mAbs 3:6, 517-527; November/December 2011; © 2011 Landes Bioscience

# Antibodies for biodefense

Jeffrey W. Froude,[1,2] Bradley Stiles,[3] Thibaut Pelat[2] and Philippe Thullier[2,*]

[1]US Army Medical Research and Material Command; [3]US Army Medical Research Institute of Infectious Diseases; Fort Detrick, MD USA;
[2]Unité de biotechnologie des anticorps et des toxines; Département de Microbiologie; Institut de Recherche Biomédicale des Armées (IRBA-CRSSA); La Tronche Cedex, France

**Key words:** antibody, anthrax, plague, smallpox, botulism, tularemia, brucellosis, hemorrhagic, ricin, SEB

**Abbreviations:** BW, biological weapons; mAbs, monoclonal antibodies isolated by cellular techniques; pAbs, polyclonal antibodies; rAbs, recombinant antibodies; i.m, intramuscular; i.p, intraperitoneal; s.c., subcutaneously; i.v., intravenously; i.n., intranasal; $MD_{50}$, mean morbidity; MTD, mean time to death; FDA, Food and Drug Administration; CDC, Centers for Disease Control and Prevention; WHO, world health organization

Potential bioweapons are biological agents (bacteria, viruses and toxins) at risk of intentional dissemination. Biodefense, defined as development of therapeutics and vaccines against these agents, has seen an increase, particularly in the US, following the 2001 anthrax attack. This review focuses on recombinant antibodies and polyclonal antibodies for biodefense that have been accepted for clinical use. These antibodies aim to protect against primary potential bioweapons or category A agents as defined by the Centers for Disease Control and Prevention (*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, botulinum neurotoxins, smallpox virus and certain others causing viral hemorrhagic fevers) and certain category B agents. Potential for prophylactic use is presented, as well as frequent use of oligoclonal antibodies or synergistic effect with other molecules. Capacities and limitations of antibodies for use in biodefense are discussed, and are generally applicable to the field of infectious diseases.

## Introduction

Biological warfare, understood as the intentional dissemination of biological agents such as viruses, bacteria or toxins to target numerous persons, can formally be traced to 1346, when plague-ridden human corpses were catapulted over the besieged walls of Caffa (now Feodosija, Ukraine).[1] Much more recently, in WWII, plague was again utilized as a biological weapon (BW),[2] and a biological bomb containing anthrax spores was developed, but not deployed.[3] At present, BWs are banned by a United Nations' convention signed in 1972.[4] However, with no verification protocol, this moral interdiction has not been sufficient to deter several countries from developing BWs.[5] These same agents can also be weapons of choice for terrorist organizations[6] due to their subjective, beyond their objective, impact. For these reasons the development of therapeutics and vaccines against BWs or biodefense, is an active field of research.

*Correspondence to: Philippe Thullier; Email: pthullier@yahoo.com
Submitted: 06/21/11; Accepted: 08/03/11
DOI: 10.4161/mabs.3.6.17621

In 1901, Emil von Behring, a German military doctor, received the first Nobel prize for the initial development of polyclonal antibodies (pAbs) against infectious diseases such as diphtheria and tetanus. Sclavo's serum, another particular example of early pAb usage, increased the survival rate of cutaneous anthrax from 76–94%.[7] At present, antibodies against infectious agents, but not against toxins, have been supplanted by antibiotics and antivirals. However, the problem of increasing bacterial resistance, with at least one known mechanism for each existing antibiotic class,[8] requires development of new therapies at a time when only five new antibiotics have been approved by the Food and Drug Administration (FDA) between 2003 and 2007 and just two from 2008–2011.[9] Given the early success of pAbs, recombinant antibodies, which are a successful class of therapeutic molecules,[10-13] may constitute some of the new anti-infective molecules that are currently needed. Recombinant antibodies (rAbs) are defined in this review as antibodies which were selected or engineered, and expressed, utilizing DNA-based molecular biology techniques. Regarding the particular case of BWs, the risk of antibiotic resistance is acute because bacteria voluntarily disseminated could first be selected or engineered, for antimicrobial resistance. The development of antibodies may be a reasonable response to this risk; existing therapeutics, when they are effective, often act by other mechanisms and may act synergistically with antibodies. Antibodies may also be utilized for prophylaxis despite their relatively short half-life (~three weeks for human antibodies), which can be increased using numerous methods.[13] In effect, antibodies bring immediate protection that could be focused on personnel at risk of being exposed to BWs. The focused protection afforded by antibodies is advantageous compared with the protection elicited by vaccines, as vaccines must elicit an immune response to be effective and maintenance of this response may require booster injections. Further, rAbs may be administered in quantities that exceed that elicited by vaccines, and thus provide a higher level of protection, which is useful because BW exposure could involve elevated levels compared with natural exposure. In the particular case of botulinum neurotoxins, which are potential BWs, vaccination could be ethically disputable. RAb production is costly, but the number of doses needed is likely to be limited compared with the supply requirements for naturally encountered infectious diseases. This high production cost can be counterbalanced

by the efficiency with which new rAbs might be isolated. It is thus no surprise that numerous efforts have been devoted to developing antibodies against BWs, particularly in the United States (US), following the 2001 anthrax letter attacks.

In this review, data corresponding to the best of our knowledge for rAbs at preclinical or clinical stages and intended for biodefense will be presented. When no such molecules were found, literature was searched for monoclonal antibodies (mAbs) at an earlier stage of development. To be complete, this study also presents pAbs approved for medical use. It is focused on antibodies developed against biological agents at the highest risk of being weaponized, known as bioterrorism categorized agents, as defined by the Centers for Disease Control and Prevention (CDC).[14] Agents are classified under category A depending on their ease in transmission, high mortality and impact on social and public health infrastructures. Antibodies against category B agents will also be reviewed when these antibodies have proven efficient in vivo; the agents epsilon toxin of *Clostridium perfringens*, *Chlamydia psittaci*, *Coxiella burnetii* and *Rickettsia prowazekii* are excluded from discussion because no such antibodies exist. Each agent will be introduced and a potential role presented for antibodies, given the limits of other therapeutics and vaccines. Priority was given to results obtained on models utilizing the pulmonary route, by which many potential BWs are highly infectious. This route, against which physical protection is difficult, may threaten large populations.

Biodefense-related agents fall into the FDA "Animal Rule"[15] where human efficacy studies are neither ethical nor feasible, and an appropriate animal model may clearly demonstrate efficacy. An animal model is appropriate if (1) it is supported by a "reasonably well-understood pathophysiological mechanism", (2) if this mechanism is not well understood, the effect must be demonstrated in "more than one animal species" or "a sufficiently well-characterized animal model for predicting the response in humans", (3) if "the animal study endpoint is clearly related to the desired benefit in humans", and (4) if "the data…allows selection of an effective dose in humans".[16] This appropriate animal model is introduced in each section. Unless otherwise stated, models were utilized where all control animals died. The numbers (n) of animals tested are indicated in parenthesis. All indicated measurements, such as affinity, are presented as in the literature and may have been obtained with different conditions and equipment. With this review, we aim to present the state of the art and promote future development of antibodies for biodefense.

## Category A Agents

Category A agents include *Bacillus anthracis*, *Yersinia pestis*, botulinum toxin, smallpox, *Francisella tularensis* and Ebola, Marburg, Lassa and Machupo viruses, and these agents cause the diseases listed in the present section.

**Anthrax.** *Bacillus anthracis* is a gram-positive, spore-forming bacterium causing cutaneous, digestive or pulmonary anthrax. This latter form can cause a mortality rate as high as 100% when left untreated; however, during the more recent 2001 anthrax attacks, this rate was reported at 45% with treatment,[17] due to a limited therapeutic window. *B. anthracis* produces several virulence factors that contribute to pathogenesis, in particular the lethal toxin (LT), which is composed of protective antigen (PA) and lethal factor (LF), the edema toxin (ET) composed of PA and edema factor (EF), and poly-γ-d-glutamic acid (γDPGA) capsule.[18] The scientific consensus[19,20] on the animal models for anthrax vaccines and therapeutics recommends rabbits and non-human primates (NHPs). The estimated lethal dose by an aerosol varies; however, the survival threshold in rabbits and NHPs is less than 10,000 spores.[21] Post-exposure treatments involve antibiotics[22,23] (fluoroquinolone, tetracycline or penicillin G), which must be continued for 60 days after inhalation,[24] during which time the spores may still germinate. Analysis of the 2001 anthrax attack showed that out of 10,000 people potentially exposed to *B. anthracis* and treated, compliance to this long treatment regimen was only 40%.[25,26]

The US development of vaccines and therapeutics against anthrax includes BioThrax® or Anthrax Vaccine Absorbed (AVA), by Emergent (Rockville, MD) which essentially consists of PA extracted from cultures of non-encapsulated *B. anthracis*. Given the existence of this approved vaccine, BW development in the US was mainly focused on therapeutic molecules, in particular to increase the therapeutic window and decrease treatment length. One method to reach both goals is administration of antibodies. Of note, to decrease the treatment length, the Advisory Committee on Immunization Practices has written that three doses of AVA at weeks 0, 2 and 4, administered with antimicrobial therapy, would be a beneficial post-exposure treatment due to the production of anti-PA antibodies.[27,28] Four rAbs and one human pAb that target PA are currently undergoing clinical development or approval (**Table 1**).

Anthrivig™, or Anthrax Immune Globulin intravenous (AIGIV) by Emergent (Rockville, MD), is a pAb therapeutic developed from plasma of healthy donors vaccinated with Emergent's BioThrax® Vaccine. Efficacy results are not published but tolerance was clinically verified under study designation NCT00845650 (www.clinicaltrials.gov).

Raxibacumab (Abthrax™) developed by Human Genome Services (Rockville, MD) is currently under final review for approval by the FDA.[29,30] Raxibacumab is a human IgG1 isolated using phage display technology[31] that binds to PA with an affinity of 2.78 nM.[29] Raxibacumab prophylactically administered at 10 or 20 mg/kg subcutaneously (s.c.) 2 days prior, or concurrently at 40 mg/kg intravenously (i.v.), to rabbits (n = 12/group) challenged with 100 $LD_{50}$ aerosolized Ames spores provided 83, 83 and 100% survival, respectively.[29] The 40 mg/kg dose prophylactically administered s.c. to NHPs (n = 10) two days prior to a challenge with 100 $LD_{50}$ aerosolized Ames spores was 90% protective.[29] Treatment was also evaluated in rabbits and NHPs exposed to 200 $LD_{50}$ of aerosolized Ames spores. After PA was detected in the serum or following a 1.1°C rise in temperature, raxibacumab (40 mg/kg) was administered i.v. to rabbits or s.c. to NHPs, providing 44% (n = 18) and 64% (n = 14) survival, respectively.[29] Raxibacumab's tolerance was also verified on human volunteers (n = 105).[32] Human Genome Services was awarded

**Table 1.** Clinical and developmental status of anthrax antibodies targeting biodefense agents

| International non-proprietary name, product name or code | Targeted antigen | Antibody type (Isolation) | Clinical phase or approval year | US Patent reference number |
|---|---|---|---|---|
| Raxibacumab/ABthrax™,[a] | anti-PA | Recombinant IgG1 mAb (naïve library) | FDA review | 7601351 |
| Valortim®/MAb-1303[b] | anti-PA | Human mAb (transgenic mice) | Phase 1 | 7456264 |
| Anthim®/ETI-204[c] | anti-PA | Chimeric deimmunized mAb (murine origin) | Phase 1 | 7446182 |
| Thravixa™/AVP-21D9[d] | anti-PA | Human mAb (hybridoma) | Phase 1 | 7438909, 7442373 |
| Anthrivig™/Anthrax Immune Globulin (AIG) | anti-PA | Polyclonal antibody from AVA human plasma | Phase 1/2 | N/A |

[a]Subramanian et al. 2005 and US Patent 7601351. [b]Vitale et al. 2006 and US Patent Number 7456264. [c]Mohamed et al. 2005 and US Patent Number 7446182. [d]Peterson et al. 2007 and US Patents 7438909 and 7442373.

a contract (HHS010020050006C) in 2006 to provide a total of 65,000 doses of Raxibacumab to the US Strategic Stockpile.

Valortim®, also designated MAb-1303 or MDX-1303, isolated by Medarex (Princeton, NJ) and developed by PharmAthene, Inc., (Annapolis, MD), is in Phase 1 clinical studies (NCT01204866, NCT01265745). It is a human anti-PA IgG1 isolated from transgenic mice.[33,34] Treatment was evaluated in rabbits and NHPs exposed to 200 $LD_{50}$ aerosolized Ames spores. In the rabbit model (n = 10), treatment with Valortim® administered i.v. as two 1 mg/kg doses, given at 1 h and three days post-infection, provided 90% survival. Later treatment still provided 89% (n = 9) protection, but with an increased dose of 10 mg/kg given twice at 24 h and 120 h post-challenge.[33,34] Treatment of NHPs (n = 6) demonstrated complete protection after a single intramuscular (i.m.) injection of 1 mg/kg Valortim® given 1 hr post-challenge.[33] Although a Phase 1 clinical study for Valortim® has been completed for tolerance, combinational studies with antimicrobial treatments (NCT00964834 and NCT00964561) are apparently on hold or terminated due to a serious adverse event.

Anthim®, previously designated ETI-204, is a chimeric IgG produced by Elusys Therapeutics, Inc. (Pine Brook, NJ) that binds to PA with an affinity of 0.33 nM.[35] Its immunogenicity was decreased by DeImmunisation®. A single 10 mg (~4 mg/kg) dose of Anthim®, prophylactically administered i.v. to rabbits (n = 8) 30–45 min prior to an exposure of 163 or 286 $LD_{50}$ aerosolized Ames spores, provided 100 and 88% protection, respectively. Treatment was evaluated in rabbits (n = 10) exposed to 172 $LD_{50}$ of aerosolized Ames spores, to which Anthim® was administered as a single i.v. dose of 10 mg at 24 or 36 h post-infection and 80% or 50% survival rates were observed, respectively.[35] Tolerance of Anthim® both alone and in combination with ciprofloxacin have completed phase I clinical trials under respective study designations NCT00829582 and NCT00138411.

Thravixa®, also designated AVP-21D9, is a human IgG1 developed by Emergent (Rockville, MD) from Epstein Barr Virus immortalized lymphocytes that were originally isolated from humans immunized with AVA. This antibody binds PA with an affinity of 82 pM.[36] Protection and treatment by Thravixa® was evaluated using both New-Zealand white and Dutch Belted Dwarf rabbits, yielding equivalent results. Ten mg/kg administered s.c. concurrently with an aerosol challenge of 87 or 100 $LD_{50}$ Ames spores were completely (n = 12/group) protective.[37] Identical protection was observed against an intranasal (i.n.) challenge of 100 $LD_{50}$.[38] Regarding treatment, s.c. administration of Thravixa® (2 mg/kg) to rabbits at 0, 24 or 36 h following an aerosol challenge by 102 $LD_{50}$ Ames spores respectively provided 100%, 66% and 33% protection.[37] Although not yet assessed for tolerance, in vivo synergistic effects were observed using combined treatments of Thravixa® with a six day regimen of ciprofloxacin in a murine model. In particular, treatment of mice (n = 10) with 16.7 mg/kg Thravixa® and 30 mg/kg/day ciprofloxacin resulted in complete survival against five nasally instilled $LD_{50}$ of Ames spores, versus 40% for the antibody alone and 60% for the antibiotic alone. Although complete survival against a similar challenge in a guinea pig model was not achieved, synergistic effects of Thravixa® and ciprofloxacin were also demonstrated (80% versus 20% and 20% respectively for mAb and antibiotic alone).[38]

The current target of mAbs that are now in clinical trials against anthrax is exclusively PA, but a multi-targeted approach could be preferred. Although not in clinical trials, the latest generations of mAbs and antibody fragments directed against LF[39,40] or γDPGA capsule[41] promisingly neutralize in vitro or afford protection in animal models.

**Plague.** *Yersinia pestis* is a gram-negative bacterium causing bubonic, septicemic and pneumonic plague. The latter form is of special biodefense interest as it is rapidly lethal.[42] F1 is the dominant surface antigen of *Y. pestis* that relies, in particular, on the low-calcium response V (LcrV) antigen for virulence. The most appropriate animals for the study of plague are mice and African Green or cynomolgus macaque monkeys, as noted in the FDA workshop Animal Models and Correlates of Protection for Plague Vaccines (http://www.fda.gov/cber/minutes/workshopmin.htm). By the pulmonary route, the $LD_{50}$ of *Y. pestis* has been observed in different primate models as 100 to 20,000 aerosolized organisms.[43,44] Although antibiotics[45] (aminoglycosides, tetracyclines and chloramphenicol) are effective against pneumonic plague, they must be given within 24 h, and the separate appearance of two antibiotic-resistant strains exemplifies the need for additional therapeutics against *Y. pestis*.[46] A recombinant F1+V protein (rF1V) vaccine is currently undergoing Phase 2 clinical trials (NCT01122784 and NCT00332956), but no antibody-based therapeutic has entered clinical trials.

Proof of antibody efficacy against *Y. pestis* was, in particular, provided by a murine anti-LcrV, mAb 7.3.[47] Mice (n = 10) given mAb 7.3 (35 μg) by the intraperitoneal (i.p.) route, 4 h prior to an aerosolized challenge of 88 $LD_{50}$ (strain GB), demonstrated complete survival. When the same mAb dose was given 24 h

post-infection, survival rates decreased to 80% (n = 10). Of note, aerosol delivery of 77.5 μg mAb 7.3 plus the same quantity of an anti-F1 mouse antibody, mAb F1-04-A-G1, 2 h post-infection increased survival to 82%, but against a lesser aerosolized challenge of 27 $LD_{50}$.[48] Similar results with i.n. delivery were observed after the use of BA5, another murine mAb directed at LcrV.[49,50] Challenges by the s.c. route gave results consistent with those observed after use of the pulmonary route.[47,49]

The rAbs m252 (anti-F1), and m253 plus m254 (anti-LcrV), are the only rAbs that provide protection in vivo in a mouse model of bubonic plague.[51] These human IgGs were isolated from a naïve library and possessed sub-nM affinity. Mice (n = 6) challenged s.c. by 25–40 $LD_{50}$ (CO92 strain) were fully protected following the i.p. administration of m252 (500 μg/mouse) at 48 h post-infection. However, in this model, the survival rate dropped to 33% if m252 was administered earlier, at 24 h post-infection. A synergistic effect was seen when m252 was administered i.p. with m253 and m254 (500 μg of each) 24 h prior to a similar infection, with 83% survival compared to 33% for m252 alone. A combination of m253 and m254 failed to provide any protection.[51]

Future experiments studying the combined efficacy of antibiotics with antibodies are expected.

**Botulism.** Botulism is a disease caused by the neurotoxins secreted by the gram-positive, spore-forming, anaerobic bacteria *Clostridium botulinum*.[52] These bacteria produce seven serotypes of botulinum neurotoxins (BoNT), from BoNT/A to BoNT/G, whose sequences share a 34–64% identity,[53] and sub-types have been described in reference 54. Four of these toxins (A, B, E and F) cause human botulism,[55] but all seven can cause botulism in NHP inhalation models.[56] BoNTs can be weaponized as aerosols as well as contaminate food and water supplies. In particular, BoNT/A is the most toxic substance on Earth, with a human $LD_{50}$ estimated at 1 ng/kg i.v., 10 ng/kg by aerosol and 1 μg/kg orally.[52] A bivalent A/B $H_C$ vaccine, rBV A/B produced by DynPort Vaccine Company LLC (Frederick, MD), has completed Phase 2 clinical trials (NCT00764634).[57] The only two non-infant therapeutic products usable for biodefense are derived from equine pAbs; specifically, these are the heptavalent BoNT $F(ab')_2$ anti-toxin (HBAT) available from the CDC (Atlanta, GA),[58] and the anti-ABE pAb Botulismus-Antitoxin Behring from Novartis (Basel, Switzerland). The neutralization capacities of anti-BoNT products are estimated in international units (IU), and one IU of anti-toxin protects a mouse against 10,000 mouse $LD_{50}$ of A–F or 1,000 $LD_{50}$ of E BoNTs. For instance, 10 mL of HBAT represents 7,500 IU, 5,500 IU, 5,000 IU, 1,000 IU, 8,500 IU, 5,000 IU and 1,000 IU of anti-toxins A–G, respectively. Ten mL of Botulismus-Antitoxin Behring represents 7,500 IU, 5,000 IU and 500 IU of anti-toxin A, B and E, respectively. In NHP models, 143 IU/kg HBAT administered 48 h prior to 6 $LD_{50}$ of inhaled BoNT/A (assuming a NHP $LD_{50}$ by this route to be 300 mouse $LD_{50}$/kg) elicited full protection.[59] Delaying treatment with the same dose of HBAT against the same challenge, at 46 h post-exposure, decreased survival of NHPs (n = 5) to 60%.[59] The pAbs-derived products are well-suited to simultaneously protect against the various serotypes of BoNTs, but oligoclonal mixtures of recombinant antibodies have been developed to recapitulate that broad capacity. Oligoclonal combinations may also synergistically neutralize a single BoNT, as in the case of other toxins.[60]

Chimeric rAbs C25, S25 and the human rAb 3D12 bind to BoNT/A with 1.69 nM, 3.9 nM and 56.2 pM affinities, respectively.[61] The potency of an equimolar combination of these antibodies was determined at 45 IU/mg. The synergistic neutralization effect of these antibodies against BoNT/A was demonstrated when a fixed quantity (50 μg in total, 25 μg of each paired antibody or 16.7 μg of each of three antibodies) was pre-mixed with various quantities of BoNT/A and injected i.p. in mice (n = 10). Although paired mAbs afforded 90% protection against 1,000 $LD_{50}$, a mixture of the three antibodies was 100% protective against 7,500 $LD_{50}$, 80% protective against 10,000 $LD_{50}$ and approximately 50% protective against 20,000 $LD_{50}$.[61]

Several other studies have demonstrated protection with combinational antibody usage. Two IgG4s, C10 and 1B6, were generated from a synthetic human single-chain variable fragment (scFv) library. Each binds to the C-terminus of the heavy chain of BoNT/A (BoNT/A-Hc) with affinities of 64.6 pM and 23.8 nM, respectively. When combined, 25 μg of each mAb, pre-mixed with 20 $LD_{50}$ BoNT/A prior i.p. administration, completely protected mice (n = 6).[62] Two IgGs against BoNT/A, 4LCA and 6A, were produced by hybridoma technology from humans immunized against pentavalent botulinum toxoid (PBT).[63,64] 4LCA and 6A bind to BoNT/A light and heavy chains, respectively, with affinities of 31 and 6.9 pM.[63,64] Pre-mixing 50 μg of 4LCA and 6A each with 1,000 $LD_{50}$ BoNT/A prior to i.v. injection in mice (n = 6) resulted in complete survival.[64] 4LCA and 6A capacities have been further enhanced by attachment to a red blood cell-targeting protein.[65]

Recently, two human IgGs, 2B18.1 and 4E17.1, that cross-neutralize multiple serotypes of BoNT and are directed against the N-terminus of the heavy chains have been developed.[66] They were isolated starting from humans immunized against PBT and with the use of shuffled libraries.[67] The parental scFv of 2B18.1 binds to BoNT/A and B with respective affinities of 62.4 and 0.64 nM, respectively.[66] Similarly, the parental scFv of 4E17.1 binds to BoNT/A, B, E and F with affinities of 0.09, 28, 0.23 and 16.8 nM, respectively. Twenty five micrograms of the rAbs 2B18.1 and 4E17.1 each, pre-mixed with 200 $LD_{50}$ of either BoNT/B or BoNT/E prior to i.p. injection in mice (n = 10), provided complete protection in both cases.

The National Institute of Allergy and Infectious Diseases (NIAID) has awarded a contract (HHSN272200800028C) to Xoma (Berkeley, CA) to produce mAbs against the major sub-types of BoNT A, B and E. Additionally, the European Union (EU) has established a collaborative AntiBotABE project to discover mAbs against the same toxins (www.antibotabe.com). The diversity of BoNTs represents a special challenge, and any single antibody able to neutralize multiple sero- or sub-types of BoNTs would significantly reduce the cost of any final oligoclonal product.

**Smallpox.** Smallpox was declared eradicated by the World Health Organization (WHO) in 1980 after a successful global vaccination campaign, which was subsequently discontinued.[68]

The smallpox virus remains a potential BW precisely because a non-vaccinated population is vulnerable to this highly infectious agent, as a single inhaled virus particle can cause disease.[69] Given this threat, live Variola virus is maintained for research purposes within only two secured labs, one in the US and the other in Russia. Smallpox virions may be encountered in two forms: intracellular mature virions (MV) or extracellular enveloped virions (EV).[70] Strong antibody responses are observed in humans receiving the vaccine Dryvax®,[71] but its side effects have raised concern.[72,73] A second generation vaccine, ACAM2000®, was developed by Acambis Inc., (Cambridge, MA) and approved in 2007, although with restrictions that will not be discussed here in reference 74. Vaccinia Immune Globulin (VIG), a pAb extracted from vaccinated humans, is a product of limited potency,[75,76] that is nonetheless approved for use against the potential side effects of the vaccine and reduces morbidity/mortality associated with the disease itself.[75] Although still under debate, the WHO advisory committee on variola virus research recommends that approval of any prophylactic or therapeutic requires testing in NHP with fully virulent smallpox virus.[77] Practically, however, given the restricted access to this virus, new antibodies are compared with VIG using a vaccinia strain. All antibodies described in this section were isolated from vaccinated or infected animals.

A humanized mAb, hB5RmAb, whose parental antibody was isolated from a rat, is directed against the B5 surface protein of EV.[78] When mice were infected i.n. with $10^7$ plaque forming units (PFU) of vaccinia, followed by treatment with the parental mAb (10 μg) administered i.p. at 5 h post-exposure, all survived and lost significantly less weight compared with controls.

Phage technology was utilized to isolate 8AH8AL, a chimeric chimpanzee/human rAb binding B5 with 0.6 nM affinity.[79] Protection was compared to VIG utilizing the mouse pneumonia models, induced with $10^5$ PFU of vaccinia (western Reserve strain or $VACV_{WR}$) administered i.n. Decreasing i.p. quantities of 8AH8AL and VIG were administered to mice (n = 5) 24 h prior to the infection, and a dose of 22.5 μg 8AH8AL provided complete protection comparable to that elicited by 5 mg VIG. At 48 h post-infection, 90 μg 8AH8AL provided complete (n = 5) protection while 5 mg of VIG did not.[79] In a second study utilizing the same approach, 6C was isolated and bound the EV protein A33 with 20 nM affinity (as measured with the parental Fab). In the same animal model, but 48 h after infection, rAb 6C was tested alone (90 μg) or in combination (45 μg each) with 8AH8AL. Full protection was observed in both cases, but weight loss was more limited with the combined treatment than with 6C only.[80]

Two human rAbs, hV26 and h101, were isolated from transgenic mice and bind to the H3 protein found at the surface of MV and to the B5 protein, respectively.[81] Each mAb was administered i.v. and compared to VIG in severe combined immunodeficiecy (SCID) mice, utilizing New York City Board of Health Vaccinia virus ($VACV_{NYCBOH}$). Twenty five μg of each mAb administered to SCID mice one day prior to challenge against 5 x $10^4$ CFU $VACV_{NYCBOH}$ elicited 50% protection, while all virus-treated controls given 1.25 mg VIG died.[81]

The WHO recommendation to test potential therapeutics against smallpox in NHP should be fulfilled for the best candidates.

**Tularemia.** *Francisella tularensis* is a gram-negative intracellular bacterium of which several subspecies, indistinguishable by serological tests, are described. The subspecies tularensis or type A (which includes the SchuS4 strain, for instance) is the most virulent and most likely to be weaponized, as opposed to subspecies holartica or type B, from which the live vaccine strain (LVS) is derived.[82] Tularemia can arise from as few as ten organisms administered either s.c.[83] or by aerosol,[84] with multiple clinical manifestations. Although there is no consensus on a small animal model, several studies[85] suggest murine models utilizing the SchuS4 strain are capable of satisfying the FDA animal rule. *F. tularensis* is generally susceptible to antibiotics (e.g., tetracycline, fluoroquinolones, aminoglycosides), but susceptibility testing is recommended,[86] and a new formulation of LVS used as a vaccine is currently in a clinical Phase 2 study (NCT01150695). In murine models, vaccination by LVS fully protects against an intradermal (i.d.), but not aerosolized, challenge of 1,000 $LD_{50}$ SchuS4,[84,87-89] Prophylaxis and treatment by antibodies were first shown by transfer of immune serum against an aerosolized challenge by *F. tularensis* LVS,[90] but only increased mean time to death (MTD) was observed after a challenge with SchuS4. All mAbs that protect against *F. tularensis* are, thus far, of murine origin.

MAb 12, an IgG2a, was developed after immunization by LVS and has been tested against both LVS and SchuS4 strains.[91] Three doses of 50 μg each, prophylactically administered at days -1, 0 and +1, provided complete survival in mice (n = 4) against an intradermal (i.d.) challenge by 7 x $10^7$ CFU of LVS. When the same three doses were administered therapeutically at 1, 3 and 5-days after infection by the same challenge, survival decreased to 50%. Regarding type A, prophylactic administration of MAb 12 did not elicit protection, but doubled MTD against a 24 CFU challenge of virulent Schu24.[91] A murine anti-LPS IgG2a, mAb 3, is the only mAb tested against an i.n. challenge of *F. tularensis*.[92] Treatment was evaluated in mice challenged against 2 x $10^4$ CFU LVS, resulting in 100% survival following the i.n. administration of mAb 3 at 50 μg within minutes of challenge (n = 5), or following the i.p. administration of mAb 3 (200 μg) at 1 h post-challenge (n = 5).

Antibodies can completely protect against LVS but not against SchuS4. This might be due to different virulence mechanisms between the strains,[93] explaining why effective protection against LVS (belonging to type B) does not guarantee protection against type A.

**Viral hemorrhagic fevers (VHF).** Viral hemorrhagic fevers (VHF) are caused by four virus families: *Filoviridae, Arenaviridae, Flaviviridae* and *Bunyaviridae*. In particular, Category A agents include Ebola and Marburg of the *Filoviridae* family, as well as Lassa and Machupo of the *Arenaviridae* family. Many VHF agents are highly infectious by aerosol, and mortality rates may be greater than 90% during natural outbreaks.[94] A consensus on the appropriate animal models of diseases caused by Filoviruses has not yet been reached due to symptom discrepancies between

models and humans, but guinea pigs and NHPs serve to date as the primary models for these diseases.[95] Ribavirin may be utilized to treat VHF, but only if caused by arenaviruses and bunyaviruses. Several DNA plasmid vaccines against Ebola and Marburg are in Phase 1 clinical studies (NCT00072605, NCT00374309, NCT00997607, NCT00605514). Immunotherapy against VHF was first demonstrated by employing crude blood transfusions during the 1995 Kikwit Ebola outbreak, with only one (n = 8) patient death following this treatment, as compared to 80% without.[96] A working consensus regarding VHF states that passive immunization strategies using rAbs should be pursued in the future.[97]

Given the complexities associated with animal models for these agents, which require BSL-4 laboratory containment, only a few antibodies directed against VHF have been tested in vivo. The first of these mAbs was KZ52, a human rAb developed from survivors, binds to the glycoprotein (GP) of Ebola Zaire and effectively neutralizes the virus in a plaque reduction assay.[98,99] Passive administration of KZ52 (25 mg/kg) in guinea pigs (n = 5) provides complete protection 1 h prior and 80% survival 1 h after s.c. challenge against 10,000 PFU of Ebola Zaire Mayinga.[100] Two i.v. doses of KZ52 (50 mg/kg) given to macaques (n = 4), one day before and 4 days after an i.m. challenge with 1,000 PFU Ebola Zaire (Kikwit), failed to affect disease progression.[101] A later study compared the in vitro inhibition mechanisms of KZ52 to J3PK11, developed from a rhesus macaque that survived an Ebola Zaire infection,[102] and found them to be distinct.[103] Although J3PK11 has only been tested in vitro, this result demonstrates that viruses responsible for Ebola and possibly other VHFs have multiple physiopathological mechanisms which may depend on the model utilized.

## Category B Agents

Category B agents include Brucella, Burkholderia, ricin, Staphyloccal enterotoxin B alphaviruses, as well as food and water safety threats, and these agents cause the diseases listed in the present section.

**Brucellosis.** Brucellosis is an anthropozoonosis caused by intracellular, gram-negative bacteria of the genus Brucella and *B. melitensis* is the major cause of the 500,000 annual cases of human brucellosis. These microbes are highly infectious, as aerosols of *B. melitensis* in NHP require only $10^2$ CFU to cause disease.[104] Mice are the primary animal model to characterize and test therapeutics against brucellosis.[105] Antibiotics (tetracyclines, aminoglycosides, rifampicin and streptomycin) are generally effective, often combined in 6-week treatments, but the risk of relapse is as high as 30%.[106] There is no licensed human vaccine for brucellosis. The last expert committee of the Food and Agriculture Organization of the United Nations/WHO in 1986 recommended development of antibody therapeutics towards brucellosis;[107] however, the only therapeutic mAbs developed to date are of murine origin.[108-110]

Prophylactic i.p. administration of anti-LPS IgG3 mAb 6B3 (100 μg) to mice (n = 3), 24 h prior to i.p. challenge with 3.6 x $10^5$ *B. melitensis* 16M, provided complete protection. In a similar challenge but with 1.8 x $10^5$ CFU *B. abortus*, 50 μg of anti-LPS IgG3 mAb 2C8 fully protected the animals (n = 3).[110]

**Melioidosis and glanders.** Glanders, a disease that primarily affects equids and melioidosis are difficult to discriminate clinically in humans. They are caused by *Burkholderia mallei* and *Burkholderia pseudomallei*, respectively.[111,112] In vivo studies using mouse models have been considered the most effective due to their adaptability to investigate different aspects of disease,[113] with BALB/c being the most susceptible to melioidosis.[114] The $LD_{50}$ in murine models is strain-dependent, but has been reported as 1.6 x $10^3$ CFU for *B. mallei* and between 20–320 CFU for *B. pseudomallei*.[115-117] Antibiotic resistance poses an issue to treat these diseases and carbapenems, as well as the trimethoprim-sulfamethoxazole association, may be recommended.[118] Currently, no vaccine effective against either of these bacteria exists.[112]

The murine anti-LPS IgG2a mAb 1G2-1D3 was developed against *Burkholderia mallei*.[119] The i.p. administration of 1G2-1D3 (1 mg) to mice (n = 6) 18 h prior to challenge with 20 $LD_{50}$ (1.9 x $10^4$ CFU) aerosolized *B. mallei* strain China 7 was completely protective. Under a similar challenge, the same administration of mAb 1G2-1D3 to mice but 18 h post-infection failed to affect disease progression.[119]

The murine IgG3 mAb Ps6F6 binds *B. pseudomallei* exopolysaccharide.[116] Three i.p. injections (3.5 μg each) of mAb Ps6F6 to mice (n = 85/group), on day -6, -3 and -2 prior to i.p. challenge against 490 CFU (1.4 $LD_{50}$) *B. pseudomallei* strain 6068 VIR, failed to achieve greater than 40% survival when 10% of controls survived.[116] In another study, seven murine mAbs demonstrated passive protection against the *B. pseudomallei* 4845 strain.[120] Protection was evaluated using 40 μg of seven antibodies (6 μg of three anti-exopolysaccharides and of three other mAbs, plus 4 μg of an anti-LPS) as an oligoclonal cocktail administered i.v. to mice (n = 10). These antibodies, given 4 h before challenge, were completely protective against $10^4$ CFU (250 mean morbidity or $MD_{50}$) by the i.p. route, but did not protect against a higher dose of $10^6$ CFU.[120]

**Intoxication by ricin.** Ricin can be easily extracted from *Ricinus communis*, a plant cultivated world-wide, also known as castor oil plant. This protein toxin consists of a B-subunit (RTB) that binds sugars on the cell surface for cytosolic entry of an A-subunit (RTA) that inhibits protein synthesis. It is lethal by the oral route, but 1,000-fold more potent by the pulmonary and parenteral routes. Ricin has an aerosolized $LD_{50}$ of 3–5 μg/kg in the mouse and 5.8–15 μg/kg in NHP models.[121,122] Two potential vaccines currently in Phase 1studies (NCT01317667, NCT00812071), protect against ricin intoxication. For treatment against ricin, there are supportive, but no specific, measures. However, in a proof of concept study utilizing direct inhalation for intoxication and treatment, pAbs against ricin were completely protective 20 min post-challenge.[123]

Two chimeric IgGs whose parental mAbs were tested in a murine model have been developed. The murine mAb 4C13,[124,125] chimerized as IgG c4C13,[126] was administered at a dose of 100 μg/mouse (n = 4) by i.p. 30 min after i.p. challenge with 2 μg ricin (10 $LD_{50}$), and all animals survived.[125] A second murine mAb, RAC18, was evaluated in an aspiration model where ricin

and the antibody are instilled in the oropharynx.[127] Mice (n = 10) challenged with 16 μg/kg (~3 $LD_{50}$) ricin before the administration of 50 μg of antibody completely survived if the antibody was administered 4 h post-intoxication, and 60% and 50% survived if the antibody was administered at 18 and 24 h post-intoxication, respectively.[127]

The latest antibodies neutralizing ricin are more human-like, as with the macaque scFv anti-RTA 43RCA.[128]

**Intoxication by Staphylococcal Enterotoxin B (SEB).** *Staphylococcus aureus* is a gram-positive bacterium that produces many different virulence factors. One of these is Staphylococcal enterotoxin B (SEB), a superantigenic toxin, and just one of more than twenty-five different staphylococcal enterotoxins (SEs) characterized to date. SEB, like many other superantigens, stimulates T cells to release levels of pro-inflammatory cytokines that can cause shock and death. Different animal models for investigating SE-induced shock, as well as potential therapeutics and vaccines, include multiple strains of mice, macaques, piglets, ferrets and shrews.[129-141] Readouts of intoxication include fever, vomiting, diarrhea, as well as lethality.

Intravenous immunoglobulin (IvIg), collected from humans, can protect against SEs and related toxic shock syndrome toxin-1 (TSST-1).[142,143] Humans are naturally in contact with *S. aureus* that grows on the skin, mucosal surfaces or in food items, and can seroconvert to *S. aureus* antigens that include the SEs. Experimentally, passive administration of pAbs (10 mg/kg), 4 h post-challenge, completely protected macaques (n = 4) against five aerosolized $LD_{50}$ of SEB.[144]

Two murine-human chimeric antibodies that bind distinct epitopes have recently been described but only tested in vitro.[145] The mAb HuMAb-154 neutralizes SEB in vitro (proinflammatory cytokine release from peripheral blood mononuclear cells) and in vivo (mouse lethality).[146] Screening of antibodies was done with a recombinantly-attenuated form of SEB used as a vaccine in various animal models.[137,138,147,148] HuMAb-154 binds to SEB with an affinity of 0.29 nM and cross-reacts with SEA, SEC1 and SED by ELISA. Pre-mixing increasing concentrations of SEB with 500 μg of HuMAb-154 prior to i.p. administration to mice (n = 5) resulted in complete protection against 5 and 10 μg (25 and 50 $LD_{50}$, respectively) SEB, and 40% protection against doses as high as 100 μg (500 $LD_{50}$). A similar dose of HuMAb-154, administered to mice (n = 30) as a therapeutic at i 0, 0.5 and 1 h post-exposure to 10 μg (50 $LD_{50}$) SEB elicited 86, 50 and 13% survival, respectively.[146]

A human phage-displayed library was used in another study to generate bivalent Fabs and full length IgG.[149] Pre-mixing 10 μg of the full length mAbs FL10 or FL9 with 2.5 μg SEB, prior to i.p administration to mice (n = 6) elicited 68 and 17% protection, respectively.[149]

In regards to antibody therapy against SEB in a biowarfare/bioterror scenario, none of mAbs discovered have been used in NHPs challenged with an SEB aerosol. Such experiments represent a high stringency test necessary to establish efficacy of therapeutic antibodies against SEB.

**Encephalitis caused by alphaviruses.** Viruses of the *Alphavirus* genus (*Togaviridae* family) cause encephalitis, and of these, Venezuelan equine encephalitis virus (VEEV) has been more extensively studied and weaponized.[150] The most relevant animal models for studying VEEV are considered mice and NHPs,[151] with the $LD_{50}$ of VEEV in mice estimated as 1–30 PFUs. Two vaccines are currently in Phase 2 clinical trials, the attenuated TC-83 (NCT00582504) and the inactivated C-84 as a booster to TC-83 (NCT00582088).[152,153] Supportive care is available for VEE, but there is no specific treatment.

The humanized mAb Hu1A3B-7 (IgG1 isotype) is broadly specific to VEEV subtypes, neutralizing type IA/B (Trinidad Donkey or TrD), type II (Fe37c) and type IIIA (Macambo BeAn8) in vitro.[154] Twenty five micrograms of Hu1A3B-7 administered i.p. to mice (n = 10) 24 h prior to s.c. challenge with 100 $LD_{50}$ TrD strain, provided complete protection. Treatment was evaluated in mice (n = 10) exposed to 10 and 100 aerosolized $LD_{50}$ TrD: 100 μg Hu1A3B-7 administered 24 h post-challenge yielded 100% and 90% survival, respectively.[154] Another humanized mAb, Hy4 IgG, binds to the E2 glycoprotein and is protective in murine models.[155] Five hundred micrograms of mAb Hy4 administered i.p. at 24 h pre-challenge against 100 i.n. $MD_{50}$ TrD 1350 PFU) protected 80% of the animals (n = 10). After i.p. challenge by 100 $LD_{50}$ TrD, the i.p. administration of 10 μg mAb Hy4 at 1 and 24 h post-infection were 90% (n = 10) or 75% (n = 20) protective.[155] A third mAb, hu1A4A1IgG1-2A, binds to the E2 glycoprotein with an affinity of 3.9 nM.[156] Treatment of mice (n = 8) with hu1A4A1IgG1-2A was evaluated against the s.c. administration of 30–50 PFU TrD and completely protected when administered i.p. (50 μg) at 24 h post-infection, but the same dose failed to protect at 72 h post-challenge. A prophylactic administration of mAb at 24 h pre-infection resulted in complete protection.[156]

Recently several human antibody fragments have been isolated but have yet to be tested as full IgGs in vivo.[157,158] Of note, the particular variability of such RNA viruses is of concern because it favors the emergence of resistance and consequently several therapeutic molecules, including Abs, may be required.

**Diseases caused by food and water safety threats.** Food and water safety threats include a diverse class of organisms that present both biodefense as well as natural outbreak concerns, exemplified by the European *E. coli* outbreak in May 2011. Thus far, only shigatoxins and *Vibrio cholerae* have been targeted by antibodies evaluated in vivo.

The chimeric mAbs HαStx1 and HαStx2 (Shigamabs®, also called cαStx1 and cαStx2), were derived from murine mAbs 13C4,[159] and 11E10 [160] and respectively target the Stx 1 and Stx 2 shigatoxins produced by *E. coli.*[161-163] They are in Phase 2 clinical trials (NCT01252199) by Thallion Pharmaceuticals (Montréal, Québec) in collaboration with Laboratoires Français de Fractionnement et des Biotechnologies (LFB; Les Ulis, France).[164] Protection was tested against Stx1 toxin with HαStx1 administered in two i.p. injections of 2.05 μg of each, at 24 h prior to challenge and simultaneously to challenge; mice (n = 5) were completely protected after i.v. intoxication with 20 $LD_{50}$ of crude Stx1.[161] Using the same administration scheme but against Stx2 toxin, HαStx2 was administered in two 0.5 μg injections against $10^{10}$ CFU of *E. coli* strain 86-24 (producing Stx2), or two 116 μg injections against $10^3$ CFU of *E. coli* strain O91:H21

(producing Stx2d[161]), resulting in complete survival against each strain.[161]

The mAb 5C12, from a transgenic mouse, binds Shiga toxin Stx 2 A-subunit with an affinity of 0.85 nM.[165] Treatment against this toxin was evaluated in piglets orally challenged with $10^{10}$ CFU of shigatoxigenic *E. coli* (STEC) 86-24 (producing Stx2), with 3 mg/kg 5C12 administered i.p. at 24 and 48 h post-challenge, resulting in 78% (n = 9) and 100% (n = 4) survival, respectively.[165] Mice (n = 10) orally challenged with $10^{10}$ CFU of another shigatoxigenic *E. coli*, B2F1 (producing Stx2) were treated with 2.1 mg/kg mAb 5C12 administered i.p. at 0, 12, 24 and 48 h post-challenge, resulting in 80, 70, 90 and 60% protection, respectively.[166]

The murine anti-LPS mAb 72.1 is directed against *Vibrio cholerae* and protects against both the Ogawa (O395) and Inaba (569B) strains.[167] Fifty μg of mAb 72.1 mixed with $10^8$ bacteria of each strain, given by oral gavage to infant mice (n = 5), provided complete protection in both cases.[167]

## Discussion

Recombinant antibodies represent a rapidly emerging class of potent therapeutics, but, contrary to the historical use of pAbs, most rAbs currently marketed do not target infectious agents. Palivizumab (Synagis®), used to prevent infections caused by respiratory syncytial virus, but with incomplete success, is the single exception.[168] The limited development of antibodies against infectious agents may be explained by existing antibiotics or antivirals, but these molecules have their own limitations. For instance, antibiotic resistance is a particularly high risk regarding bioweapons, and the available therapeutic window is often narrow. Such limitations explain that, perhaps particularly after the deliberate dissemination of *B. anthracis* spores in the US in 2001, antibodies against anthrax and other bioweapons were increasingly developed. Regarding anthrax, the current stockpiling of an effective rAb,[29] and existence of three competitors,[33,35,37] showcase the capacity to isolate efficient rAbs when microbial pathogenesis is understood. However, the situation regarding anthrax has no equivalent so far, perhaps because oligoclonal rAbs may be necessary to effectively target a single agent, such as *Y. pestis*[51] or *B. pseudomallei*,[120] thus increasing the complexity of antibody development. This may also be the reason why, despite impressive scientific results, no combination of antibodies targeting the various botulinum toxins has yet entered clinical trials. In fact, the narrow specificity of antibodies limits their efficacy, although it conversely limiting their reactivity with human tissues, which confers an advantage regarding clinical tolerance. Antibodies developed against smallpox represent one of the several possibilities of antibody prophylaxis.[78-81] Indeed, antibodies are often more efficient for prophylaxis versus therapy of infectious diseases, as discussed in this review and also exemplified by palivizumab.[168] There are extreme cases, such as brucellosis, where only prophylactic use is effective.[110] Of note, while early treatment is almost always better, fast diagnosis is imperative, and antibodies certainly have a role in biodefense for the development of rapid tests.[42,169,170]

Targeting toxins, viruses or intra-cellular bacteria before internalization is more effective for antibodies, which usually do not enter cells, but antibodies might offer possibilities that are otherwise scarcely exploited. These possibilities include administration by aerosol, exemplified by antibodies targeting ricin,[123] which could serve as an example for new clinical developments. Additionally, the effective use of antibodies against Alphaviruses may exemplify antibody capacity to cross the blood-brain barrier,[171] and could prompt clinical developments targeting other central nervous system diseases.

All results were not as positive as expected, such as in the case of protection against Ebola virus,[103] which is probably an example of where more fundamental research is needed. In effect, antibody inhibition of infectious mechanisms may be model-dependent and perhaps not always relevant to humans. More research might also be needed regarding the most virulent type A strains of *F. tularensis*, against which no antibody effectively protects. Being efficacious against several extremely virulent pathogens, antibodies for biodefense highlight their capacities against infectious diseases,[172] and thus should lead investigators toward new clinical developments and research. Antibodies do have intrinsic limits, but these should not be confused with the limits of our knowledge.


### Acknowledgments

J.W.F. II is part of a formal Engineer-Scientist Exchange Program established between the governments of France and the USA. All opinions are solely those of the authors and do not reflect official government views.


### References


1. Wheelis M. Biological warfare at the 1346 siege of Caffa. Emerg Infect Dis 2002; 8:971-5.
2. Rutecki GW. A revised timeline for biological agents: revisiting the early years of the germ theory of disease. Med Hypotheses 2007; 68:222-6.
3. Berche P. L'histoire secrète des guerres biologiques: Mensonges et crimes d'Etat. Paris: Robert Laffont 2009.
4. Henderson DA. The looming threat of bioterrorism. Science 1999; 283:1279-82.
5. Harris ED. Chemical and Biological Weapons: Possession and Programs Past and Present. The Nonproliferation Review 2004; 86-109.
6. Sugishima M. Aum Shinrikyo and the Japanese law on bioterrorism. Prehosp Disaster Med 2003; 18:179-83.
7. Legge TM. The Milroy Lectures ON INDUSTRIAL ANTHRAX: Delivered before the Royal College of Physicians of London. Br Med J 1905; 1:589-93.
8. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 2005; 36:697-705.
9. Hughes JM. Preserving the lifesaving power of antimicrobial agents. Jama 2011; 305:1027-8.
10. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov 2010; 9:767-74.
11. Drugs@FDA. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/. Silver Spring MD 2011.
12. Reichert JM. Probabilities of success for antibody therapeutics. MAbs 2009; 1:387-9.
13. Arnett SO, Teillaud JL, Wurch T, Reichert JM, Dunlop C, Huber M. IBC's 21st Annual Antibody Engineering and 8th Annual Antibody Therapeutics International Conferences and 2010 Annual Meeting of The Antibody Society December 5–9, 2010, San Diego, CA USA. MAbs 2011; 3:133-52.
14. Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, Hughes JM. Public health assessment of potential biological terrorism agents. Emerg Infect Dis 2002; 8:225-30.
15. New drug and biological drug products; evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. Final rule. Fed Regist 2002; 67:37988-98.
16. FDA. Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, guidance for industry. Animal Models—Essential Elements to Address Efficacy Under the Animal Rule 2009.

17. Jernigan DB, Raghunathan PL, Bell BP, Brechner R, Bresnitz EA, Butler JC, et al. Investigation of bioterrorism-related anthrax, United States 2001: epidemiologic findings. Emerg Infect Dis 2002; 8:1019-28.
18. Friedlander AM, Little SF. Advances in the development of next-generation anthrax vaccines. Vaccine 2009; 27:28-32.
19. FDA. Transcripts of Workshop, Anthrax: Bridging Correlates of Protection in Animals to Immunogenicity in Humans 2007.
20. FDA. Pathway to Licensure for Protective Antigen-based Anthrax Vaccines for a Post-exposure Prophylaxis Indication Using the Animal Rule. Vaccines and Related Biological Products Advisory Committee Meeting 2010.
21. Coleman ME, Thran B, Morse SS, Hugh-Jones M, Massulik S. Inhalation anthrax: dose response and risk analysis. Biosecur Bioterror 2008; 6:147-60.
22. Meyerhoff A, Albrecht R, Meyer JM, Dionne P, Higgins K, Murphy D. US Food and Drug Administration approval of ciprofloxacin hydrochloride for management of postexposure inhalational anthrax. Clin Infect Dis 2004; 39:303-8.
23. FDA. Prescription drug product: doxycycline and penicillin G procaine administration for inhalational anthrax (post-exposure). Federal Reg 2001; 66:55679-82.
24. Update: Investigation of bioterrorism-related anthrax 2001. MMWR Morb Mortal Wkly Rep 2001; 50:1008-10.
25. Update: Investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. MMWR Morb Mortal Wkly Rep 2001; 50:973-6.
26. Shepard CW, Soriano-Gabarro M, Zell ER, Hayslett J, Lukacs S, Goldstein S, et al. Antimicrobial post-exposure prophylaxis for anthrax: adverse events and adherence. Emerg Infect Dis 2002; 8:1124-32.
27. Wright JG, Quinn CP, Shadomy S, Messonnier N. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2010; 59:1-30.
28. Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Anthrax as a biological weapon 2002: updated recommendations for management. Jama 2002; 287:2236-52.
29. Migone TS, Subramanian GM, Zhong J, Healey LM, Corey A, Devalaraja M, et al. Raxibacumab for the treatment of inhalational anthrax. N Engl J Med 2009; 361:135-44.
30. Rosen CA, Laird MW, Gentz R. Antibodies against protective antigen. United States: Human Genome Sciences, Inc. 2009.
31. Zhang X, Askins J, Fleming R, Sturm B, Poortman C, Viriassov P, et al. Selection of potent neutralizing human monoclonal antibodies to protective antigen of *Bacillus anthracis*. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy 2003.
32. Subramanian GM, Cronin PW, Poley G, Weinstein A, Stoughton SM, Zhong J, et al. A phase 1 study of PAmAb, a fully human monoclonal antibody against *Bacillus anthracis* protective antigen, in healthy volunteers. Clin Infect Dis 2005; 41:12-20.
33. Vitale L, Blanset D, Lowy I, O'Neill T, Goldstein J, Little SF, et al. Prophylaxis and therapy of inhalational anthrax by a novel monoclonal antibody to protective antigen that mimics vaccine-induced immunity. Infect Immun 2006; 74:5840-7.
34. Keler T, Lowy I, Vitale L, Blanset D, Srinivasan M. Human monoclonal antibodies against *Bacillus anthracis* protective antigen. United States Patent Office. United States: Medarex, Inc. 2008.
35. Mohamed N, Clagett M, Li J, Jones S, Pincus S, D'Alia G, et al. A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized *Bacillus anthracis* spore challenge. Infect Immun 2005; 73:795-802.
36. Sawada-Hirai R, Jiang I, Wang F, Sun SM, Nedellec R, Ruther P, et al. Human anti-anthrax protective antigen neutralizing monoclonal antibodies derived from donors vaccinated with anthrax vaccine adsorbed. J Immune Based Ther Vaccines 2004; 2:5.
37. Peterson JW, Comer JE, Baze WB, Noffsinger DM, Wenglikowski A, Walberg KG, et al. Human monoclonal antibody AVP-21D9 to protective antigen reduces dissemination of the *Bacillus anthracis* Ames strain from the lungs in a rabbit model. Infect Immun 2007; 75:3414-24.
38. Peterson JW, Comer JE, Noffsinger DM, Wenglikowski A, Walberg KG, Chatuev BM, et al. Human Monoclonal Anti-Protective Antigen Antibody Completely Protects Rabbits and Is Synergistic with Ciprofloxacin in Protecting Mice and Guinea Pigs against Inhalation Anthrax. Infect Immun 2006; 74:1016-24.
39. Pelat T, Hust M, Laffly E, Condemine F, Bottex C, Vidal D, et al. High-affinity, human antibody-like antibody fragment (single-chain variable fragment) neutralizing the lethal factor (LF) of *Bacillus anthracis* by inhibiting protective antigen-LF complex formation. Antimicrob Agents Chemother 2007; 51:2758-64.
40. Chen Z, Moayeri M, Crown D, Emerson S, Gorshkova I, Schuck P, et al. Novel chimpanzee/human monoclonal antibodies that neutralize anthrax lethal factor and evidence for possible synergy with anti-protective antigen antibody. Infect Immun 2009; 77:3902-8.
41. Chen Z, Schneerson R, Lovchik J, Lyons CR, Zhao H, Dai Z, et al. Pre- and postexposure protection against virulent anthrax infection in mice by humanized monoclonal antibodies to *Bacillus anthracis* capsule. Proc Natl Acad Sci USA 2011; 108:739-44.
42. Bertherat E, Thullier P, Shako JC, England K, Kone ML, Arntzen L, et al. Lessons Learned about Pneumonic Plague Diagnosis from 2 Outbreaks, Democratic Republic of the Congo. Emerg Infect Dis 2011; 17:778-84.
43. Speck RS, Wolochow H. Studies on the experimental epidemiology of respiratory infections. VIII. Experimental pneumonic plague in Macacus rhesus. J Infect Dis 1957; 100:58-69.
44. Ehrenkranz NJ, Meyer KF. Studies on immunization against plague. VIII. Study of three immunizing preparations in protecting primates against pneumonic plague. J Infect Dis 1955; 96:138-44.
45. Prevention of plague: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1996; 45:1-15.
46. Galimand M, Carniel E, Courvalin P. Resistance of *Yersinia pestis* to antimicrobial agents. Antimicrob Agents Chemother 2006; 50:3233-6.
47. Hill J, Copse C, Leary S, Stagg AJ, Williamson ED, Titball RW. Synergistic protection of mice against plague with monoclonal antibodies specific for the F1 and V antigens of *Yersinia pestis*. Infect Immun 2003; 71:2234-8.
48. Hill J, Eyles JE, Elvin SJ, Healey GD, Lukaszewski RA, Titball RW. Administration of antibody to the lung protects mice against pneumonic plague. Infect Immun 2006; 74:3068-70.
49. Quenee LE, Berube BJ, Segal J, Elli D, Ciletti NA, Anderson D, et al. Amino acid residues 196–225 of LcrV represent a plague protective epitope. Vaccine 2010; 28:1870-6.
50. Eisele NA, Anderson DM. Dual-function antibodies to *Yersinia pestis* LcrV required for pulmonary clearance of plague. Clin Vaccine Immunol 2009; 16:1720-7.
51. Xiao X, Zhu Z, Dankmeyer JL, Wormald MM, Fast RL, Worsham PL, et al. Human anti-plague monoclonal antibodies protect mice from *Yersinia pestis* in a bubonic plague model. PLoS One 2010; 5:13047.
52. Dembek ZF, Smith LA, Rusnak JM. Botulism: cause, effects, diagnosis, clinical and laboratory identification and treatment modalities. Disaster Med Public Health Prep 2007; 1:122-34.
53. Lacy DB, Stevens RC. Sequence homology and structural analysis of the clostridial neurotoxins. J Mol Biol 1999; 291:1091-104.
54. Smith TJ, Hill KK, Foley BT, Detter JC, Munk AC, Bruce DC, et al. Analysis of the neurotoxin complex genes in *Clostridium botulinum* A1-A4 and B1 strains: BoNT/A3/Ba4 and/B1 clusters are located within plasmids. PLoS One 2007; 2:1271.
55. Centers for Disease Control and Prevention: Botulism in the United States 1899–1996. Handbook for Epidemiologists, Clinicians and Laboratory Workers. Atlanta, GA: Centers for Disease Control and Prevention 1998.
56. Middlebrook JL, Franz DR. Botulinum toxins. In: Slidell FR, Takafuji ET, Franz DR, Eds. Textbook of Military Medicine, Part 1: Warfare, Weaponry and Then Casualty: Medical Aspects of Chemical and Biological Warfare. Washington DC: Borden Institute, Walter Reed Army Medical Center 1997; 642-54.
57. Rusnak JM, Smith LA. Botulinum neurotoxin vaccines: Past history and recent developments. Hum Vaccin 2009; 5:794-805.
58. Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigational botulinum antitoxin E. MMWR Morb Mortal Wkly Rep 2010; 59:299.
59. Franz DR, Pitt LM, Clayton MA, Hanes MA, Rose KJ. Efficacy of Prophylactic and Therapeutic Administration of Antitoxin for Inhalation Botulism. In: DasGupta BR, Ed. Botulinum and Tetanus Neurotoxins. New York: Plenum Press 1993; 473-6.
60. Demarest SJ, Hariharan M, Elia M, Salbato J, Jin P, Bird C, et al. Neutralization of *Clostridium difficile* toxin A using antibody combinations. MAbs 2010; 2.
61. Nowakowski A, Wang C, Powers DB, Amersdorfer P, Smith TJ, Montgomery VA, et al. Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody. Proc Natl Acad Sci USA 2002; 99:11346-50.
62. Yu R, Wang S, Yu YZ, Du WS, Yang F, Yu WY, et al. Neutralizing antibodies of botulinum neurotoxin serotype A screened from a fully synthetic human antibody phage display library. J Biomol Screen 2009; 14:991-8.
63. Adekar SP, Jones RM, Elias MD, Al-Saleem FH, Root MJ, Simpson LL, et al. Hybridoma populations enriched for affinity-matured human IgGs yield high-affinity antibodies specific for botulinum neurotoxins. J Immunol Methods 2008; 333:156-66.
64. Adekar SP, Takahashi T, Jones RM, Al-Saleem FH, Ancharski DM, Root MJ, et al. Neutralization of botulinum neurotoxin by a human monoclonal antibody specific for the catalytic light chain. PLoS One 2008; 3:3023.
65. Adekar SP, Segan AT, Chen C, Bermudez R, Elias MD, Selling BH, et al. Enhanced neutralization potency of botulinum neurotoxin antibodies using a red blood cell-targeting fusion protein. PLoS One 2011; 6:17491.
66. Garcia-Rodriguez C, Geren IN, Lou J, Conrad F, Forsyth C, Wen W, et al. Neutralizing human monoclonal antibodies binding multiple serotypes of botulinum neurotoxin. Protein Eng Des Sel 2010; 24:321-31.
67. Lou J, Geren I, Garcia-Rodriguez C, Forsyth CM, Wen W, Knopp K, et al. Affinity maturation of human botulinum neurotoxin antibodies by light chain shuffling via yeast mating. Protein Eng Des Sel 2010; 23:311-9.
68. Fenner F, Henderson DA, Jezek A, Ladnyi ID. Smallpox and Its Eradication. Geneva, Switzerland: World Health Organization 1988.
69. Nicas M, Hubbard AE, Jones RM, Reingold AL. The Infectious Dose of Variola (Smallpox) Virus. Applied Biosafety 2004; 9:118-27.
70. Sodeik B, Krijnse-Locker J. Assembly of vaccinia virus revisited: de novo membrane synthesis or acquisition from the host? Trends Microbiol 2002; 10:15-24.
71. Amanna IJ, Slifka MK, Crotty S. Immunity and immunological memory following smallpox vaccination. Immunol Rev 2006; 211:320-37.

72. Beachkofsky TM, Carrizales SC, Bidinger JJ, Hrncir DE, Whittemore DE, Hivnor CM. Adverse events following smallpox vaccination with ACAM2000 in a military population. Arch Dermatol 2010; 146:656-61.
73. Nalca A, Zumbrun EE. ACAM2000: the new smallpox vaccine for United States Strategic National Stockpile. Drug Des Devel Ther 2010; 4:71-9.
74. Monath TP, Caldwell JR, Mundt W, Fusco J, Johnson CS, Buller M, et al. ACAM2000 clonal Vero cell culture vaccinia virus (New York City Board of Health strain)—a second-generation smallpox vaccine for biological defense. Int J Infect Dis 2004; 8:31-44.
75. Hopkins RJ, Lane JM. Clinical efficacy of intramuscular vaccinia immune globulin: a literature review. Clin Infect Dis 2004; 39:819-26.
76. Wittek R. Vaccinia immune globulin: current policies, preparedness and product safety and efficacy. Int J Infect Dis 2006; 10:193-201.
77. WHO. Report of the Twelth Meeting "Scientific Review of Variola Virus Research 1999–2010". WHO Advisory Committee on Variola Virus Research. Geneva, Switzerland 2010.
78. Kinet JP, Jouvin MH. Smallpox Monoclonal Antibody. In: Office USP, Ed. United States 2008.
79. Chen Z, Earl P, Americo J, Damon I, Smith SK, Zhou YH, et al. Chimpanzee/human mAbs to vaccinia virus B5 protein neutralize vaccinia and smallpox viruses and protect mice against vaccinia virus. Proc Natl Acad Sci USA 2006; 103:1882-7.
80. Chen Z, Earl P, Americo J, Damon I, Smith SK, Yu F, et al. Characterization of chimpanzee/human monoclonal antibodies to vaccinia virus A33 glycoprotein and its variola virus homolog in vitro and in a vaccinia virus mouse protection model. J Virol 2007; 81:8989-95.
81. McCausland MM, Benhnia MR, Crickard L, Laudenslager J, Granger SW, Tahara T, et al. Combination therapy of vaccinia virus infection with human anti-H3 and anti-B5 monoclonal antibodies in a small animal model. Antivir Ther 2010; 15:661-75.
82. Oyston PC, Sjostedt A, Titball RW. Tularaemia: bioterrorism defence renews interest in *Francisella tularensis*. Nat Rev Microbiol 2004; 2:967-78.
83. Saslaw S, Eigelsbach HT, Wilson HE, Prior JA, Carhart S. Tularemia vaccine study. I. Intracutaneous challenge. Arch Intern Med 1961; 107:689-701.
84. Saslaw S, Eigelsbach HT, Prior JA, Wilson HE, Carhart S. Tularemia vaccine study. II. Respiratory challenge. Arch Intern Med 1961; 107:702-14.
85. Conlan JW, Zhao X, Harris G, Shen H, Bolanowski M, Rietz C, et al. Molecular immunology of experimental primary tularemia in mice infected by respiratory or intradermal routes with type A *Francisella tularensis*. Mol Immunol 2008; 45:2962-9.
86. Dennis DT, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Tularemia as a biological weapon: medical and public health management. Jama 2001; 285:2763-73.
87. Twine SM, Petit MD, Fulton KM, House RV, Conlan JW. Immunoproteomics analysis of the murine antibody response to vaccination with an improved *Francisella tularensis* live vaccine strain (LVS). PLoS One 2010; 5:10000.
88. McCrumb FR. Aerosol Infection of Man with Pasteurella Tularensis. Bacteriol Rev 1961; 25:262-7.
89. Hornick RB, Eigelsbach HT. Aerogenic immunization of man with live Tularemia vaccine. Bacteriol Rev 1966; 30:532-8.
90. Kirimanjeswara GS, Golden JM, Bakshi CS, Metzger DW. Prophylactic and therapeutic use of antibodies for protection against respiratory infection with *Francisella tularensis*. J Immunol 2007; 179:532-9.
91. Savitt AG, Mena-Taboada P, Monsalve G, Benach JL. *Francisella tularensis* infection-derived monoclonal antibodies provide detection, protection and therapy. Clin Vaccine Immunol 2009; 16:414-22.
92. Lu Z, Roche MI, Hui JH, Unal B, Felgner PL, Gulati S, et al. Generation and characterization of hybridoma antibodies for immunotherapy of tularemia. Immunol Lett 2007; 112:92-103.
93. Crane DD, Warner SL, Bosio CM. A novel role for plasmin-mediated degradation of opsonizing antibody in the evasion of host immunity by virulent, but not attenuated, *Francisella tularensis*. J Immunol 2009; 183:4593-600.
94. Outbreak of Marburg virus hemorrhagic fever—Angola, October 1, 2004–March 29, 2005. MMWR Morb Mortal Wkly Rep 2005; 54:308-9.
95. Leffel EK, Reed DS. Marburg and Ebola viruses as aerosol threats. Biosecur Bioterror 2004; 2:186-91.
96. Mupapa K, Massamba M, Kibadi K, Kuvula K, Bwaka A, Kipasa M, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. J Infect Dis 1999; 179:18-23.
97. Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, Jahrling PB, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. Jama 2002; 287:2391-405.
98. Maruyama T, Parren PW, Sanchez A, Rensink I, Rodriguez LL, Khan AS, et al. Recombinant human monoclonal antibodies to Ebola virus. J Infect Dis 1999; 179:235-9.
99. Maruyama T, Rodriguez LL, Jahrling PB, Sanchez A, Khan AS, Nichol ST, et al. Ebola virus can be effectively neutralized by antibody produced in natural human infection. J Virol 1999; 73:6024-30.
100. Parren PW, Geisbert TW, Maruyama T, Jahrling PB, Burton DR. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. J Virol 2002; 76:6408-12.
101. Oswald WB, Geisbert TW, Davis KJ, Geisbert JB, Sullivan NJ, Jahrling PB, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. PLoS Pathog 2007; 3:9.
102. Meissner F, Maruyama T, Frentsch M, Hessell AJ, Rodriguez LL, Geisbert TW, et al. Detection of antibodies against the four subtypes of ebola virus in sera from any species using a novel antibody-phage indicator assay. Virology 2002; 300:236-43.
103. Shedlock DJ, Bailey MA, Popernack PM, Cunningham JM, Burton DR, Sullivan NJ. Antibody-mediated neutralization of Ebola virus can occur by two distinct mechanisms. Virology 2010; 401:228-35.
104. Mense MG, Borschel RH, Wilhelmsen CL, Pitt ML, Hoover DL. Pathologic changes associated with brucellosis experimentally induced by aerosol exposure in rhesus macaques (*Macaca mulatta*). Am J Vet Res 2004; 65:644-52.
105. Silva TM, Costa EA, Paixao TA, Tsolis RM, Santos RL. Laboratory animal models for brucellosis research. J Biomed Biotechnol 2011; 2011:518323.
106. Solera J. Update on brucellosis: therapeutic challenges. Int J Antimicrob Agents 2010; 36:18-20.
107. WHO. Technical Report Series 740, Sixth Report. Joint FAO/WHO Expert Committee on Brucellosis. Geneva, Switzerland: World Health Organization 1986.
108. Vizcaino N, Fernandez-Lago L. Protection and suppression of the humoral immune response in mice mediated by a monoclonal antibody against the M epitope of Brucella. FEMS Immunol Med Microbiol 1994; 8:133-9.
109. Elzer PH, Jacobson RH, Jones SM, Nielsen KH, Douglas JT, Winter AJ. Antibody-mediated protection against *Brucella abortus* in BALB/c mice at successive periods after infection: variation between virulent strain 2308 and attenuated vaccine strain 19. Immunology 1994; 82:651-8.
110. Laurent TC, Mertens P, Dierick JF, Letesson JJ, Lambert C, De Bolle X. Functional, molecular and structural characterisation of five anti-Brucella LPS mAb. Mol Immunol 2004; 40:1237-47.
111. Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R, et al. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J Clin Microbiol 2003; 41:2068-79.
112. Inglis TJ, Sousa AQ. The public health implications of melioidosis. Braz J Infect Dis 2009; 13:59-66.
113. Galyov EE, Brett PJ, DeShazer D. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. Annu Rev Microbiol 2010; 64:495-517.
114. Tan GY, Liu Y, Sivalingam SP, Sim SH, Wang D, Paucod JC, et al. *Burkholderia pseudomallei* aerosol infection results in differential inflammatory responses in BALB/c and C57Bl/6 mice. J Med Microbiol 2008; 57:508-15.
115. Russell P, Eley SM, Ellis J, Green M, Bell DL, Kenny DJ, et al. Comparison of efficacy of ciprofloxacin and doxycycline against experimental melioidosis and glanders. J Antimicrob Chemother 2000; 45:813-8.
116. Bottex C, Gauthier YP, Hagen RM, Finke EJ, Splettstosser WD, Thibault FM, et al. Attempted passive prophylaxis with a monoclonal anti-Burkholderia pseudomallei exopolysaccharide antibody in a murine model of melioidosis. Immunopharmacol Immunotoxicol 2005; 27:565-83.
117. Gauthier YP, Hagen RM, Brochier GS, Neubauer H, Splettstoesser WD, Finke EJ, et al. Study on the pathophysiology of experimental *Burkholderia pseudomallei* infection in mice. FEMS Immunol Med Microbiol 2001; 30:53-63.
118. Bricaire F, Bossi P. Maladie du charbon. Bioterrorisme. Paris: Elsevier 2003; 22-8.
119. Trevino SR, Permenter AR, England MJ, Parthasarathy N, Gibbs PH, Waag DM, et al. Monoclonal antibodies passively protect BALB/c mice against *Burkholderia mallei* aerosol challenge. Infect Immun 2006; 74:1958-61.
120. Jones SM, Ellis JF, Russell P, Griffin KF, Oyston PC. Passive protection against *Burkholderia pseudomallei* infection in mice by monoclonal antibodies against capsular polysaccharide, lipopolysaccharide or proteins. J Med Microbiol 2002; 51:1055-62.
121. Roy CJ, Hale M, Hartings JM, Pitt L, Duniho S. Impact of inhalation exposure modality and particle size on the respiratory deposition of ricin in BALB/c mice. Inhal Toxicol 2003; 15:619-38.
122. Wannamacher RJA. Inhalation ricin: aerosol procedures, animal toxicology and therapy. In: Salem H, Ed. Inhalation Toxicology. Boca Raton, FL: CRC Press 2005; 973-9.
123. Poli MA, Rivera VR, Pitt ML, Vogel P. Aerosolized specific antibody protects mice from lung injury associated with aerosolized ricin exposure. Toxicon 1996; 34:1037-44.
124. Guo JW, Shen BF, Feng JN, Sun YX, Yu M, Hu MR. A novel neutralizing monoclonal antibody against cell-binding polypeptide of ricin. Hybridoma (Larchmt) 2005; 24:263-6.
125. Guo J, Shen B, Sun Y, Yu M, Hu M. A novel neutralizing monoclonal antibody against both ricin toxin A and ricin toxin B, and application of a rapid sandwich enzyme-linked immunosorbent assay. Hybridoma (Larchmt) 2006; 25:225-9.
126. Wang Y, Guo L, Zhao K, Chen J, Feng J, Sun Y, et al. Novel chimeric anti-ricin antibody C4C13 with neutralizing activity against ricin toxicity. Biotechnol Lett 2007; 29:1811-6.
127. Pratt TS, Pincus SH, Hale ML, Moreira AL, Roy CJ, Tchou-Wong KM. Oropharyngeal aspiration of ricin as a lung challenge model for evaluation of the therapeutic index of antibodies against ricin A-chain for post-exposure treatment. Exp Lung Res 2007; 33:459-81.
128. Pelat T, Hust M, Hale M, Lefranc MP, Dubel S, Thullier P. Isolation of a human-like antibody fragment (scFv) that neutralizes ricin biological activity. BMC Biotechnol 2009; 9:60.

129. Lowell GH, Colleton C, Frost D, Kaminski RW, Hughes M, Hatch J, et al. Immunogenicity and efficacy against lethal aerosol staphylococcal enterotoxin B challenge in monkeys by intramuscular and respiratory delivery of proteosome-toxoid vaccines. Infect Immun 1996; 64:4686-93.
130. Stiles BG, Bavari S, Krakauer T, Ulrich RG. Toxicity of staphylococcal enterotoxins potentiated by lipopolysaccharide: major histocompatibility complex class II molecule dependency and cytokine release. Infect Immun 1993; 61:5333-8.
131. Mattix ME, Hunt RE, Wilhelmsen CL, Johnson AJ, Baze WB. Aerosolized staphylococcal enterotoxin B-induced pulmonary lesions in rhesus monkeys (*Macaca mulatta*). Toxicol Pathol 1995; 23:262-8.
132. Vlach KD, Boles JW, Stiles BG. Telemetric evaluation of body temperature and physical activity as predictors of mortality in a murine model of staphylococcal enterotoxic shock. Comp Med 2000; 50:160-6.
133. DaSilva L, Welcher BC, Ulrich RG, Aman MJ, David CS, Bavari S. Humanlike immune response of human leukocyte antigen-DR3 transgenic mice to staphylococcal enterotoxins: a novel model for superantigen vaccines. J Infect Dis 2002; 185:1754-60.
134. Miethke T, Wahl C, Holzmann B, Heeg K, Wagner H. Bacterial superantigens induce rapid and T cell receptor V beta-selective downregulation of L-selectin (gp90Mel-14) in vivo. J Immunol 1993; 151:6777-82.
135. Krakauer T, Buckley M. Dexamethasone attenuates staphylococcal enterotoxin B-induced hypothermic response and protects mice from superantigen-induced toxic shock. Antimicrob Agents Chemother 2006; 50:391-5.
136. Huzella LM, Buckley MJ, Alves DA, Stiles BG, Krakauer T. Central roles for IL-2 and MCP-1 following intranasal exposure to SEB: a new mouse model. Res Vet Sci 2009; 86:241-7.
137. Boles JW, Pitt ML, LeClaire RD, Gibbs PH, Torres E, Dyas B, et al. Generation of protective immunity by inactivated recombinant staphylococcal enterotoxin B vaccine in nonhuman primates and identification of correlates of immunity. Clin Immunol 2003; 108:51-9.
138. Boles JW, Pitt ML, LeClaire RD, Gibbs PH, Ulrich RG, Bavari S. Correlation of body temperature with protection against staphylococcal enterotoxin B exposure and use in determining vaccine dose-schedule. Vaccine 2003; 21:2791-6.
139. Inskeep TK, Stahl C, Odle J, Oakes J, Hudson L, Bost KL, et al. Oral vaccine formulations stimulate mucosal and systemic antibody responses against staphylococcal enterotoxin B in a piglet model. Clin Vaccine Immunol 2010; 17:1163-9.
140. Wright A, Andrews PL, Titball RW. Induction of emetic, pyrexic and behavioral effects of *Staphylococcus aureus* enterotoxin B in the ferret. Infect Immun 2000; 68:2386-9.
141. Hu DL, Omoe K, Shimoda Y, Nakane A, Shinagawa K. Induction of emetic response to staphylococcal enterotoxins in the house musk shrew (*Suncus murinus*). Infect Immun 2003; 71:567-70.
142. Yanagisawa C, Hanaki H, Natae T, Sunakawa K. Neutralization of staphylococcal exotoxins in vitro by human-origin intravenous immunoglobulin. J Infect Chemother 2007; 13:368-72.
143. Kato K, Sakamoto T, Ito K. Gamma-globulin inhibits superantigen-induced lymphocyte proliferation and cytokine production. Allergol Int 2007; 56:439-44.
144. LeClaire RD, Hunt RE, Bavari S. Protection against bacterial superantigen staphylococcal enterotoxin B by passive vaccination. Infect Immun 2002; 70:2278-81.
145. Tilahun ME, Rajagopalan G, Shah-Mahoney N, Lawlor RG, Tilahun AY, Xie C, et al. Potent neutralization of staphylococcal enterotoxin B by synergistic action of chimeric antibodies. Infect Immun 2010; 78:2801-11.
146. Drozdowski B, Zhou Y, Kline B, Spidel J, Chan YY, Albone E, et al. Generation and characterization of high affinity human monoclonal antibodies that neutralize staphylococcal enterotoxin B. J Immune Based Ther Vaccines 2010; 8:9.
147. Ulrich RG, Olson MA, Bavari S. Development of engineered vaccines effective against structurally related bacterial superantigens. Vaccine 1998; 16:1857-64.
148. Stiles BG, Garza AR, Ulrich RG, Boles JW. Mucosal vaccination with recombinantly attenuated staphylococcal enterotoxin B and protection in a murine model. Infect Immun 2001; 69:2031-6.
149. Larkin EA, Stiles BG, Ulrich RG. Inhibition of toxic shock by human monoclonal antibodies against staphylococcal enterotoxin B. PLoS One 2010; 5:13253.
150. Berstein B. The birth of the US biological-warfare program. Sci Am 1987; 256:116-21.
151. Steele KE, Twenhafel NA. REVIEW PAPER: pathology of animal models of alphavirus encephalitis. Vet Pathol 2010; 47:790-805.
152. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalomyelitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. J Infect Dis 1977; 136:354-9.
153. Bennett AM, Lescott T, Phillpotts RJ. Improved protection against Venezuelan equine encephalitis by genetic engineering of a recombinant vaccinia virus. Viral Immunol 1998; 11:109-17.
154. Goodchild SA, O'Brien LM, Steven J, Muller MR, Lanning OJ, Logue CH, et al. A humanised murine monoclonal antibody with broad serogroup specificity protects mice from challenge with Venezuelan equine encephalitis virus. Antiviral Res 2011.
155. Hunt AR, Frederickson S, Hinkel C, Bowdish KS, Roehrig JT. A humanized murine monoclonal antibody protects mice either before or after challenge with virulent Venezuelan equine encephalomyelitis virus. J Gen Virol 2006; 87:2467-76.
156. Hu WG, Phelps AL, Jager S, Chau D, Hu CC, O'Brien LM, et al. A recombinant humanized monoclonal antibody completely protects mice against lethal challenge with Venezuelan equine encephalitis virus. Vaccine 2010; 28:5558-64.
157. Kirsch MI, Hulseweh B, Nacke C, Rulker T, Schirrmann T, Marschall HJ, et al. Development of human antibody fragments using antibody phage display for the detection and diagnosis of Venezuelan equine encephalitis virus (VEEV). BMC Biotechnol 2008; 8:66.
158. Hunt AR, Frederickson S, Maruyama T, Roehrig JT, Blair CD. The first human epitope map of the alphaviral E1 and E2 proteins reveals a new E2 epitope with significant virus neutralizing activity. PLoS Negl Trop Dis 2010; 4:739.
159. Strockbine NA, Marques LR, Holmes RK, O'Brien AD. Characterization of monoclonal antibodies against Shiga-like toxin from *Escherichia coli*. Infect Immun 1985; 50:695-700.
160. Perera LP, Marques LR, O'Brien AD. Isolation and characterization of monoclonal antibodies to Shiga-like toxin II of enterohemorrhagic *Escherichia coli* and use of the monoclonal antibodies in a colony enzyme-linked immunosorbent assay. J Clin Microbiol 1988; 26:2127-31.
161. Edwards AC, Melton-Celsa AR, Arbuthnott K, Stinson JR, Schmitt CK, Wong HC, et al. Vero Cell Neutralization and Mouse Protective Efficacy of Humanized Monoclonal Antibodies Against Escherichia Coli Toxins Stx1 and Stx2. In: Kaper JB, O'Brien AD, Eds. *Escherichia coli* O157:H7 and Other Shiga Toxin Producing E coli Strains. Washington DC American Society for Microbiology 1998; 388-92.
162. Smith MJ, Carvalho HM, Melton-Celsa AR, O'Brien AD. The 13C4 monoclonal antibody that neutralizes Shiga toxin Type 1 (Stx1) recognizes three regions on the Stx1 B subunit and prevents Stx1 from binding to its eukaryotic receptor globotriaosylceramide. Infect Immun 2006; 74:6992-8.
163. Smith MJ, Melton-Celsa AR, Sinclair JF, Carvalho HM, Robinson CM, O'Brien AD. Monoclonal antibody 11E10, which neutralizes shiga toxin type 2 (Stx2), recognizes three regions on the Stx2 A subunit, blocks the enzymatic action of the toxin in vitro, and alters the overall cellular distribution of the toxin. Infect Immun 2009; 77:2730-40.
164. Bitzan M, Poole R, Mehran M, Sicard E, Brockus C, Thuning-Roberson C, et al. Safety and pharmacokinetics of chimeric anti-Shiga toxin 1 and anti-Shiga toxin 2 monoclonal antibodies in healthy volunteers. Antimicrob Agents Chemother 2009; 53:3081-7.
165. Sheoran AS, Chapman-Bonofiglio S, Harvey BR, Mukherjee J, Georgiou G, Donohue-Rolfe A, et al. Human antibody against shiga toxin 2 administered to piglets after the onset of diarrhea due to *Escherichia coli* O157:H7 prevents fatal systemic complications. Infect Immun 2005; 73:4607-13.
166. Sheoran AS, Chapman S, Singh P, Donohue-Rolfe A, Tzipori S. Stx2-specific human monoclonal antibodies protect mice against lethal infection with *Escherichia coli* expressing Stx2 variants. Infect Immun 2003; 71:3125-30.
167. Dharmasena MN, Krebs SJ, Taylor RK. Characterization of a novel protective monoclonal antibody that recognizes an epitope common to *Vibrio cholerae* Ogawa and Inaba serotypes. Microbiology 2009; 155:2353-64.
168. Hu J, Robinson JL. Treatment of respiratory syncytial virus with palivizumab: a systematic review. World J Pediatr 2010; 6:296-300.
169. Attree O, Guglielmo-Viret V, Gros V, Thullier P. Development and comparison of two immunoassay formats for rapid detection of botulinum neurotoxin type A. J Immunol Methods 2007; 325:78-87.
170. Thullier P, Griffiths G. Broad recognition of ricin toxins prepared from a range of Ricinus cultivars using immunochromatographic tests. Clin Toxicol (Phila) 2009; 47:643-50.
171. Hunt AR, Bowen RA, Frederickson S, Maruyama T, Roehrig JT, Blair CD. Treatment of mice with human monoclonal antibody 24 h after lethal aerosol challenge with virulent Venezuelan equine encephalitis virus prevents disease but not infection. Virology 2011; 414:146-52.
172. Zhang MY, Borges AR, Ptak RG, Wang Y, Dimitrov AS, Alam SM, et al. Potent and broad neutralizing activity of a single chain antibody fragment against cell-free and cell-associated HIV-1. MAbs 2010; 2:266-74.