax: the Investigation of a Deadly Outbreak*, University of California Press
27 Henderson, D.W. *et al.* (1956) Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. *J. Hyg.* 54, 28–36
28 Altboum, Z. *et al.* (2002) Postexposure prophylaxis against anthrax: evaluation of various treatment regimens in intranasally infected guinea pigs. *Infect. Immun.* 70, 6231–6241
29 Jefferds, M.D. *et al.* (2002) Adherence to antimicrobial inhalational anthrax prophylaxis among postal workers, Washington, D.C., 2001. *Emerg. Infect. Dis.* 8, 1138–1144
30 Shepard, C.W. *et al.* (2002) Antimicrobial post-exposure prophylaxis for anthrax: adverse events and adherence. *Emerg. Infect. Dis.* 8, 1124–1132
31 Brookmeyer, R. and Blades, N. (2002) Prevention of inhalation anthrax in the US outbreak. *Science* 295, 1861
32 Inglesby, T.V. *et al.* (2000) Plague as a biological weapon: medical and public health management. *J.A.M.A.* 283, 2281–2290
33 Smith, M.D. *et al.* (1995) *In vitro* antimicrobial susceptibilities of strains of *Yersinia pestis*. *Antimicrob. Agents Chemother.* 39, 2153–2154
34 Byrne, W.R. *et al.* (1998) Antibiotic treatment of experimental pneumonic plague in mice. *Antimicrob. Agents Chemother.* 42, 675–681
35 Crook, L.D. and Tempest, B. (1992) Plague: a clinical review of 27 cases. *Arch. Intern. Med.* 152, 1253–1256
36 Russell, P. *et al.* (1998) The efficacy of ciprofloxacin and doxycycline against experimental tularaemia. *J. Antimicrob. Chemother.* 41, 461–465
37 Kuberski, T. *et al.* (2003) A case of plague successfully treated with ciprofloxacin and sympathetic blockade for treatment of gangrene. *Clin. Infect. Dis.* 36, 521–523
38 Galimand, M. *et al.* (1997) Multidrug resistance in *Yersinia pestis* mediated by a transferable plasmid. *New Engl. J. Med.* 337, 677–680
39 Williamson, E.D. (2001) Plague vaccine research and development. *J. Appl. Microbiol.* 91, 606–608
40 Nierengarten, M.B. and Lutwick, L. (2002) Vaccine development for plague. *Medscape Infectious Diseases* 4, 2
41 Williamson, E.D. *et al.* (1995) A new improved subunit vaccine for plague - the basis of protection. *FEMS Immunol. Med. Microbiol.* 12, 223–230
42 Friedlander, A.M. *et al.* (1995) Relationship between virulence and immunity as revealed in recent studies of the F1 capsule of *Yersinia pestis*. *Clin. Infect. Dis.* 21(Suppl. 2), S178–S181
43 Heath, D.G. *et al.* (1998) Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine. *Vaccine* 16, 1131–1137
44 Grosfeld, H. *et al.* (2003) Effective protective immunity to *Yersinia pestis* infection conferred by DNA vaccine coding for derivatives of the F1 capsular antigens. *Infect. Immun.* 71, 374–383
45 Ellis, J. *et al.* (2002) Tularemia. *Clin. Microbiol. Rev.* 15, 631–646
46 Dennis, D.T. *et al.* (2002) Tularemia as a biological weapon. In *Bioterrorism: Guidelines for Medical and Public Health Management* (Henderson, D.A. *et al.*, eds), pp. 167–189, American Medical Association Press
47 Perez-Castillon, J.L. *et al.* (2001) Tularemia epidemic in northwestern Spain: clinical description and therapeutic response. *Clin. Infect. Dis.* 33, 573–576
48 Nierengarten, M.B. and Lutwick, L. (2002) Developing new tularemia vaccines. *Medscape Infectious Diseases* 4, 1
49 Dreisbach, V.C. *et al.* (2000) Purified lipopolysaccharide from *Francisella tularensis* live vaccine strain (LVS) induces protective immunity against LVS infection that requires B cells and gamma interferon. *Infect. Immun.* 68, 1988–1996
50 Fulop, M. *et al.* (2001) Role of antibody to lipopolysaccharide in protection against low- and high-virulence strains of *Francisella tularensis*. *Vaccine* 19, 4465–4472
51 Surcel, H.M. *et al.* (1989) Membrane proteins of *Francisella tularensis* LVS differ in ability to induce proliferation of lymphocytes from tularemia-vaccinated individuals. *Microb. Pathog.* 7, 411–419
52 Sjostedt, A. *et al.* (1992) Humoral and cell-mediated immunity in mice to a 17-kilodalton lipoprotein of *Francisella tularensis* expressed by *Salmonella typhimurium*. *Infect. Immun.* 60, 2855–2862
53 Fulop, M. *et al.* (1996) Role of two outer membrane antigens in the induction of protective immunity against *Francisella tularensis* strains of different virulence. *FEMS Immunol. Med. Microbiol.* 13, 245–247
54 Arnon, S.S. *et al.* (2002) Botulinum toxin as a biological weapon. In *Bioterrorism. Guidelines for Medical and Public Health Management.* (Henderson, D.A. *et al.*, eds), pp. 141–165, American Medical Association Press
55 Byrne, M.P. and Smith, L.A. (2000) Development of vaccines for prevention of botulism. *Biochimie* 82, 955–966
56 Nierengarten, M.B. and Lutwick, L. (2003) Vaccine development for botulism. *Medscape Infectious Diseases* 5, 1