Chapter 21

MEDICAL COUNTERMEASURES

JANICE M. RUSNAK, MD[*]; ELLEN F. BOUDREAU, MD[†]; MATTHEW J. HEPBURN, MD[‡]; JAMES W. MARTIN, MD, FACP[§]; AND SINA BAVARI, PhD[¥]

[*]*Lieutenant Colonel, US Air Force (Ret); Research Physician, Special Immunizations Program, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702; formerly, Deputy Director of Special Immunizations Program, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland*

[†]*Chief, Special Immunizations Program, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702*

[‡]*Major, Medical Corps, US Army; Infectious Diseases Physician, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702*

[§]*Colonel, Medical Corps, US Army; Chief, Operational Medicine Department, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702*

[¥]*Chief, Department of Immunology, Target Identification and Translational Research, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702*

INTRODUCTION

Countermeasures against bioterrorism to prevent or limit the number of secondary infections or intoxications include (*a*) early identification of the bioterrorism event and persons exposed, (*b*) appropriate decontamination, (*c*) infection control, and (*d*) medical countermeasures. The initial three countermeasures are nonmedical and discussed in other chapters. This chapter will be restricted to medical countermeasures, which include interventions such as active immunoprophylaxis (ie, vaccines), passive immunoprophylaxis (ie, immunoglobulins and antitoxins), and chemoprophylaxis (ie, postexposure antibiotic prophylaxis) (Tables 21-1 and 21-2). Medical countermeasures may be initiated either before an exposure (if individuals are identified as being at high risk for exposure) or after a confirmed exposure event. Because medical countermeasures

TABLE 21-1

VACCINES, VACCINE DOSAGE SCHEDULES, AND POSTVACCINATION PROTECTION

Vaccine	Primary Series	Protection	Booster Doses
Anthrax (0.5 mL SQ)	Days 1, 14, 28 Months 6, 12, 18	3 weeks after 3rd vaccine dose	Annual boosters after dose 6 of vaccine
Tularemia[*],[†] (15 punctures PC)	Day 0	"Take" after vaccination	Every 10 years[†]
Q fever[‡] (0.5 mL SQ)	Day 0	3 weeks after vaccination	None
VEE C-83[*],[§] (0.5 mL SQ)	Day 0	Titer $\geq$ 1:20	None (boost with TC-84)[¥]
VEE TC-84[§] (0.5 mL SQ)	Day 0	Titer $\geq$ 1:20	As needed per titer[¥]
EEE[¶] (0.5 mL SQ)	Days 0, 7, 28	Titer $\geq$ 1:40	As needed per titer[¥]
WEE[¶]	Days 0, 7, 28	Titer $\geq$ 1:40	As needed per titer[¥]
Yellow fever[*] (0.5 mL SQ)	Day 0	4 weeks after vaccination	Every 10 years
Smallpox[*],[**] (3 punctures PC for primary vaccination)	Day 0	Evidence of a "take" (vesiculo-papular response); Scab resolved (day 21-28 after vaccination)	1, 3, or 10 years[**]
RVF (1 mL SQ)	Days 0, 7, 28, 180	Titer $\geq$ 1:40 after dose 3	As needed per titer[¥]
Junin[*],[††] (0.5 mL IM)	Day 0	4 weeks after vaccination	None
TBE[§§] (0.5 mL SQ)	Days 0, 30	2 weeks after 2nd vaccine dose	Every 3 years
PBT[¥¥] (0.5 mL SQ)	Days 0, 14, 84, and month 6	Potential protection within 4 weeks of 3rd vaccine dose (antitoxin titers no longer obtained)	Booster dose at 12 months and then yearly

[*] Live vaccine.
[†] Investigational live attenuated tularemia NDBR 101 vaccine. Booster doses currently recommended every 10 years, although immunity may persist longer.
[‡] Investigational inactivated freeze-dried Q Fever NDBR 105 vaccine.
[§] Investigational live attenuated TC-83 NDBR 102 VEE vaccine is given as a one-time injection. $PRNT_{80}$ titers were obtained after vaccination and yearly to assess for adequate titers. If $PRNT_{80}$ titers fell below a predetermined level, another investigational vaccine, the inactivated C-84 TSI-GSD-205 VEE vaccine, was given to boost titers.
[¥] $PRNT_{80}$ titers. Titers are obtained within 28 days of the primary series and yearly afterward to assess immune response. Booster doses for EEE were administered as 0.1 mL intradermally.
[¶] Investigational inactivated TSI-GSD-104 EEE and TSI-GSD-210 WEE vaccines.
[**] Booster doses are administered as 15 punctures PC, given every 10 years, but may be recommended more frequently if high risk of exposure (ie, smallpox outbreak, laboratory workers). Laboratory workers are given booster doses every 3 years if working with monkeypox and yearly if working with variola (variola research only at CDC).
[††] Investigational live attenuated AHF virus vaccine (Candid 1).
[§§] Investigational FSME-IMMUN inject vaccine.
[¥¥] Investigational botulinum pentavalent (ABCDE) botulinum toxoid.
CDC: Centers for Disease Control and Prevention; EEE: eastern equine encephalitis; IM: intramuscular; MA: microagglutination titer; PBT: pentavalent botulinum toxoid; PC: percutaneous; $PRNT_{80}$: 80% plaque reduction neutralization titer; RVF: Rift Valley fever; SQ: subcutaneous; TBE: tick-borne encephalitis; VEE: Venezuelan equine encephalitis; WEE: western equine encephalitis

TABLE 21-2

POSTEXPOSURE ANTIBIOTIC PROPHYLAXIS REGIMENS

Agent	Antibiotic	Duration of Treatment
Bacillus anthracis[*]	Ciprofloxacin, doxycycline, or penicillin (if sensitive)	Vaccinated: 30 days (aerosol) Unvaccinated: 60 days (aerosol)
Yersinia pestis	Doxycycline or ciprofloxacin	7 days
Francisella tularensis	Doxycycline or ciprofloxacin	14 days
Burkholderia mallei	Doxycycline, trimethoprim-sulfamethoxazole, augmentin, or ciprofloxacin	14 days (consider 21 days)[†]
B pseudomallei	Doxycycline, trimethoprim-sulfamethoxazole (possibly ciprofloxacin)	14 days (consider 21 days)[†]
Brucella	Doxycycline plus rifampin	21 days
Coxiella burnetii	Doxycycline	7 days (not to be given before day 8 after exposure because it may only prolong the incubation period)

[*]Advisory Committee on Immunization Practices membership notes no data on postexposure prophylaxis for preventing cutaneous anthrax but suggests 7- to 14-day course of antibiotics may be considered.
[†]No clinical data to support

may be associated with adverse events, the recommendation for their use must be weighed against the risk of exposure and disease. Vaccines, both investigational and approved by the Food and Drug Administration (FDA), are available for some bioterrorism agents. In the event of a bioterrist incident, preexposure vaccination, if safe and available, may modify or eliminate the need for postexposure chemoprophylaxis. However, preexposure vaccination may not be possible or practical in the absence of a known or expected release of a specific bioterrorist agent, particularly with vaccinations that require booster doses to maintain immunity. In these cases, chemoprophylaxis after identifying an exposure may be effective in preventing disease. Any effective bioterrorism plan should address the logistics of maintaining adequate supplies of drugs and vaccines, as well as personnel to coordinate and dispense needed supplies to the affected site.

Although the anthrax and smallpox vaccines are both FDA approved, potential bioterrorism agents have only investigational vaccines that were developed and manufactured over 30 years ago. These vaccines have demonstrated efficacy in animal models and safety in at-risk laboratory workers; however, they did not qualify for FDA approval because studies to demonstrate their efficacy in humans were deemed unsafe and unethical. Although these vaccines can be obtained under investigational new drug (IND) protocols at limited sites in the United States, the vaccines are in extremely limited supply and are declining in immunogenicity with age.

Under the FDA animal rule instituted in 2002, approval of vaccines can now be based on demonstration of efficacy in animal models alone, if efficacy studies in humans would be unsafe or unethical. This rule has opened the opportunity to develop many new and improved vaccines, with the ultimate goal of FDA licensure. Vaccine development generally is a long process, requiring 3 to 5 years to identify a potential vaccine candidate and conduct animal studies to test for vaccine immunogenicity and efficacy, with an additional 5 years of clinical trials for FDA approval and licensure. FDA vaccine approval then takes from 7 to 10 years, so vaccine replacements are not expected to be available in the near future.

BACTERIAL AND RICKETTSIAL DISEASES

Anthrax

Anthrax is caused by *Bacillus anthracis,* a spore-forming, gram-positive bacillus. Associated disease may occur in wildlife such as deer and bison in the United States but occurs most frequently in domestic animals such as sheep, goats, and cattle, which acquire spores by ingesting contaminated soil. Humans can become infected through skin contact, ingestion, or inhalation of *B anthracis* spores from infected animals or animal products. Anthrax is not transmissible from person to person. The infective dose for inhalational anthrax based on nonhuman primate studies is estimated to be 8,000 to 50,000 spores.[1,2] The 2001 anthrax

incident suggests that inhalational anthrax may result from inhalation of relatively few spores with exposure to small particles of aerosolized anthrax.[3] The stability and prolonged survival of the spore stage makes *B anthracis* an ideal agent for bioterrorism.

Vaccination

History of the anthrax vaccine. In 1947 a factor isolated from the edema fluid of cutaneous *B anthracis* lesions was noted to successfully vaccinate animals.[4] This factor, identified as the protective antigen (PA), was subsequently recovered from incubating *B anthracis* in special culture medium.[5,6] This led to the development in 1954 of the first anthrax vaccine, which was derived from an alum-precipitated cell-free filtrate of an aerobic culture of *B anthracis.*[7]

This early version of the anthrax vaccine was demonstrated to protect small laboratory animals[8] and nonhuman primates from inhalational anthrax.[7] The vaccine also demonstrated protection against cutaneous anthrax infections in employees working in textile mills processing raw imported goat hair.[8] During this study, only 3 cases of cutaneous anthrax occurred in 379 vaccinated employees, versus 18 cases of cutaneous anthrax and all 5 cases of inhalational anthrax that occurred in the 754 nonvaccinated employees. Based on these results, the vaccine efficacy for anthrax was determined to be 92.5%. The vaccine failures were noted in a person who had received only two doses of vaccine, a second person who had received the initial three doses of vaccine but failed to receive follow-up doses at 6 and 12 months (infection at 13 months), and a third person who was within a week of the fourth vaccine dose (the 6-month dose), a period when titers are known to be lower. Local reactions were noted in 35% of vaccinees, but most reactions were short-lived (generally resolving within 24 to 48 hours), with severe reactions occurring in only 2.8% in the vaccinated population.

Anthrax vaccine adsorbed. The current FDA-approved anthrax vaccine adsorbed (AVA) was derived through improvements of the early alum-precipitated anthrax vaccine and involved (*a*) using a *B anthracis* strain that produced a higher fraction of PA, (*b*) growing the culture under microaerophilic instead of aerobic conditions, and (*c*) substituting an aluminum hydroxide adjuvant in place of the aluminum potassium salt adjuvant.[9,10] Originally produced by the Michigan Department of Public Health, AVA is now manufactured by BioPort Corporation in Lansing, Michigan. AVA is derived from a sterile cell-free filtrate (with no dead or live bacteria) from cultures of an avirulent, nonencapsulated strain of *B anthracis* (toxinogenic, nonencapsulated V770-NP1-R), that produces predominantly PA in relative absence of other toxin components such as lethal factor or edema factor.[9,11] The filtrate used to produce AVA is adsorbed to aluminum hydroxide (Amphogel [Wyeth Laboratories, Madison, NJ]) as an adjuvant and contains PA, formaldehyde, and benzethonium chloride, with trace lethal factor and edema factor components.[11]

AVA is given as subcutaneous injections (in the upper deltoid muscle) of 0.5 mL at 0, 2, and 4 weeks, followed by injections at 6, 12, and 18 months, and then yearly boosters. Vaccine breakthroughs have been reported in persons who received only two doses of vaccine, but infections in those who received all three initial doses (and are current on subsequent primary and booster doses) are uncommon. The few published reports of breakthroughs occurred with use of the earlier, alum-precipitated anthrax vaccine and within days before the scheduled 6-month vaccine dose (dose 4), when antibody titers have been demonstrated to be low.[8,12]

Evidence suggests that both humoral and cellular immune responses against PA are critical to protection against disease after exposure.[9,13,14] Vaccinating rhesus macaques with one dose of AVA elicited anti-PA immunoglobulin (Ig) M titers peaking at 2 weeks after vaccination, IgG titers peaking at 4 to 5 weeks, and PA-specific lymphocyte proliferation present at 5 weeks.[15] Approximately 95% of vaccinees seroconvert with a 4-fold rise in anti-PA IgG titer after three doses of vaccine.[13,16] Although animal studies have demonstrated transfer of passive immunity from polyclonal antibodies,[17] the correlation of protection against anthrax infection with a specific antibody titer has not yet been defined.[13]

Both the alum-precipitated vaccine and AVA demonstrated efficacy in animal models against aerosol challenge.[6,7,10,13-15,18-20] A total of 52 of 55 monkeys (95%) given two doses of anthrax vaccine survived lethal aerosol challenge without antibiotics.[21] Because spore forms of *B anthracis* may persist for over 75 days after an inhalational exposure, vaccination against anthrax may provide more prolonged protection than postexposure antibiotic prophylaxis alone.[22,23] However, vaccination after exposure alone was not effective in preventing disease from inhalational anthrax. Vaccination of rhesus monkeys at days 1 and 15 after aerosol exposure did not protect against inhalational anthrax (4 x 10^5 spores, which is 8 median lethal doses) resulting in death in 8 of the 10 monkeys. However, all rhesus monkeys given 30 days of doxycycline in addition to postexposure vaccination survived.[24] Recent studies indicate that a short course of postexposure antibiotics (14 days) in conjunction with vaccination provides

significant protection against high dose aerosol challenge in nonhuman primates.[25]

Vaccine adverse events. Adverse reactions in 6,985 persons who received a total of 16,435 doses of AVA (9,893 initial series doses and 6,542 annual boosters) were primarily local reactions.[26] Local reactions (edema or induration) were severe (> 12 cm) in less than 1% vaccinations, moderate (3–12 cm) in 3% vaccinations, and mild (< 3 cm) in 20% vaccinations. Systemic reactions were uncommon, occurring in less than 0.06% of vaccines, and included fever, chills, body aches, or nausea.

Data from the Vaccine Adverse Event Reporting System from 1990 to 2000, after nearly 2 million doses of vaccine were distributed, showed approximately 1,500 adverse events reported from the vaccine. The most frequently reported events were injection site hypersensitivity (334), edema at the injection site (283), pain at the injection site (247), headache (239), arthralgia (232), asthenia (215), and pruritus (212). Only 76 events (5%) were serious, including the reporting of anaphylaxis in two cases.[27]

In an anthrax vaccine study conducted in laboratory workers and maintenance personnel at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) over 25 years, females were found to be more likely than males to have injection site reactions, edema, and lymphadenopathy.[28] Initial data also showed a decrease in the rate of local reactions if the time interval between the first and second dose was extended or if the vaccine was administered intramuscularly. No decrease in seroconversion rates or anti-PA IgG geometric mean titers was noted with either of these modifications of administration. Delay of the second vaccine dose to 4 weeks (instead of 2 weeks) was associated with induration in only 1 of 10 females (10%) and subcutaneous nodules in only 4 of 10 females (40%), versus 10 of 18 (56%) and 15 of 43 (83%), respectively, when the second vaccine dose was given at 2 weeks.[29] When AVA was administered intramuscularly at 0 and 4 weeks, none of the 10 persons exhibited induration or subcutaneous nodules, and only one person developed erythema. The Centers for Disease Control and Prevention (CDC) is conducting a large study to confirm these results.

Protocols for managing vaccine adverse events have not yet been evaluated in randomized trials. However, individuals with local adverse events may be managed with ibuprofen or acetaminophen for pain, second-generation antihistamines if localized itching is a dominant feature, and ice packs for severe swelling extending below the elbow. In special cases, to alleviate future discomfort for patients with large or persistent injection-site reactions after subcutaneous injection, the US Army Medical Command policy for troops allows intramuscular injection to be considered if the provider (*a*) believes intramuscular injection will provide appropriate protection and reduce side effects, and (*b*) informs the patient that intramuscular injection is not FDA approved.[30]

Additional anthrax vaccination is contraindicated in persons who have experienced an anaphylactic reaction to the vaccine or any of the vaccine components.[22] It is also contraindicated in persons with a history of anthrax infection because of previous observations of an increase in severe adverse events.[22] The vaccine may be given in pregnancy only if the benefit outweighs the risk.

Other anthrax vaccines. An attenuated live anthrax vaccine given by scarification or subcutaneous injection is used in the former Soviet Union. The vaccine is reported to be protective in mass field trials, in which anthrax occurred less commonly in vaccinated persons (2.1 cases per 100,000 persons), a risk reduction of cutaneous anthrax by a factor of 5.4 in the 18 months after vaccination.[31,32] A PA-based anthrax vaccine, made by alum precipitation of a cell-free culture filtrate of a derivative of the attenuated *B anthracis* Sterne strain, is currently licensed in the United Kingdom.[19,33]

New vaccine research. The ability to prepare purified components of anthrax toxin by recombinant technology has presented the possibility of new anthrax vaccines. New vaccine candidates may be PA toxoid vaccines or PA-producing live vaccines that elicit partial or complete protection against anthrax infection.[19] A recombinant PA vaccine candidate given intradermally or intranasally was demonstrated to provide complete protection in rabbits and nonhuman primates against aerosol challenge with anthrax spores.[34]

Recent research has shown toxin neutralization approaches to be protective in animal models. Inter-alpha inhibitor protein (IαIp), an endogenous serine protease inhibitor in human plasma, given to BALB/c mice 1 hour before intravenous challenge to a lethal dose of *B anthracis*, was associated with a 71% survival rate at 7 days compared to no survivors in the control groups.[35] One potential mechanism of action for IαIp is through the inhibition of furin, an enzyme required for assembling lethal toxin in anthrax pathogenesis.

Chemoprophylaxis

Antibiotics. Antibiotics are effective only against the vegetative form of *B anthracis* (not effective against the spore form). However, in the nonhuman primate model of inhalational anthrax, spores have been shown to survive for months (< 1% at 75 days and trace spores present at 100 days) without germination.[22-24]

Prolonged spore survival has not been observed for other routes of exposure.

Ciprofloxacin, doxycycline, and penicillin G procaine have been FDA approved for prophylaxis of inhalational anthrax.[2,11,22,24,36] Ciprofloxacin, doxycycline, and penicillin have been demonstrated in nonhuman primates to reduce the incidence or progression of disease after aerosol exposure to *B anthracis*.[22,24,36] Macaques exposed to 240,000 to 560,000 anthrax spores (8 median lethal doses) and given postexposure antibiotic prophylaxis with 30 days of penicillin, doxycyline, or ciprofloxacin resulted in survival of 7 of 10, 9 of 10, and 8 of 9 monkeys, respectively.[24] All animals survived while on prophylaxis, but three monkeys treated with penicillin died between days 39 and 50 postexposure, one monkey treated with doxycycline died day 58 postexposure, and one monkey treated with ciprofloxacin died day 36 postexposure. This phenomenon is attributed to delayed vegetation of spores that may persist in lung tissue after inhalational exposure.

To avoid toxicity in children and pregnant or lactating women exposed to penicillin-susceptible strains, amoxicillin given three times daily is an option. However, it is not recommended as a first-line treatment because it lacks FDA approval and its efficacy and ability to achieve adequate therapeutic levels at standard doses are uncertain. Because strains may be resistant to penicillin, amoxicillin should not be used until sensitivity testing has been performed.[22]

Duration of antibiotic prophylaxis. The optimal duration of postexposure antibiotic prophylaxis after aerosol exposure to *B anthracis* in unvaccinated individuals is 60 days, which is based on the results of the animal studies described above.[22,24,37] Spore survival in the lung tissue of Macaques exposed to 4 median lethal doses was estimated to be 15% to 20% at 42 days, 2% at 50 days, and less than 1% at 75 days.[22-24] The 1979 outbreak of inhalational anthrax after an accidental release of spores from a Soviet biological weapons production facility (the Sverdlovsk outbreak) suggests that lethal spores persisted after the initial exposure because cases of human anthrax developed as late as 43 days after the release.[38] Current recommendations for treating unvaccinated persons after aerosol exposure to *B anthracis* from the CDC, Advisory Committee for Immunization Practices (ACIP), and Occupational Safety and Health Administration, are for 60 days of either ciprofloxacin (500 mg twice daily) or doxycycline (100 mg twice daily).[22,37] Tetracycline may be a possible alternative for doxycycline, but it has not been well studied.

Adverse events of chemoprophylaxis. Adverse events associated with the prolonged, 60-day, antibiotic prophylaxis regimen have had a significant impact on compliance. Compliance was reported to be as low as 42% among the 10,000 persons in the 2001 incident at the Brentwood Post Office and Senate office building who were recommended to receive the regimen.[39] Adverse events reported by the 3,428 postal workers receiving postexposure prophylaxis with ciprofloxacin were primarily gastrointestinal symptoms of nausea, vomiting, or abdominal pain (19%); fainting, dizziness, or light-headedness (14%); heartburn or acid reflux (8%); and rash, hives, or itchy skin (7%).[40] Reasons for early discontinuation of ciprofloxacin included adverse events (3%), fear of possible adverse events (1%), and belief that the drug was unnecessary (1%). Other adverse events that can occur with quinolones but not reported in this survey include headache, tremors, restlessness, confusion, and Achilles tendon rupture.[40] Adverse events associated with tetracycline and amoxicillin were predominantly gastrointestinal symptoms.

Postexposure Vaccination With Chemoprophylaxis

Vaccination alone after exposure to *B anthracis* was not protective in preventing inhalational anthrax in nonhuman primates; therefore, AVA is not currently licensed for postexposure prophylaxis. Both the ACIP and CDC endorse making anthrax vaccine available for unvaccinated persons identified as at risk for inhalational exposure in a three-dose regimen (0, 2, and 4 weeks) in combination with antimicrobial postexposure prophylaxis under an IND application.[41] However, there is insufficient data to determine the duration of antibiotic prophylaxis when initiated with vaccination. Based on antibody titers peaking at 14 days after the third dose of AVA,[42] a recommendation of 30 days was suggested in persons already fully or partially immune, and perhaps 7 to 14 days after the third vaccine dose when the vaccine was initiated in conjunction with postexposure prophylaxis. Doxycycline given for 30 days after aerosol exposure resulted in survival of 9 of 10 monkeys, and doxycycline given for 30 days after aerosol exposure in conjunction with two doses of anthrax vaccine was protective in 9 of 9 monkeys challenged with *B anthracis*.[24] The addition of the vaccine may suggest a possible benefit, but the difference was not statistically different ($P = 0.4$) for this study.[24] However, recent nonhuman primate studies indicated that a 14-day course of oral ciprofloxacin in combination with AVA vaccination may significantly reduce the duration of postexposure prophylaxis, from 30 days to 14 days with a statistical significance of $P = 0.011$.[25] In this study, vaccine was provided on days 0, 14, and 30, with 100% protection (10/10) of nonhuman primates receiving a 14-day course of oral

ciprofloxacin and three doses of AVA vaccine. Because there are no prolonged spore stages with percutaneous and gastrointestinal exposures, the CDC does not recommend postexposure prophylaxis in these instances. However, the ACIP noted that there are no controlled studies of this issue and suggested a course of 7 to 14 days as prophylaxis for both cutaneous and gastrointestinal anthrax provided no inhalational exposure is suspected.[41,43]

Clinical Indications for Vaccine or Postexposure Antibiotic Prophylaxis

Evaluation for inhalational exposure to *B anthracis* includes a physical examination, laboratory tests, and chest radiograph, as indicated, to exclude active infection. Nasal swabs may be used for epidemiological purposes, but should not be used as a primary determinate for the initiation or cessation of postexposure antibiotic prophylaxis[44,45]; a negative nares culture does not exclude inhalational exposure to the organism. However, if an individual has a positive nares culture, postexposure antibiotic prophylaxis should be initiated.

Antibiotic prophylaxis should be initiated upon possible aerosol exposure to *B anthracis* and should be continued until *B anthracis* exposure has been excluded. If exposure is confirmed or cannot be excluded, prophylaxis should continue for 60 days duration in unvaccinated persons. In unvaccinated individuals who subsequently undergo vaccination, antibiotic prophylaxis should be continued for 7 days after the third dose of vaccine is administered. For persons with a history of anthrax vaccination who are within 1 year of their annual booster, a 30-day course of antibiotics should be sufficient. Individuals should be monitored for symptoms throughout the incubation period, lasting 1 to 7 days after percutaneous exposure or ingestion, and potentially up to 90 days following aerosol exposures.

Tularemia

Francisella tularensis, a highly infectious bacterial pathogen responsible for serious illness, and occasionally death, has long been recognized as a potential biological weapon.[46] Humans can acquire tularemia through (*a*) contact of skin or mucous membranes with the tissues or body secretions of infected animals; (*b*) bites of infected arthropeds (deerflies, mosquitoes, or ticks); (*c*) ingestion of contaminated food or water (less commonly); or (*d*) inhalation of aerosolized agent from infected animal secretions. Tularemia is not transmissible person to person. Because of the low infective dose (10–50 organisms) of *F tularensis*, disease may

readily develop when exposure is by the pulmonary route. This disease was the most common laboratory-acquired infection (153 cases) during the 25 years of the US Biological Warfare Program. These tularemia infections were acquired mainly from aerosol exposures.[12] Outbreaks of tularemia in nonendemic areas should alert officials to the possibility of a bioterrorism event.

Vaccination

Investigational live tularemia vaccine. No FDA-licensed vaccine protecting against tularemia is currently available. However, an investigational live attenuated vaccine given to at-risk researchers at Fort Detrick, Maryland, has been available since 1959. This vaccine is only available at USAMRIID under an IND protocol.

Vaccination of at-risk laboratory personnel with an inactivated phenolized tularemia vaccine (Foshay vaccine) during the US offensive biological warfare program at Fort Detrick before 1959 ameliorated disease but did not prevent infection.[47–49] A sample of the Soviet live tularemia vaccine (known as strain 15), which was used in millions of persons during epidemics of type B tularemia beginning in the 1930s, was made available to Fort Detrick in 1956.[48] Both a gray-variant and blue-variant colony were cultivated from this vaccine (colonies were blue when illuminated with oblique light under a dissecting microscope). The blue-variant colony was proven to be both more virulent and more immunogenic than the gray-variant colony. To improve protection against the virulent *F tularensis* SCHU S4 strain, the blue-variant colony was passaged through white mice to potentiate its virulence and immunogenicity. These passages subsequently resulted in the derivative vaccine strain known as the live vaccine strain (LVS). The strain was used to prepare a lyophilized preparation known as the live tularemia vaccine, which was composed of 99% blue-variant and 1% gray-variant colonies.

Beginning in 1959, the live attenuated tularemia vaccine, LVS, was administered to at-risk laboratory personnel in the offensive biological warfare program at Fort Detrick until closure of the program in 1969 (Figure 21-1).[47] Before vaccination, tularemia was the most frequently diagnosed laboratory-acquired infection, with mainly typhoidal/pneumonic and ulceroglandular disease manifestations. After vaccination, the incidence of typhoidal/pneumonic tularemia decreased from 5.7 to 0.27 cases per 1,000 at-risk employee–years. Although no decrease in ulceroglandular tularemia was noted during this time, the vaccine did ameliorate symptoms from ulceroglandular tularemia,
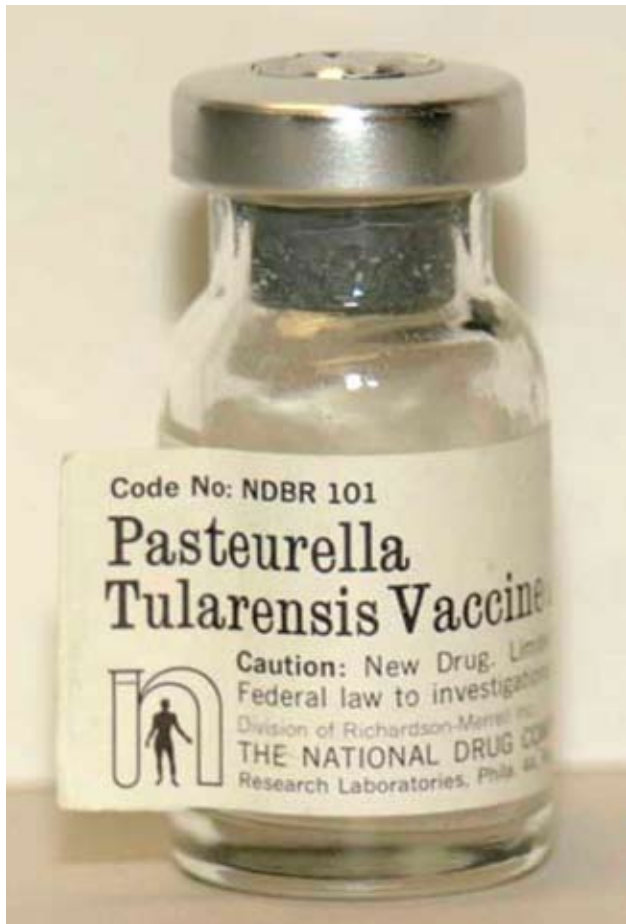
Fig. 21-1. Live attenuated NDBR 101 tularemia vaccine. Vaccination of at-risk laboratory workers, beginning in 1959, resulted in a decreased incidence of typhoidal tularemia from 5.7 to 0.27 cases per 100 at-risk employee–years, and ameliorated symptoms from ulceroglandular tularemia. The vaccine is administered by scarification with 15 to 30 pricks on the forearm, using a bifurcated needle.

and vaccinated persons no longer required hospitalization. The occurrence of ulceroglandular tularemia in vaccinated persons was consistent with the observation that natural disease also failed to confer immunity to subsequent infections of ulceroglandular tularemia. In 1961 commercial production of LVS was initiated by the National Drug Company, Swiftwater, Pennsylvania, under contract to the US Army Medical Research and Materiel Command; this vaccine was designated NDBR 101. The vaccine continues to be given as an investigational drug to at-risk laboratory workers in the US Biodefense Program.

The live attenuated NDBR 101 tularemia vaccine is supplied as a lyophilized preparation and reconstituted with sterile water before use, resulting in

approximately 7×10^8 viable organisms per mL. The vaccine is administered by scarification, with 15 to 30 pricks to the ulnar side of the forearm using a bifurcated needle and a droplet (approximately 0.1 mL) of the vaccine. The individual is examined after vaccination for a "take," similar to the examination done after smallpox vaccination. A take with tularemia vaccine is defined as the development of an erythematous papule, vesicle, and/or eschar with or without induration at the vaccination site; however, the postvaccination skin lesion is markedly smaller and has less induration than generally seen in vaccinia vaccinations. Although a take is related to immunity, its exact correlation has not yet been determined (Figure 21-2). Studies measuring cell-mediated immunity to tularemia in vaccinees are being undertaken to determine the duration of immunity from the vaccine.

Protective immunity against *F tularensis* is considered to be primarily cell mediated. Cell-mediated immunity has been correlated with a protective effect, and lack of cell-mediated immunity has been correlated with decreased protection.[50,51] Cell-mediated immunity responses occur within 1 to 4 weeks after naturally occurring infection or after LVS vaccination and reportedly last a long time (10 years or longer).[50,52–59] Absolute levels of agglutinating antibodies in persons vaccinated with aerosolized LVS could not be correlated with immunity, although the presence of agglutination antibodies in vaccinated persons suggested that they were more resistant to infection than the unvaccinated control group.[60] A similar experience was observed in

Fig. 21-2. "Take" from the live attenuated NDBR 101 tularemia vaccine at day 7 postvaccination.
Photograph: Courtesy of Special Immunizations Program, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

studies of the inactivated Foshay tularemia vaccine, in which antibodies were induced by the vaccine but were not protective against tularemia.[47,49] Although nearly all vaccinees develop a humoral response, with microagglutination titers appearing between 2 and 4 weeks postvaccination,[50,57,61] a correlation could not be demonstrated between antibody titers and the magnitude of lymphocyte proliferative responses.[51,59,62,63] An explanation for this discrepancy may be that the two types of immune responses are directed toward different antigenic determinants of the organism, with a protein determinant responsible for the cell-mediated immune response and a carbohydrate determinant causing the humoral response.[62]

Vaccine adverse events. The local skin lesion after vaccination (known as a take) is an expected occurrence and may result in the formation of a small scar. At the site of inoculation, a slightly raised erythematous lesion appears, which may become papular or vesicular and then form a scab lasting approximately 2 to 3 weeks. Local axillary lymphadenopathy is not uncommon, reported in 20% to 36% of persons. Systemic reactions are uncommon (< 1%) and may include mild fever, malaise, headache, myalgias, arthralgias, and nausea. Mild elevation of liver function tests was noted in some vaccinees but not determined to be vaccine related. The main contraindications of the vaccine are prior tularemia infection, immunodeficiency, liver disease, and pregnancy.

Other vaccines. The current US IND tularemia vaccine was derived from the Soviet live attenuated vaccine dating from the 1930s. Research is ongoing to develop a new LVS tularemia vaccine (using the National Drug Company's LVS as a starting material) as well as subunit vaccines against tularemia.[64]

Chemoprophylaxis

Prophylaxis with tetracycline given as a 1-g dose twice daily within 24 hours of exposure for 14 days was demonstrated to be highly effective for preventing tularemia in humans exposed to aerosols of 25,000 *F tularensis* SCHU-S4 spores, with none of the eight exposed persons becoming ill.[65] However, decreasing the tetracycline dose to only 1 g daily was not as effective in preventing tularemia, with 2 of 10 persons becoming ill. The failure of once daily tetracycline to prevent tularemia may be due to considerable fluctuations in tissue levels, as demonstrated in monkeys given once daily tetracycline, which ameliorated symptoms but did not prevent tularemia.[65]

Whereas streptomycin for 5 days successfully prevented tularemia in humans after intradermal challenge with an inoculation of *F tularensis*, neither chlor-amphenicol nor tetracycline given in a 5-day course was effective as postexposure prophylaxis.[66] *F tularensis* is an intracellular pathogen that is cleared slowly from the cells, even in the presence of bacterostatic antibiotics. Tetracyclines, even in high concentrations, merely suppress multiplication of the organisms,[64] which may explain the requirement for a prolonged 14-day course of bacterostatic antibiotics.

Based on the above studies, 100 mg of doxycycline orally twice a day or 500 mg of tetracycline orally four times a day for 14 days is recommended for postexposure prophylaxis to *F tularensis*. A 500-mg dose of ciprofloxacin orally twice a day may be considered as an alternative regimen.

Plague

Plague is an acute bacterial disease caused by a nonmotile, gram-negative bacillus known as *Yersinia pestis*.[67] Naturally occurring disease is generally acquired from bites of infected fleas, resulting in lymphatic and blood infections (bubonic and septicemia plague). Less commonly, plague may occur from direct handling of skins of dead animals, by inhalation of aerosols from infected animal tissues, or by ingestion of infected animal tissues. Pneumonic plague may be acquired by inhaling droplets emitted from an infected person or by inhaling *Y pestis* as an aerosolized weapon, or it may occur as a result of secondary hematogenous seeding from plague septicemia. As the causative agent of pneumonic plague, *Y pestis* is a candidate for use as biological warfare or terrorism agent, with symptoms occurring within 1 to 4 days after aerosol exposure.

Vaccination

Formalin-killed plague vaccine. The US-licensed formalin-killed whole bacillus vaccine (Greer Laboratories, Inc, Lenoir, NC) for preventing bubonic plague was discontinued in 1999. Although this vaccine demonstrated efficacy in the prevention or amelioration of bubonic plague based on retrospective indirect evidence in vaccinated military troops, it had not been proven effective for pneumonic plague.[68–75] Vaccine efficacy against aerosolized plague was demonstrated to be poor in animal models, with at least two persons developing pneumonic plague despite vaccination.[69-75]

Other vaccines. A live attenuated vaccine made from an avirulent strain of *Y pestis* (the EV76 strain) has been available since 1908. This vaccine offers protection against both bubonic and pneumonic plague in animal models, but it is not fully avirulent and has resulted in disease in mice.[70] For safety reasons, this vaccine is not used for humans in most countries.

New vaccine research. Because of safety issues with live vaccine, recent efforts have focused on the development of a subunit vaccine using virulence factors from the surface of the plague bacteria to induce immunity.[69,76] Two virulence factors were found to induce immunity and provide protection against plague in animal models, identified as the fraction 1 (F1) capsular antigen and the virulence (V) antigen. At USAMRIID the first new plague vaccine was developed by fusing the F1 capsular antigen with the V antigen to make the recombinant F1-V vaccine. The F1-V vaccine candidate has been shown to be protective in mice and rabbits against both pneumonic and bubonic plague. In nonhuman primates during aerosol challenge experiments, it provided better protection than either the F1 or V antigen alone.[77,78]

Chemoprophylaxis

Postexposure prophylaxis with ciprofloxacin for 5 days was highly effective as prophylaxis in mice, when administered within 24 hours after aerosol exposure.[79,80] However, if ciprofloxacin was administered after the onset of disease, approximately 48 hours postexposure, most studies resulted in high rates of treatment failure.[79,80] Doxycycline was relatively ineffective as prophylaxis in one mouse model study, even if given within 24 hours after aerosol exposure with mean inhibitory concentrations (MICs) ranging from 1 to 4 mg/L.[79,80] The effectiveness of doxycycline, a bacterostatic drug, generally requires antibiotic levels to be 4 times the MIC. The treatment failure may be related in part to increased metabolism of doxycycline in mice, because tetracycline has been used successfully in humans to treat or prevent pneumonic plague and because doxycycline was able to stabilize the bacterial loads in spleens of mice infected with *Y pestis* strains with lower MICs ($\le$ 1 mg/L).[81]

Recommendations for postexposure prophylaxis after a known or suspected *Y pestis* exposure are doxycycline (100 mg twice daily), tetracycline (500 mg four times daily), or ciprofloxacin (500 mg twice daily) for 7 days or until exposure has been excluded.[67,79,80,82,83] Postexposure prophylaxis should be given to persons exposed to aerosols of *Y pestis* and to close contacts of persons with pneumonic plague (within 6.5 feet). It should be administered as soon as possible because of the short incubation of plague (1 to 4 days). Sulfonamides have been used in the past to successfully treat plague, but they are less effective than tetracycline and are not effective against pneumonic plague. Therefore, use of trimethoprim-sulfamethoxazole (TMP-SMZ) (1.6–3.2 g of the trimethoprim component per day given twice daily) has been suggested for prophylaxis only in persons with contraindications to tetracyclines

or ciprofloxacin.[84] Chloramphenicol (25 mg/kg orally four times a day) is an alternative in individuals who cannot take tetracyclines or quinolones, but has the risk of aplastic anemia.[67] Antibiotic sensitivity testing should be performed to assess for resistant strains.

Glanders and Melioidosis

Glanders and melioidosis are zoonotic diseases caused by gram-negative bacteria, *Burkholderia mallei* and *B pseudomallei*, respectively.[85–87] The natural reservoirs for *B mallei* are equines. Infection with *B mallei* in horses may be systemic with prominent pulmonary involvement (known as glanders), or may be characterized by subcutaneous ulcerative lesions and lymphatic thickening with nodules (known as farcy). Glanders in humans is not common and has generally been associated with contact with equines. The mode of acquisition is believed to be primarily from inoculation with infectious secretions of the animal through broken skin or the nasal mucosa, and less commonly from inhalation, with onset of symptoms 10 to 14 days after aerosol exposure.

B pseudomallei is a natural saprophyte that can be isolated from soil, stagnant waters, rice paddies, and market produce in endemic areas such as Thailand. Infection in humans is generally acquired through soil contamination of skin abrasions, but may also be acquired from ingesting or inhaling the organism. Although symptoms of *B pseudomallei* infection are variable, the pulmonary form of the disease is the most common and may occur as a primary pneumonia or from secondary hematogenous seeding. The incubation period may be as short as 2 days, but the organism may remain latent for a number of years before symptoms occur. Both *B mallei* and *B pseudomallei* have been studied in the past as potential biowarfare agents, and the recent increase of biodefense concerns has renewed research interest in these organisms.

Vaccination

No vaccines are currently available for preventing glanders or melioidosis.

Chemoprophylaxis

Data are currently lacking on the efficacy of postexposure chemoprophylaxis for either *B mallei* or *B pseudomallei* in humans. A recent publication noted that 13 laboratory workers, identified as having high-risk exposure to *B pseudomallei* from sniffing of culture plates and/or performing routine laboratory procedures such as subculturing and inoculation of the organism outside a biosafety cabinet (before the

organism was identified), were given postexposure prophylaxis with a 2-week course of TMP-SMZ.[88] None of the 13 individuals developed illness or antibodies to *B pseudomallei* over the following 6 weeks; however, this response may reflect the low risk of laboratory-acquired illness from the organism as opposed to the effectiveness of antibiotic prophylaxis.[89,90] Chemoprophylaxis recommendations are based on animal studies and in-vitro data.

Animal studies with *B pseudomallei*. Postexposure prophylaxis with 10 days of quinolones or TMP-SMZ, when given within 3 hours of subcutaneous exposure to 10^5 organisms of *B pseudomallei*, was found to be completely effective for preventing disease in white rats (verified by autopsy at 2 months postexposure).[91] Another study demonstrated protection of hamsters with both doxycycline and ciprofloxacin (administered twice daily for 5 or 10 days duration) if started 48 hours before or immediately after intraperitoneal challenge with *B pseudomallei*, but relapses occurred in a few animals within 4 weeks after discontinuation of antibiotics.[92] However, delay of antibiotic prophylaxis initiation to 24 hours after the exposure provided minimal protection, resulting only in a delay of infection that occurred 5 weeks or later after the discontinuation of antibiotics.[92] The differences in results between the two animal models may be related to the higher susceptibility of hamsters to melioidosis.

Animal studies with *B mallei*. Doxycycline or ciprofloxacin for 5 days initiated 48 hours before or immediately after intraperitoneal challenge with 2.9 x 10^7 colony-forming units of *B mallei* had a protective effect in hamsters.[92] However, the effect was temporary in some animals, with disease occurring after discontinuing the antibiotics. Relapses were associated with ciprofloxacin beginning at day 18 and with doxycycline beginning at day 28 after challenge. Necropsies of fatalities revealed splenomegaly with splenic abscesses from *B mallei*, and necropsies of the surviving animals revealed splenomegaly with an occasional abscess.[92] However, hamsters are highly susceptible to infection from *B mallei*, and the protective effect of chemoprophylaxis in humans may be greater. Delay of ciprofloxacin or doxycycline prophylaxis initiation to 24 hours after the exposure resulted in a delay of disease, with relapses occurring in hamsters within 4 weeks of the challenge.

In-vitro susceptibility tests. Both *B pseudomallei* and *B mallei* have demonstrated sensitivity on in-vitro susceptibility testing to TMP-SMZ, tetracyclines, and augmentin, with *B mallei* also sensitive to rifampin, quinolones, and macrolides (only a few *B mallei* quinolone-resistant strains are known).[86,93,94] *B pseudomallei* is resistant to ciprofloxacin on in-vitro testing, with MICs exceeding achievable serum drug levels.[95,96]

Ciprofloxacin may achieve intracellular concentrations 4 to 12 times greater than that achieved in the serum, and it has been successful in treating some patients with melioidosis in spite of reported in-vitro resistance.[97,98] Most isolates of *B pseudomallei* are resistant to rifampin,[96] and 20% of isolates in Thailand are now resistant to TMP-SMZ.

Chemoprophylaxis recommendations. Recommendations for postexposure prophylaxis are based on in-vitro and animal data, with limited or no supportive data in humans. Drugs that may be considered for chemoprophylaxis for melioidosis may include doxycycline (100 mg twice daily), tetracycline (500 mg four times daily), TMP-SMZ (one double-strength tablet twice daily), or ciprofloxacin (500 mg twice daily). For glanders, chemoprophylaxis may consist of doxycycline (100 mg twice daily), TMP-SMZ (one double-strength tablet twice daily), augmentin 500/125 (one tablet twice daily), or possibly ciprofloxacin (500 mg twice daily). The duration of treatment should be at least 14 days, but a 21-day course of therapy may be considered, based on relapses occurring in animals receiving antibiotics for 5 to 10 days following exposure. Treatment of disease requires two drugs; it is not known if a chemoprophylaxis regimen of two drugs will reduce the risk of relapse. Postexposure prophylaxis with TMP-SMZ for 21 days was given to 16 of 17 laboratory workers who had manipulated cultures of *B pseudomallei* (77% were assessed as high-risk exposures), and no individuals developed subsequent disease or seroconversion.[99] Chemoprophylaxis regimens should be adjusted based on results of sensitivity testing. Individuals who start prophylaxis, particularly if more than 24 hours after exposure, must be carefully monitored after completion of antibiotic therapy because delayed chemoprophylaxis in animal studies failed to provide protection; it only delayed the onset of symptoms.

Brucellosis

Brucellosis is a zoonotic disease caused by infection with one of six species of *Brucellae,* a group of intracellular, gram-negative coccobacilli.[100] The natural reservoirs for this organism are sheep, cattle, and goats. Infection is transmitted to humans by direct contact with infected animals or their carcasses, or from ingestion of unpasteurized milk or milk products. Brucellosis is not transmissible person to person. *Brucella* are highly infectious by aerosol and are still one of the most common causes of laboratory-acquired exposure,[12,101] with an infective dose of only 10 to 100 organisms.[100] Symptoms generally occur within 7 to 21 days of exposure, but may occur as late as 8 weeks or longer postexposure.

Vaccination

Live animal vaccines have eliminated brucellosis in most domestic animal herds in the United States, but no licensed human vaccine is available.

Chemoprophylaxis

No FDA-approved chemoprophylaxis exists for brucellosis. A 6-week course of both rifampin (600 mg orally once daily) and doxycycline (100 mg twice daily) has been effective in the treatment of brucellosis, with relapse rates less than 5% to 10%.[102,103] Although a 3- to 6-week course of rifampin and doxycycline may be considered as chemoprophylaxis in high-risk exposures, there are no animal or human data to support this regimen other than its effectiveness in brucellosis treatment. However, one study reported prophylaxis using doxycycline (200 mg daily) and rifampin (600 mg daily) administered to nine asymptomatic laboratory workers who seroconverted after exposure to *B abortus* serotype 1 atypical strain (a strain with low virulence).[104] These individuals subsequently developed symptoms of fever, headache, and chills that lasted a few days. This was in contrast to three persons who did not receive prophylaxis and had symptoms of fever, headache, and chills for 2 to 3 weeks, in addition to symptoms of anorexia, malaise, myalgia, or arthralgia lasting an additional 2 weeks. No relapses occurred in the nine persons who received antibiotic prophylaxis, which may be a result of either the low virulence of this particular strain in humans or the early administration of antibiotic prophylaxis. In another hospital laboratory incident, six laboratory workers were identified as having had a high-risk exposure to *B melitensis* because they had sniffed and manipulated cultures outside a biosafety cabinet.[105] Five individuals were given postexposure prophylaxis for 3 weeks (four individuals received doxycycline 100 mg twice daily plus rifampin 600 mg daily, and one pregnant laboratory worker received TMP-SMZ 160 mg/800 mg twice daily). One individual declined prophylaxis and subsequently developed brucellosis (confirmed by culture). The five individuals who received postexposure prophylaxis remained healthy and did not seroconvert.

Other combinations of drugs that may be considered for chemoprophylaxis are TMP-SMZ with doxycycline (if the patient cannot take rifampin) and ofloxacin with rifampin (if the patient cannot take doxycycline).[106,107] Quinolones have been demonstrated to have in-vitro activity, but clinical experience with quinolones is limited, and initial experience suggests they may not be as effective as the other drugs.[104,108]

Q Fever

Q fever is a zoonotic disease caused by a rickettsia, *Coxiella burnetii*. The natural reservoirs for this organism are sheep, cattle, and goats.[109,110] Humans acquire Q fever infection by inhaling aerosols contaminated with the organisms, with infections resulting from as few as 1 to 10 organisms.[100] Q fever is not transmissible person to person. The incubation period is generally between 15 and 26 days, but has been reported to be as long as 40 days with exposures to low numbers of organisms.[111] Although this agent is deemed a category B biological warfare agent because it cannot cause massive fatalities, its low infective dose, the significant complications resulting from chronic infection (endocarditis), and its known environment stability (it may remain viable in the soil for weeks) make *C burnetii* a potential biowarfare agent.

Vaccination

C burnetii has two major antigens, known as phase I and phase II antigens. Strains in phase I have been propagated mainly in mammalian hosts, whereas strains in phase II have been adapted to yolk sacs or embryonated eggs. Although early vaccines were made from phase II egg-adapted strains, the later vaccines were made from phase I strains and demonstrated protective potencies in guinea pigs 100 to 300 times greater than vaccines made from phase II strains.[112] No FDA-approved vaccine is currently available for vaccination against Q fever in the United States. However, a vaccine approved in Australia (Q-Vax, manufactured by CSL Ltd, Parkville, Victoria, Australia) has been demonstrated to be safe and effective for preventing Q fever, and a similar IND vaccine (NDBR 105) has been used in at-risk researchers at Fort Detrick since 1965. The latter vaccine is available only at USAMRIID on an investigational basis.

Q-Vax. Q fever can be prevented by vaccination. The Q-Vax vaccine, currently licensed in Australia, was demonstrated to be protective in abattoir workers in Australia. Q-Vax is a formalin-inactivated, highly purified *C burnetii* whole-cell vaccine derived from the Henzerling strain, phase I antigenic state.[113,114] Over 4,000 abattoir workers were vaccinated subcutaneously with 0.5 mL (30 μg) of the vaccine from 1981 to 1988. In an analysis of data through August 1989, only eight vaccinated persons developed Q fever, with all infections occurring within 13 days of vaccination (before vaccine-induced immunity) versus 97 cases in unvaccinated persons (approximately 2,200 unvaccinated individuals but the exact number is not known).[113]

The protective effect of the vaccine has been virtually 100%, with only two cases of Q fever occurring in 2,555 vaccinated abattoir workers between 1985 and 1990, with both cases occurring within a few days of vaccination (before immunity developed).[115] Over 32,000 Australian abattoir workers have been vaccinated since 1981, reducing the incidence of Q fever in this high-risk group to virtually zero. Skin test postvaccination was not a useful indicator of immunogenicity, with only 31 of 52 vaccinees (60%) converting to skin test positive.[116] However, conversion from a negative to a positive lymphoproliferative response (indicating cell-mediated immunity) was observed in 11 of 13 subjects (85%) in this same study, occurring between days 9 to 13 postvaccination.[116] The main adverse event noted with this vaccine was the risk of severe necrosis at the vaccine site in vaccinees who had prior exposure to Q fever.[113,117] Therefore, a skin test with 0.02 mg of the vaccine is required before vaccination. The exclusion from vaccination of individuals who tested positive on the skin test (denoting previous exposure to *C burnetii*) has eliminated sterile abscesses (Figure 21-3).[118,119]

NDBR 105 Q fever vaccine. The NDBR 105 (IND 610) Q fever vaccine is an inactivated, lyophilized vaccine that has a preparation similar to Q-Vax. The vaccine originates from chick fibroblast cultures derived from specific pathogen-free eggs infected with the phase I Henzerling strain.

The NDBR 105 Q fever vaccine was demonstrated to be effective in animal studies.[118,120,121] The vaccine also prevented further cases of Q fever in at-risk laboratory

Fig. 21-3. Positive Q fever skin test. Skin testing, performed by injecting 0.1 mL of skin test antigen intradermally in the forearm, is required before vaccination against Q fever to identify persons with prior exposure. Vaccination is contraindicated in individuals with a positive skin test because they are at risk for severe necrosis at the vaccine site.
Photograph: Courtesy of Dr Herbert Thompson, MD, MPH.

workers in the Fort Detrick offensive biological warfare program during the final 4 years of the program (1965–1969), compared to an average of three cases per year before the vaccine availability.[12,122] There has been only one case of Q fever (mild febrile illness with serologic confirmation) with use of the vaccine in the 35 years of the subsequent biodefense research program at Fort Detrick, attributed to a high-dose exposure from a breach in the filter of a biosafety cabinet.[123] The vaccine may have ameliorated symptoms of disease in this individual.

Skin testing is required before vaccination to identify persons with prior exposure to Q fever, performed by injecting 0.1 mL of skin-test antigen (1:1500 dilution of the vaccine with sterile water) intradermally in the forearm. A positive skin test is defined as erythema of 30 mm (or greater) or induration of 20 mm (or greater) at day 1 or later after the skin test, or erythema and induration of 5 mm (or greater) on day 7 after the test. These persons are considered to be naturally immune and do not require vaccination. Because of the risk of severe necrosis at the vaccine site, vaccination with Q fever is contraindicated in persons with a positive skin test.

The vaccine is administered by injecting 0.5 mL subcutaneously in the upper outer aspect of the arm, and is given only once. Protection against Q fever is primarily cell-mediated immunity. Markers to determine vaccine immunity to the NDBR 105 vaccine have been studied (ie, cell-mediated immunity studies, skin testing, and antibody studies pre- and postimmunization), but reliable markers have not yet been identified for the NDBR 105 vaccine. After vaccination with Q-Vax (similar to the NDBR 105 Q fever vaccine), skin test seroconversion occurred in only 31 of 52 persons (60%),[113,116,119,124,125] but lymphoproliferative responses to *C burnetii* antigens were demonstrated to persist for at least 5 years in 85% to 95% of vaccinated persons.[113,124] Vaccine breakthroughs have been rare in vaccinated persons.

Adverse events from the NDBR 105 vaccine were reported by 72 of 420 skin-test–negative vaccinees (17%) and were mainly local reactions, including erythema, induration, or sore arm. Most local reactions were classified as mild or moderate, but one person required prednisone secondary to erythema extending to the forearm. Some vaccinees experienced self-limited systemic adverse events, but these were uncommon and generally characterized by headache, chills, malaise, fatigue, myalgia, and arthralgia.[126]

Other vaccines. The Soviet Union studied a live vaccine with an avirulent variant of Grita strain (M-44). Vaccinating guinea pigs with the M-44 attenuated

vaccine was associated with both persistence of the organism and mild lesions in the heart, spleen, and liver.[127] Because of the risk of endocarditis in persons with valvular heart disease, this vaccine or the pursuit of development of other attenuated vaccines for human use has not been considered safe.[127–129]

Current vaccine research has concentrated on efforts to develop a vaccine that induces protective immunity but allows for administration without screening for prior immunity. Partially purified subunit protein vaccines have demonstrated protection in mice and guinea pigs.[130–132] However, the proteins of these two vaccines were not cloned or well characterized to identify a single protective protein. Although DNA vaccines have been associated with strong cell-mediated immune responses, development of a DNA vaccine against Q fever is difficult because no protective antigen has been identified.[130]

Chemoprophylaxis

Prophylaxis with oxytetracycline (in a 3-g loading dose followed by 0.75 g every 6 hr) for 5 to 6 days was demonstrated to be effective for preventing disease in humans, if started 8 to 12 days after exposure.[111] Initiation of prophylaxis earlier than 7 days postexposure may only delay the onset of symptoms. Four of five men given oxytetracycline (for 5 to 6 days) within 24 hours after exposure to a small quantity of *C burnetii* only delayed disease for 8 to 10 days longer than seen in the control group who were not given chemoprophylaxis, with disease occurring approximately 3 weeks after discontinuation of therapy.[111] Based on these studies, doxycycline (100 mg orally twice daily) or tetracycline (500 mg 4 times daily for 7 days) beginning 8 to 12 days after the exposure may be considered for postexposure chemoprophylaxis to *C burnetii*.

VIROLOGY

Vaccination is the mainstay of medical countermeasures against viral agents of bioterrorism. Both FDA-approved vaccines (eg, smallpox, yellow fever) and investigational vaccines (eg, Rift Valley fever vaccines and Venezuelan, eastern, and western equine encephalitis viruses) are available in the United States. Although antiviral agents and immunotherapy may be given postexposure, many of these therapies are investigational drugs with associated toxicities, and they may be in limited supply.

Alphaviruses

Venezuelan, eastern, and western equine encephalitis (VEE, EEE, and WEE) viruses are ribonucleic acid viruses of the family *Togaviridae*. Infections from these encephalitic viruses may manifest with fever, chills, headache, myalgias, vomiting, and encephalitis. Infections are naturally acquired through the bite of infected mosquitoes, but infections may also be acquired from aerosolized virus (such as in a bioterrorism event).

Vaccination

Licensed vaccinations are available for equines, but the only vaccines available for humans against VEE, EEE, and WEE are investigational. Both a live attenuated VEE vaccine (TC-83) and an inactivated VEE vaccine (C-84) are available under IND status at USAMRIID. Formalin-inactivated vaccines for both EEE and WEE viruses are also available on an IND basis at USAMRIID. These vaccines have demonstrated efficacy in animal models and have been used in at-risk laboratory workers at the institute for more than 30 years. Because of their investigational status and limited supply, use of these vaccines in a bioterrorism event would be extremely limited.

The Venezuelan equine encephalitis TC-83 vaccine. Laboratory infections with VEE became problematic soon after the discovery of the agent in 1938. In 1943 eight cases of occupationally acquired VEE were reported.[133] Attempts to produce an effective and safe vaccine against VEE in the 1950s at Fort Detrick failed. As a result of live virus remaining in a poorly inactivated vaccine preparation, 14 cases of clinical illness and eight virus isolations occurred in 327 subjects who had received 1,174 vaccinations.[134]

Live attenuated VEE TC-83 vaccine (IND 142, NDBR 102) was manufactured at the National Drug Company in Swiftwater, Pennsylvania, in 1965 using serial propagation of the Trinidad strain (subtype I-AB) of VEE in fetal guinea pig heart cells. The virus was plaqued once in chick embryo fibroblasts. Several VEE viral plaques were then picked and inoculated by the intracranial route into mice. The plaques that did not kill the mice were judged attenuated. One of the nonlethal plaques of VEE was used as seed stock to propagate in the 81st passage in fetal guinea pig heart cells.[135]

The TC-83 designation refers to the 83 passages in cell culture. The seed stock (81-2-4) was provided by Fort Detrick and diluted in a 1:100 ratio. Five lots were produced. The bulk vaccine was stored at −80°C in 2- to 3-liter quantities at the National Drug Company (Swiftwater, Pa). In 1971 the bulk was diluted in a ratio of 1:400 with modified Earle's medium and 0.5% human serum albumin, then lyophilized. The freeze-

dried product was then distributed under vacuum into 6-mL vials to provide convenient 10-dose vials at 0.5 mL per dose.

Lot release testing was performed in animals, including a guinea pig safety test, mouse safety test, and guinea pig protection (potency) tests. The initial safety test challenge in the animals was a 0.5 mL (intraperitoneally) dose of the vaccine (containing approximately 10^6 virions). All animals survived. Additional rabbit, suckling mouse, mouse virulence, and monkey neurovirulence testing was conducted. The vaccine was protective against both subcutaneous and aerosol challenge in mice and hamsters. There was inconsistent protection in the monkey model after aerosol exposure. Postrelease potency analyses have been performed periodically over the past 35 years, showing that infectivity for all lots seems to have declined by one to two logs from the original data in the IND 142 submitted in 1965.[136]

At-risk laboratory workers at Fort Detrick have received the TC-83 vaccine since 1963. VEE TC-83 lot 4-3 vaccination of at-risk USAMRIID laboratory workers from 2002 to 2005 was associated with an acceptable postvaccination 80% plaque reduction neutralization titer ($PRNT_{80}$) of 1:20 or greater in 136 of 169 individuals (80%). Because the vaccine is derived from epizootic strains, the vaccine may not protect against enzootic strains of VEE (subtypes II through VI) and may not adequately protect against distantly related VEE subtype I-AB variants.[123]

The components of the TC-83 vaccine include 0.5% human serum albumin and 50 μg/mL each of neomycin and streptomycin. The vaccine is administered as a 0.5-mL subcutaneous injection (approximately 10^4 plaque-forming units per dose) in the deltoid area of the arm.

TC-83 vaccine adverse events. The severity and frequency of adverse events from the VEE TC-83 vaccine varied with the vaccine lot. Of all lot 4-2 VEE TC-83 vaccine recipients, 40% developed mild-to-moderate systemic reactions, primarily fever, fatigue, neck pain, upper back pain, sore throat, headache, muscle ache, nausea, vomiting, and loss of appetite. In another 5% of vaccine recipients, these symptoms were severe enough to require bed rest or time off from work. The onset of these symptoms was usually abrupt. The fever lasted 24 to 48 hours, and symptoms persisted up to 3 days. The occurrence of these symptoms often had two phases, occurring initially 2 to 3 days after vaccination and recurring 7 to 18 days after vaccination. These reactions resolved without permanent effects. A change of lot of VEE TC-83 vaccine occurred in January 2002. Although the rate of mild-to-moderate reactions remained stable at 42% (32/76 vaccine recipients) with

lot 4-3, the rate of severe reactions observed was higher, occurring in 16% (12/76 subjects). No person-to-person transmission of VEE has been documented after vaccination with TC-83.[137] Local reactions are rarely seen.

The association of diabetes mellitus with VEE TC-83 vaccine is uncertain. Three cases of diabetes have been recognized after receipt of the vaccine at USAMRIID, occurring in two individuals with a strong family history of diabetes. In a study conducted after a VEE epidemic caused by virulent Trinidad strain,[138] an increased risk of developing insulin-dependent diabetes was noted, but because the size of the observed population group was limited, statistical significance was not observed. Studies involving the induction of diabetes after VEE infection in animal models were inconclusive,[139–141] and no animal model of VEE virus induction of acute, insulin-dependent diabetes exists. However, the vaccine is not given to individuals with a family history of diabetes in first-degree relatives.

The VEE TC-83 vaccine has never been evaluated in pregnant women. In 1975 one spontaneous abortion occurred as a probable complication of TC-83 vaccination. In 1985 a severe fetal malformation in a stillborn infant occurred in a woman whose pregnancy was unidentified at the time of vaccination.[142] There are many animal models in which this kind of event can be reproduced. Rhesus monkey fetuses were inoculated with VEE vaccine virus by direct intracerebral route at approximately 100 days gestation. Congenital microcephaly, hydrocephalus, and cataracts were found in all animals and porencephaly in 67% of the cases. The virus replicated in the brain and other organs of the fetus.[143] VEE vaccine virus is teratogenic for nonhuman primates and must be considered a potential teratogen of humans. The wild-type VEE virus is known to cause fetal malformations, abortions, and stillbirths.[144]

The Venezuelan equine encephalitis C-84 vaccine. The VEE C-84 formalin inactivated vaccine (IND 914, TSI-GSD 205) is made from the TC-83 production seed and has undergone one more passage through chick embryo fibroblasts (the number 84 refers to the number of passages). The vaccine is then inactivated with formalin and the resultant product freeze-dried.

The VEE C-84 vaccine was protective against subcutaneous challenge but not against aerosol challenge in hamsters or cynomolgus monkeys, and protection against aerosol challenge in BALB/c mice was short-lived (less than 6 months).[145–149] VEE-specific IgA was detected less frequently in mice vaccinated with the inactivated VEE C-84 vaccine than with the live attenuated VEE TC-83 vaccine. This was noted particularly in the bronchial and nasal washings, suggesting that VEE-specific IgA in the mucosal secretions may be important in protection against aerosolized VEE virus.

Therefore, the C-84 vaccine has not been used for primary vaccination against VEE, but it has been used in at-risk laboratory workers at Fort Detrick as a booster for those individuals who had received the VEE TC-83 vaccine and had either (*a*) an inadequate initial response with a $PRNT_{80}$ of less than or equal to 1:20 or (*b*) had an adequate response to the VEE TC-83, but $PRNT_{80}$ levels subsequently dropped below 1:20. The inactivated VEE C-84 vaccine demonstrated immunogenicity, with a positive response ($PRNT_{80} \geq 1:20$) following a booster dose with the vaccine observed in 87% (N=581) of individuals receiving the vaccine (1987–2001).

The components of the VEE C-84 vaccine are neomycin and streptomycin at a concentration of 50 µg/mL, sodium bisulfite, chicken eggs, and formalin. The vaccine is administered as a 0.5-mL subcutaneous injection above the triceps area. The current protocol allows for a maximum of four doses a year if postvaccination titers are not adequate. From 2002 to 2006 at USAMRIID, 8% to 33% of individuals receiving C-84 as a booster have reported a discernible adverse event. Most reactions were mild and self-limiting local reactions of swelling, tenderness, and erythema at the vaccine site. Systemic reactions were uncommon and consisted of headache, arthralgia, fatigue, malaise, influenza-like symptoms, and myalgia. All resolved without sequelae.

The western equine encephalitis vaccine. The inactivated western equine encephalitis vaccine (IND 2013, TSI-GSD 210) is a lyophilized product originating from the supernatant harvested from primary chicken fibroblast cell cultures.[150] The vaccine was prepared from specific pathogen-free eggs infected with the attenuated CM4884 strain of WEE virus. The supernatant was harvested and filtered, and the virus was inactivated with formalin. The residual formalin was neutralized by sodium bisulfite. The medium contains 50 µg each of neomycin and streptomycin and 0.25% (weight/volume) of human serum albumin (US Pharmacopeia). The freeze-dried vaccine must be maintained at − 25°C (± 5°C) in a designated vaccine storage freezer. The inactivated WEE vaccine was originally manufactured by the National Drug Company. The current product, lot 2-1-91, was manufactured at the Salk Institute, Government Services Division (Swiftwater, Pa) in 1991. Potency tests have been conducted every 2 to 3 years since then, initially at the Salk Institute and then at Southern Research Institute (Frederick, Md).

Animal studies showed the vaccine to be effective against intracerebral challenge with WEE in 19 of 20 mice (95%).[151] Hamsters were protected against intraperitoneal challenge with WEE when vaccinated intraperitoneally at days 0 and 7.[152] Vaccination of horses at days 0 and 21 resulted in protection in all 17

animals against intradermal challenge at 12 months after vaccination, even in the absence of detectable WEE protective neutralizing antibodies.[153] This suggests that the vaccine may also provide protection in the absence of detectable antibody levels.

Human subjects administered WEE vaccine subcutaneously (either 0.5 mL at days 0 and 28 or 0.5 mL at day 0 and 0.25 mL at day 28) showed similar serologic responses.[150] Neutralizing antibody titers did not occur until day 14 after the first dose of vaccine in each group. The mean log neutralization index was 1.7 and 1.8, respectively, at day 28 after the first dose. The antibody levels remained at acceptable levels through day 360 in 14 of 15 volunteers. Side effects from the vaccine were minimal, consisting primarily of headache, myalgias, malaise, and tenderness at the vaccination site.

The inactivated WEE vaccine has been administered to at-risk personnel at Fort Detrick since the 1970s. Pittman et al evaluated the vaccine for its immunogenicity and safety in 363 at-risk workers enrolled in evaluation trials at USAMRIID between 1987 and 1997.[154] All volunteers were injected subcutaneously with 0.5 mL of the inactivated WEE vaccine (lot 81-1), in an initial series of three doses, administered up to day 42 (the intended schedule was 0, 7, and 28 days). For individuals whose $PRNT_{80}$ fell below 1:40, a booster dose (0.5 mL) was given subcutaneously. Serum samples for neutralizing antibody assays were collected before vaccination and approximately 28 days after the last dose of the initial series and each booster dose.

Of these vaccinees, 151 subjects (41.6%) responded with a $PRNT_{80}$ of greater than or equal to 1:40. Seventy-six of 115 initial nonresponders (66%) were converted to responder status after the first booster dose. A vaccination regimen of three initial doses and one booster dose provided protection lasting for 1.6 years in 50% of initial responders.

Passive collection of local and systemic adverse events from the inactivated WEE vaccine was the method used from 1987 to 1997. Of the 363 vaccinees who received three initial injections, only five reported local or systemic reactions. These reactions usually occurred between 24 and 48 hours after vaccine administration. Erythema, pruritus, and induration were reported after just one of the initial vaccinations. Two volunteers also reported influenza-like symptoms after the initial dose. All reactions were self-limited. No reactions were reported after 153 booster doses.

Recent active collection of adverse events from 2002 through 2006 in the Special Immunizations Clinic at USAMRIID revealed a reaction rate of 15% to 20% following the primary series. The reaction rate was less for booster doses than for primary series doses.

The majority of these symptoms were systemic and consisted of headache, sore throat, nausea, fatigue, myalgia, low-grade fever, and malaise. The duration of these adverse events was less than 72 hours. The vaccine has not been tested for teratogenicity or abortogenicity in any animal model, nor has it been tested in pregnant women; therefore, the vaccination of pregnant women is not advisable.

The eastern equine encephalitis vaccine. The formalin-inactivated EEE vaccine (TSI-GSD 104) was manufactured in 1989 by the Salk Institute.[155] The seed for the EEE virus was passaged twice in adult mice, twice in guinea pigs, and nine times in embryonated eggs.[156] The final EEE vaccine was derived from supernatant fluids bearing virus accumulated from three successive passages on primary chick embryo fibroblast cell cultures prepared from specific pathogen-free eggs infected with the attenuated PI-6 strain of virus. The supernatant was harvested and filtered, and the virus then inactivated with formalin. The product was then lyophilized for storage at $-20°C$.

The EEE vaccine contains 50 $\mu g/mL$ of both neomycin and streptomycin and 0.25% (weight/volume) of human serum albumin. The initial vaccine dose is given as a 0.5-mL injection subcutaneously above the triceps area. A postvaccination $PRNT_{80}$ of 1:40 or greater is considered adequate. Should the titer fall below 1:40, a booster dose of 0.1 mL should be given intradermally on the volar surface of the forearm. Booster doses must be given at least 8 weeks apart.

Animal studies demonstrated that the EEE vaccine is 95% protective against intracerebral challenge in guinea pigs, with survival correlating to serum neutralizing antibody titers.[157] Vaccination of horses was also protective against intradermal challenge at 12 months postvaccination, even with absence of detectable neutralizing antibody titers in 16 of the 17 animals, suggesting the vaccine may also provide protection in this species in the absence of detectable antibody levels.[153] The vaccine has been given to at-risk laboratory workers at Fort Detrick for over 25 years. The response rate of 255 volunteers who received two primary vaccinations between 1992 and 1998 was 77.3% (197 individuals), with a response defined as a $PRNT_{80}$ of 1:40 or greater. Intradermal vaccination with EEE resulted in an adequate titer in 66% of the initial nonresponders.

Adverse events from the EEE vaccine occurred in approximately 20% individuals, consisting of headache, myalgias, and light-headedness. All symptoms subsided within several days. Mild and self-limiting local reactions of induration, erythema, pruritus, or pain at the vaccine site have also been reported.

Postexposure Prophylaxis

No treatment has been shown to alter the course of VEE, WEE, or EEE disease in humans once disease has been contracted. The treatment is limited to supportive care; no currently known antiviral drug is effective.

New Vaccine Research

The live attenuated VEE vaccine candidate V3526 was scheduled to replace the 40-year-old VEE TC-83 IND vaccine. The newer-generation VEE vaccine candidate had improved activity against VEE enzootic strains. However, because of high rates of severe neurologic adverse events in clinical phase I trials, further development of this product was halted. This was unexpected with the new V3526 vaccine candidate because it demonstrated less reactogenicity in nonhuman primate studies than the VEE TC-83 product. Recently, the V3526 vaccine candidate was inactivated and transferred to the National Institute of Allergy and Infectious Diseases for future preclinical and clinical development as a multidose primary series. Many of the existing equine encephalitis vaccines have been under IND status for over 30 years, yet because of funding shortfalls, these products have never been transitioned from development to licensure.

Smallpox

Smallpox is caused by variola virus, of the genus *Orthopoxvirus*. Smallpox is recognized to have occurred in ancient Egypt, China, and India, and for centuries was the greatest infectious cause of human mortality. The disease was declared eradicated in 1980, after an intensive vaccination program. Subsequently, all known stocks of variola virus were destroyed, with the exception of stock at two World Health Organization collaborating centers, the CDC, and the Russian State Research Center of Virology and Biotechnology. Smallpox has been designated a category A biothreat agent because of its high mortality, high transmissibility, and past history of massive weaponization by the former Soviet Union.

Vaccination

History of smallpox vaccination. Vaccination with smallpox was recorded in 1,000 BCE in India and China, where individuals were inoculated with scabs or pus from smallpox victims (either in the skin or nasal mucosa), producing disease that was milder than naturally occurring smallpox. In the 18th century in Europe, scratching and inoculation of the skin with

pock material, known as variolation, was performed, resulting in a 90% reduction in mortality and long-lasting immunity. Variolation performed in Boston in 1752 resulted in a smallpox death rate of 1% (2,124 persons) compared to a death rate of 10% in unvaccinated persons (5,545 persons).

In 1796 Edward Jenner noticed that milkmaids rarely had smallpox scars, and subsequently discovered that inoculation of the skin with cowpox taken from a milkmaid's hand resulted in immunity. In 1845 the smallpox vaccine was manufactured in calfskin. Production of the vaccine became regulated in 1925, with use of the New York City Board of Health strain of vaccinia as the primary US vaccine strain. Vaccination eventually led to eradication of the disease, with the last known case of naturally occurring smallpox reported in 1977. Routine vaccination of US children ceased in 1971, and vaccination of hospital workers ceased in 1976. Finally, vaccination of military personnel was discontinued in 1989. Because of the recent risk of bioterrorism, vaccination of smallpox in at-risk military personnel was resumed in 2003.

The smallpox vaccine. Dryvax, the smallpox vaccine, manufactured by Wyeth Laboratories (Marietta, Pa), is a live-virus preparation of vaccinia virus made from calf lymph. The calf lymph is purified, concentrated, and lyophilized. The diluent for the vaccine contains 50% glycerin and 0.25% phenol in US Pharmacopeia sterile water, with no more than 200 viable bacterial organisms per mL in the reconstituted product. Polymyxin B sulfate, dihydrostreptomycin sulfate, chlortetracycline hydrochloride, and neomycin sulfate are added during the processing of the vaccine, and small amounts of these antibiotics may be present in the final product. The reconstituted vaccine contains approximately 100 million infectious vaccinia viruses per mL, and it is intended only for administration into the superficial layers of the skin by multiple puncture technique.

The vaccine is administered by scarification with a bifurcated needle, by applying three punctures to scarify the epidermis on the upper arm for primary vaccination, and 15 punctures for booster vaccinations. The individual is followed after vaccination to document a take, which indicates immunity against smallpox. Six to 8 days after the primary vaccination, a primary major reaction to the vaccine develops, with a clear vesicle or pustule of approximately 1 cm diameter. The site then scabs over by the end of the second week, with the scab drying and separating by day 21 to 28 (Figure 21-4). In individuals with prior vaccination, an immune response is generally observed. The immune response is an accelerated response, with a pruritic papule appearing between days 1 and 3 post-vaccination. Individuals who do not exhibit either a primary major reaction or an immune response (ie, individuals with erythema, pruritus, or induration but no papule or vesicle) require revaccination. If no primary reaction is noted after revaccination (and ensuring proper technique in vaccine administration was used), these individuals are considered immune. At some point in the future, which may be years, the immunity of these individuals may wane, and revaccination at that time may result in a take.

Smallpox vaccine has been demonstrated to be effective in prevention of smallpox. Protection against smallpox is from both humoral and cell-mediated immunity; the latter provides the main protection. Humoral responses of neutralizing and hemagglutination inhibition antibodies to the vaccine appear between days 10 and 14 after primary vaccination, and within 7 days after secondary vaccination. Health officials recommend vaccination with confirmation of a take every 3 years for those who are likely to be exposed to the virus (ie, a smallpox outbreak). However, individuals working with variola in the laboratory are recommended to have a yearly smallpox vaccination.

Secondary attack rates from smallpox in unvaccinated persons have generally ranged from 36% to 88%, with an average rate of 58%. Household contacts in close proximity to the smallpox case for 4 hours or longer are at higher risk for acquiring infection. In an outbreak recorded in the Shekhupura District of Pakistan during the smallpox era, the secondary attack rate in vaccinated persons was only 4% in persons
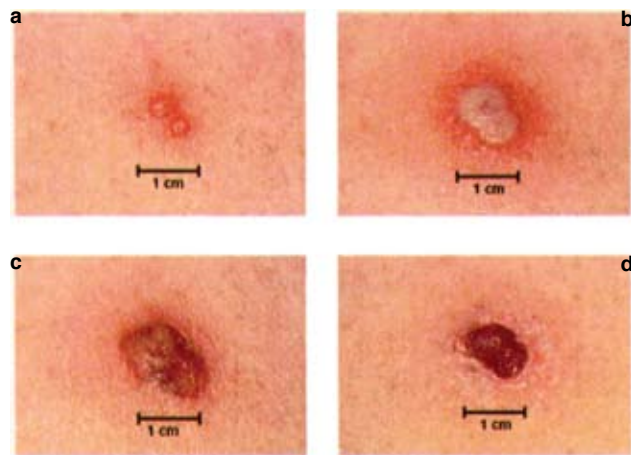

Fig. 21-4. Primary reaction to smallpox vaccination, at (**a**) day 4, (**b**) day 7, (**c**) day 14, and (**d**) day 21.
Reproduced from: Centers for Disease Control and Prevention Web site. Available at: http://www.bt.cdc.gov/agent/smallpox/smallpox-images/vaxsit5a.htm. Accessed March 26, 2007.

vaccinated within the previous 10 years (5/115) and 12% in persons vaccinated over 10 years before (8/65), compared to 96% in unvaccinated persons (26/27).[158,159] Estimates of vaccine protection from imported cases of variola major between 1950 and 1971 in Western countries, where immunity from smallpox would be expected to be mainly from vaccination, showed a case fatality rate of only 1.4% in individuals who had received the smallpox vaccine within the previous 10 years, compared to a 52% mortality rate in individuals who had never received the vaccine, 7% mortality in individuals vaccinated 11 to 20 years before, and 11% mortality in individuals vaccinated over 20 years before. Postexposure vaccination resulted in 27% less mortality when compared (retrospectively) with smallpox patients who were never vaccinated.[158] However, postexposure vaccination was only helpful if given within 7 days of the exposure. Postexposure vaccination is most effective if given within 3 days of exposure (preferably within 24 hours), but may still be effective if given within 7 days.[160]

Contraindications and adverse events. Smallpox vaccination is contraindicated in the preoutbreak setting for individuals who

- have a history of atopic dermatitis (eczema);
- have active acute, chronic, or exfoliative skin conditions disruptive of the epidermis or have Darier disease (keratosis follicularis);
- are pregnant or breastfeeding;
- are immunocompromised;
- have a serious allergy to any of the vaccine components; or
- are younger than 1 year old.[161]

The CDC has recently recommended underlying cardiac disease (history of ischemic heart disease, myocarditis, or pericarditis) or significant cardiac risk factors as relative contraindications to the vaccine. The ACIP also does not recommend vaccination of persons younger than 18 years old in the preoutbreak setting.[161] Vaccination is also contraindicated in persons with household members who have a history of eczema or active skin conditions as described above, are immunosuppressed, or are pregnant. Although the presence of an infant in the household is not a contraindication for vaccination of the adult member, the ACIP recommends deferring vaccination of individuals with households that have infants younger than 1 year old because of data indicating a higher risk for adverse events among primary vaccinees in this age group.[161] Because skin lesions resulting from the varicella vaccine may be confused with vaccinia lesions, simultaneous administration of the smallpox and varicella vaccine is not recommended. However, in an outbreak situation, there are no contraindications to vaccination for any person who has been exposed to smallpox (Tables 21-3 and 21-4).

Smallpox vaccine adverse reactions are diagnosed by clinical exam. Most reactions can be managed with observation and supportive measures. Self-limited reactions include fever, headache, fatigue, myalgia, chills, local skin reactions, nonspecific rashes, erythema multiforme, lymphadenopathy, and pain at the vaccination site. Adverse reactions that require further evaluation and possible therapeutic intervention include inadvertent inoculation involving the eye, generalized vaccinia, eczema vaccinatum, progressive vaccinia, postvaccinial central nervous system disease, and fetal vaccinia (Tables 21-5 and 21-6).[162,163]

Vaccinia can be transmitted from a vaccinee's unhealed vaccination site to other persons by close contact and can lead to the same adverse events as intentional vaccination (Figure 21-5). Incidence of transmission to contacts during the most recent military vaccination experience was 47 per million vaccinees. Additionally, vaccinees may inoculate themselves and cause infection in areas such as the eye, which is associated with significant morbidity (Figure 21-6). Incidence of inadvertent self-inoculation in the military was 107 per million vaccines.[162] To avoid inadvertent transmission, vaccinees should wash their hands with soap and water or use antiseptic hand rubs immediately after touching the vaccination site and after dressing changes. Vaccinia-contaminated dressings should be placed in sealed plastic bags and disposed in household trash.

Inadvertent inoculation generally results in a condition that is self-limited unless the inoculation involves the eye or eyelid, which requires evaluation by an ophthalmologist (see Figure 21-6).[164] Topical treatment with trifluridine (Viroptic; Catalytica Pharmaceuticals, Inc, Greenville, NC) or vidarabine (Vira-A) is often recommended, although treatment of ocular vaccinia with either of these drugs is not specifically approved by the FDA.[165] Most published experience is with use of vidarabine, but this drug is no longer manufactured. Vaccinia immune globulin (VIG) may be recommended in severe cases of ocular vaccinia, but it is contraindicated in individuals with vaccinal keratitis because of the risk of corneal clouding. Corneal clouding was observed in 4 of 22 persons with vaccinal keratitis who received VIG.[166] A subsequent study in rabbits showed that treatment of vaccinal keratitis with VIG was associated with both corneal scarring and persistent and larger satellite lesions compared to control animals.[167]

Generalized vaccinia is characterized by a disseminated maculopapular or vesicular rash, frequently on an erythematous base and typically occurring 6 to 9

TABLE 21-3

CONTRAINDICATIONS TO SMALLPOX VACCINATION (PRE-EVENT VACCINATION PROGRAM)*

Condition	Contraindication
Allergies to vaccine components *Each Dryvax (Wyeth Laboratories; Marietta, Pa) vaccine lot contains antibiotics and preservatives. Specific allergies to these products may occur. Appropriate history of such allergies should be obtained and may negate vaccine administration when smallpox is not present.* Current Dryvax contains following antibiotics: • polymyxin B sulfate • streptomycin sulfate • chlortetracycline hydrochloride • neomycin sulfate	If smallpox is present and the risk of contact is great, the vaccine should be administered with subsequent use of an appropriate antihistamine or other medication.
Pregnancy	Do not administer if pregnant and advise vaccinee not to become pregnant for 1 month after vaccination.
Infancy	Younger than 1 year old
Immunodeficiency	Includes any disease with immunodeficiency (congenital or acquired) as a component: • HIV infection • AIDS • Many cancers
Immunosuppressive therapy *Immunosuppression from some medications may last for up to 3 months after discontinuation*	• Cancer treatments • Some treatments for autoimmune diseases • Organ transplant maintenance • Steroid therapy (equivalent to 2 mg/kg or greater of prednisone daily, or 20 mg/day, if given for 14 days or longer)
Eczema or atopic dermatitis or Darier's disease (keratosis follicularis)	History or presence of eczema or atopic dermatitis or Darier's disease. (Even patients with "healed" eczema or atopic dermatitis may manifest complications. They should not be vaccinated, and they should avoid contact with a recent vaccinee.)
Skin disorders *The size and extent of the non-eczema/atopic skin disorder may be sufficiently small that vaccination can be safely performed. However, all such patients must be counseled to take great care to avoid any transfer from the primary site to the affected skin. Persons with conditions or injuries that cause extensive breaks in the skin should not be vaccinated until the condition resolves.*	Disruptive or eruptive conditions: • Severe acne • Burns • Impetigo • Contact dermatitis or psoriasis • Chickenpox
Cardiovascular disease	Reports of myopericarditis and cardiovascular disease have resulted in recent exclusion of individuals with history of these disorders.

*Vaccine contraindicated if listed condition exists either in the potential vaccinee, or if condition exists in household contact or other close physical contact of the vaccinee (excluding history of vaccine allergy or known cardiovascular disease in contacts). During a smallpox outbreak, the risk of vaccination must be weighed against the risk of disease. (During the smallpox era, there was no absolute contraindication to vaccination.)
HIV: human immunodeficiency virus
AIDS: acquired immunodeficiency syndrome
Adapted from: Centers for Disease Control and Prevention. Smallpox vaccination and adverse events training module. 2002. Available at: http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/contraindications.html. Accessed March 23, 2007.

TABLE 21-4

PRECAUTIONS FOR SMALLPOX VACCINATION (PRE-EVENT VACCINATION PROGRAM)

Condition	Precaution
Active eye disease of the conjunctiva or cornea	Persons with inflammatory eye diseases may be at increased risk for inadvertent inoculation due to touching or rubbing of the eye.
Inflammatory eye disease requiring steroid treatment	The Advisory Committee for Immunization Practices recommends that persons with inflammatory eye diseases requiring steroid treatment defer vaccination until the condition resolves and the course of therapy is complete.
Moderately or severely ill at the time of vaccination	Ill persons should usually not be vaccinated until recovery.
Breastfeeding	Whether the virus transmitted in breast milk is unknown. Close contact may also increase chance of transmission to infant. The product label of the smallpox vaccine recommends individuals not breastfeed after vaccination (Dryvax [Package insert]. Marietta, Pa: Wyeth Laboratories, 1994)

Adapted from: Centers for Disease Control and Prevention. Smallpox vaccination and adverse events training module. 2002. Available at: http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/contraindications.html. Accessed March 23, 2007.

days after primary vaccination (Figure 21-7). Lane reported 242.5 cases per million primary vaccinations and 9.0 cases per million revaccinations in a 1968 10-state survey of smallpox vaccination complications.[168] The rash usually resolves without therapy. Treatment with VIG is restricted to those who are systemically ill or have an immunocompromising condition. Contact precautions should be used to prevent further transmission and nosocomial infection. Generalized vaccinia must be distinguished from other postvaccination exanthems, such as erythema multiforme and roseola vaccinatum (Figure 21-8).

Eczema vaccinatum may occur in individuals with a history of atopic dermatitis, regardless of current disease activity, and can be a papular, vesicular, or pustular rash (Figures 21-9 and 21-10). Historically, eczema vaccinatum occurred at a rate of 14.1 and 3.0 per million primary and revaccinations, respectively[168]; however, in more recent military experience, there were no cases of eczema vaccinatum in 450,293 smallpox vaccinations (of which 70.5% were primary vaccinations).[163] The rash may be generalized or localized with involvement anywhere on the body, with a predilection for areas of previous atopic dermatitis lesions. Individuals with eczema vaccinatum are generally systemically ill and require immediate therapy with VIG. The mortality rate of individuals with eczema vaccinatum was 7% (9/132), even with VIG therapy. A measurable antibody response developed in 55 of the 56 survivors who had antibody titers obtained after VIG administration.[169] No antibody response was detected in five persons with fatal eczema vaccinatum cases who had post-VIG antibody titers measured.

TABLE 21-5

ADVERSE EVENTS AFTER SMALLPOX VACCINATION

Event Type	US Department of Defense Rate per Million Vaccinees* (95% confidence interval)	US Civilian Historical Rate per Million Vaccinees
Generalized vaccinia, mild	80 (63–100)	45–212[†]
Inadvertent self-inoculation	107 (88–129)[§]	606[†]
Vaccinia transfer to contact	47 (35–63)	8–27[†]
Encephalitis	2.2 (0.6–7.2)	2.6–8.7[†]
Acute myopericarditis	82 (65–102)	100[‡]
Eczema vaccinatum	0 (0–3.7)	2–35[†]
Progressive vaccinia	0 (0–3.7)	1–7[†]
Death	0 (0–3.7)	1–2[†]

* US military vaccinations from December 13, 2002, through May 28, 2003.
[†] Based on adolescent and adult smallpox vaccinations from 1968 studies (both primary vaccination and revaccination).
[‡] Based on case series in Finnish military recruits vaccinated with the Finnish strain of vaccinia.
[§] Includes 38 inadvertent inoculations of the skin and 10 of the eye.
Data source: Grabenstein JD, Winckenwerder W. US military smallpox vaccination program experience. *JAMA.* 2003;289:3278–3282.

TABLE 21-6

VACCINIA IMMUNE GLOBULIN ADMINISTRATION FOR COMPLICATIONS OF SMALLPOX (VACCINIA) VACCINATION

Indicated	Not Recommended
• Inadvertent inoculation (only for extensive lesions or ocular implantations without evidence of vaccinia keratitis) • Eczema vaccinatum • Generalized vaccinia (only if severe or recurrent) • Progressive vaccinia	• Inadvertent inoculation (mild instances) • Generalized vaccinia (mild or limited—most instances) • Erythema multiforme • Postvaccination encephalitis • Isolated vaccinia keratitis (may produce severe corneal opacities)

Adapted from: Centers for Disease Control and Prevention. Smallpox vaccination and adverse events training module. 2002. Available at: http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/contraindications.html. Accessed March 23, 2007.

Fig. 21-5. Accidental autoinoculation. This 22-month-old child presented after having autoinoculated his lips and cheek 9 days postvaccination**.** Autoinoculation involves the spread of the vaccinia virus to another part of the vaccinee's body, caused by touching the vaccination site and then touching another part of the body. Image 4655.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Allen W Mathies, MD, and John Leedom, MD, California Emergency Preparedness Office, Immunization Branch. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-6. Ocular vaccinia. This 2-year-old child presented with a case of ocular vaccinia from autoinoculation. Ocular vaccinia is an eye infection that can be mild to severe and can lead to a loss of vision. It usually results from touching the eye when the vaccinia virus is on the hand. Image 5219.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Allen W Mathies, MD, and John Leedom, MD, California Emergency Preparedness Office, Immunization Branch. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-7. Generalized vaccinia. This 8-month-old infant developed a generalized vaccinia reaction after having been vaccinated. Generalized vaccinia is a widespread rash, which involves sores on parts of the body away from the vaccination site resulting from vaccinia virus traveling through the blood stream. Image 4644.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Allen W Mathies, MD, California Emergency Preparedness Office, Immunization Branch. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-9. Eczema vaccinatum. This 8-month-old boy developed eczema vaccinatum after he had acquired vaccinia from a sibling recently vaccinated for smallpox. Eczema vaccinatum is a serious complication that occurs in people with atopic dermatitis who come in contact with the vaccinia virus. These individuals are at special risk of implantation of vaccinia virus into the diseased skin. 1969. Image 3311.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Arthur E Kaye, Centers for Disease Control and Prevention. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-8. This child displays a generalized erythematous eruption called roseola vaccinatum after receiving a primary smallpox vaccination. Eruptions such as this one are common after vaccination and, although often dramatic in appearance, these are largely benign. There is no evidence of systemic or cutaneous spread of the vaccinia virus, and live virions cannot be recovered from the involved sites. The older literature from the compulsory vaccination era used an imprecise nosology for a wide range of benign post vaccination exanthems. Terms such as generalized vaccinia and erythema multiforme that occur in the older literature must be interpreted cautiously because on retrospective analysis, it is clear that they encompassed much more than those specific entities.
Data source: Lewis FS, Norton SA, Bradshaw RD, Lapa J, Grabenstein JD. Analysis of cases reported as generalized vaccinia during the US military smallpox vaccination program, December 2002 to December 2004. *J Am Acad Dermatol*. 2006;55:23–31. (Personal communication, Colonel Scott A. Norton, MD, MPH, former Chief of Dermatology, Walter Reed Army Medical Center.) Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Arthur E Kaye, Centers for Disease Control and Prevention. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Contact precautions should be used to prevent further transmission and nosocomial infection.

Progressive vaccinia is a rare, severe, and often fatal

Fig. 21-11. Progressive vaccinia. This patient with progressive vaccinia required a graft to correct the necrotic vaccination site. One of the most severe complications of smallpox vaccination, progressive vaccinia is almost always life threatening. Persons who are immunosuppressed are most susceptible to this condition. Image 4624.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Allen W Mathies, MD, California Emergency Preparedness Office, Immunization Branch. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-10. Eczema vaccinatum. This 28-year-old woman with eczema vaccinatum contracted it from her vaccinated child. She had a history of atopic dermatitis, and her dermatitis was inactive when her child was vaccinated. As a therapy, she was given vaccinia immune globulin, idoxuridine eye drops, and methisazone, resulting in healed lesions, no scarring, and no lasting ocular damage. Image 4621.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of Allen W Mathies, MD, California Emergency Preparedness Office, Immunization Branch. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

complication of vaccination that occurs in individuals with immunodeficiency conditions. It is characterized by painless progressive necrosis at the vaccination site with or without metastases to distant sites (Figures 21-11, 21-12, and 21-13). This condition carries a high mortality rate and should be aggressively treated with VIG, debridement, intensive monitoring, and tertiary medical center level support. Those at highest risk include persons with congenital or acquired immunodeficiencies, human immunodeficiency virus infection/acquired immunodeficiency syndrome, cancer, or autoimmune

disease, or who have undergone organ transplantation or immunosuppressive therapy. Historical rates of progressive vaccinia ranged from 1 to 3 per million vaccinees historically,[168] no cases in 450,293 US military vaccines,[163] and no cases (that met case definition) in 38,440 US civilian vaccinees in 2003.[170] Anecdotal experience has shown that despite treatment with VIG, individuals with cell-mediated immunity defects have a poorer prognosis than those with humoral defects. A recent animal study showed that both topical and intravenous cidofovir were effective in treating vaccinia necrosis in mice deficient in cell-mediated immunity.[171] Topical cidofovir was more effective than intravenous cidofovir, and the administration of both cidofovir preparations was superior to either preparation alone. Infection control measures should include contact and respiratory precautions to prevent transmission and nosocomial infection.

Central nervous system disease, which includes postvaccinial encephalopathy and postvaccinial encephalomyelitis, although rare, is the most frequent cause of

Fig. 21-12. Progressive vaccinia. This patient presented with progressive vaccinia after having been vaccinated for smallpox. Progressive vaccinia is one of the most severe complications of smallpox vaccination and is almost always life threatening. Although it was rare in the past, the condition may be a greater threat today because of the larger proportion of susceptible persons in the population. Image 4592. Reproduced from: Centers for Disease Control and Prevention Public Health Image Library Web site. Photograph: Courtesy of California Department of Health Services. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

death related to smallpox vaccination.[168] Postvaccinial encephalopathy occurs more frequently than encephalomyelitis, typically affects infants and children younger than 2 years old, and reflects vascular damage to the central nervous system. Symptoms typically occur 6 to 10 days postvaccination and include seizures, hemiplegia, aphasia, and transient amnesia. Histopathologic findings include cerebral edema, lymphocytic meningeal inflammation, ganglion degeneration, and perivascular hemorrhage. Patients with postvaccinial encephalopathy who survive can be left with cerebral impairment and hemiplegia. Postvaccinial encephalomyelitis, which generally affects individuals aged 2 years or older, is characterized by abrupt onset of fever, vomiting, malaise, and anorexia occurring approximately 11 to 15 days postvaccination.[164,172] Neff's 1963 national survey detected 12 cases of postvaccinial encephalitis among 14,014 vaccinations.[173] Symptoms progress to amnesia, confusion, disorientation, restlessness, delirium,

Fig. 21-14. Fetal vaccinia. Image 3338.
Photograph: Courtesy of the Centers for Disease Control and Prevention. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

Fig. 21-13. Progressive vaccinia after debridement. Image 4594. Reproduced from: Centers for Disease Control and Prevention. Available at: http://phil.CDC.gov. Accessed June 14, 2006.

drowsiness, and seizures. The cerebral spinal fluid has normal chemistries and cell count. Histopathologic findings include demyelination and microglial proliferation in demyelinated areas with lymphocytic infiltration without significant edema. The cause for central nervous system disease is unknown, and no specific therapy exists. Intervention is limited to anticonvulsant therapy and intensive supportive care.[174,175]

Fetal vaccinia, which results from vaccinial transmission from mother to fetus, is a very rare but serious complication of smallpox vaccination during or immediately before pregnancy (Figure 21-14). Fewer than 40 cases have been documented in the world's literature.[162]

Myopericarditis, although previously reported as a rare complication of vaccination using vaccinia strains other than the New York City Board of Health strain, was not well recognized until reported during active surveillance of the Department of Defense's 2002–2003 vaccination program (Figure 21-15).[176,177] The mean time from vaccination to evaluation for myopericarditis was 10.4 days, with a range of 3 to 25 days. Sixty-seven symptomatic cases were reported among 540,824 vaccinees, for a rate of 1.2 per 10,000 vaccinations. Reports of myocarditis in 2003 vaccinees raised concerns about carditis and cardiac deaths in individuals undergoing smallpox vaccination. Of 36,217 vaccinees, 21 cases of myopericarditis were reported with 19 cases (90%) occurring in revaccinees. The median age of the affected vaccinees was 48 years, and there was a predominance of females. Ele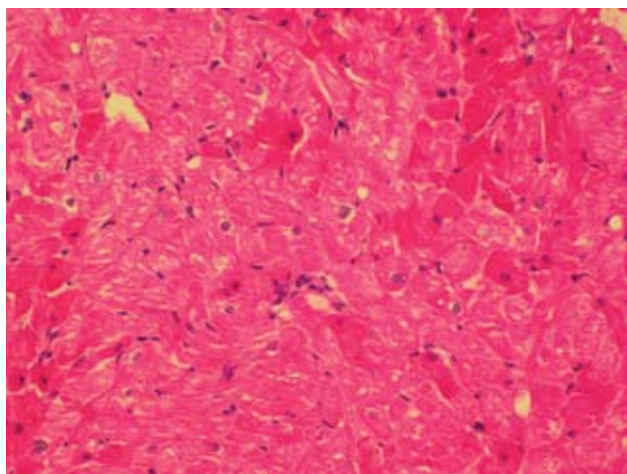ven of the individuals were hospitalized, but there were no fatalities. The military experience included 37 cases of myopericarditis of 440,293 vaccinees, for a rate of 82 per million vaccines.[163] Additionally, ischemic cardiac events, including fatalities, have been reported following vaccination with the vaccinia vaccine (Dryvax). Although no clear association has been found, history of ischemic heart disease and the presence of significant cardiac risk pose relative contraindications for smallpox vaccination. Consequently, individuals with a history of myocarditis, pericarditis, or ischemic heart disease should not be vaccinated.[176–178]

In a smallpox release from a bioterrorism event, individuals would be vaccinated according to the current national policy, which recommends vaccination initially of higher-risk groups: individuals directly exposed to the agent, household contacts or individuals with close contact to smallpox cases, and medical and emergency transport personnel. Ring vaccination of contacts and contact of the contacts in concentric rings around an identified active case is the strategy that was used to control smallpox during the final years of the eradication campaign. There are no absolute contraindications to vaccination for an individual with high-risk exposure to smallpox. Persons at greatest risk of complications of vaccination are those for whom smallpox infection poses the greatest risk. If relative contraindications exist for an exposed individual, then risks of adverse complications from vaccination must be weighed against the risk of a potentially fatal smallpox infection.

New Vaccine Research

To develop a replacement vaccine for Dryvax and other first-generation live vaccines, researchers must produce a vaccine safe enough by current standards for widespread clinical use in a population with large segments of immunosuppressed individuals, but still induces an adequate cell-mediated immune response. Dryvax and other first-generation vaccines are manufactured from the lymph collected from the skin of live animals scarified with vaccinia virus. Because of risks from adventitious viruses and subpopulations of virus with undesirable virulence properties, the manufacture of a cell culture-derived (second-generation) vaccine is preferable to the animal-derived product.[179,180] Advances in technology and the ability to replicate vaccinia in high concentrations in a variety of cell cultures make such second-generation vaccines possible.

ACAM 2000, a candidate smallpox vaccine, is a cell-culture replicated product derived from Dryvax.[181,182] ACAM 1000 was one of six clones of *vaccinia* obtained by serial passage and plaque picking at terminal dilution, selected for its similar immunogenicity to Dryvax in animal testing and lower neurovirulence in mice and

Fig. 21-15. Histopathology of vaccine-related myocarditis showing a nonspecific lymphocytic infiltrate.
Reproduced with permission of Department of Pathology, Brooke Army Medical Center, Texas.

monkeys. The ACAM 1000 pilot production vaccine was produced in MRC-5 human diploid lung fibroblast cells. To overcome production capacity problems, the African green monkey (Vero) cell line was used after 10 passages to produce the ACAM 2000 Vero cell vaccine. Animal studies have confirmed high degrees of similarity among the ACAM 1000 master virus seed, the ACAM 2000 production vaccine, and Dryvax. Neurovirulence profiles for the ACAM 1000 and ACAM 2000 vaccine were similar, but lower than the profile for Dryvax. Phase 2 and 3 clinical trials have revealed that like Dryvax, ACAM 2000 is associated with myopericarditis. The significance of ACAM 2000's cardiac adverse effects remains to be determined.[180]

Other approaches to developing a safe vaccine have used "non-replicating" and genetically modified "defective" viruses. Modified vaccinia ankara (MVA), a nonreplicating vaccinia virus, was produced by 574 serial passages in chicken embryo fibroblasts, resulting in a vaccinia strain safe for use in immunocompromised individuals. MVA was safely given to 150,000 persons.[183] MVA's main problem is that production in chicken embryos does not have an optimal safety profile. Production batches may consist of hundreds of eggs, which carry a risk of contamination with adventitious viruses, a problem that cannot be corrected with viral inactivation procedures. MVA can be replicated in mammalian cells, but the passage in permanent mammalian cell lines risks production of a viral strain with increased mammalian virulence. Defective vaccinia viruses have been developed by deleting a gene essential for viral replication (uracil DNA glycosylase). One such vaccine candidate, defective vaccinia virus Lister, is blocked in late gene expression from replication in any but the complementing permanent cell line. MVA and defective vaccinia virus Lister have similar safety and immunogenicity profiles.[179]

Immunoprophylaxis

There are limited studies on the effect of VIG in conjunction with the smallpox vaccine for preventing smallpox in contact cases.[184–186] A 1961 study by Kempe[184] demonstrated a statistically significant difference in smallpox cases among exposed contacts. Smallpox occurred in 5.5% of contacts (21/379) who received the smallpox vaccine alone compared to 1.5% of contacts (5/326) who received both the smallpox vaccine and VIG therapy.[184] Research published a year later by Marennikova studied the effect of antivaccinia gamma globulin given to 13 of 42 persons who had been in close contact with smallpox patients.[185] None of the 13 persons developed smallpox. Only 4 of the 13 individuals had a history of prior smallpox

vaccination, and all but 3 of the patients were not revaccinated until day 4 after the contact. Thirteen of the 29 persons not given antivaccinia gamma globulin developed smallpox. However, there are no clinical trials providing evidence that giving VIG in conjunction with the smallpox vaccine as prophylaxis has a greater survival benefit than vaccination alone.[187,188] There are currently two VIG preparations: (1) an intravenous and (2) an intramuscular formulation. The intravenous formulation recently received FDA approval and has become the formulation of first choice.[189] Intravenous VIG has the advantage of immediate and higher antibody levels (2.5 times the level obtained with the intramuscular VIG), and has a similar side effect profile as intramuscular VIG.[189] Supplies of VIG are limited and are used primarily for complications from the smallpox vaccine. VIG does not currently have a role in smallpox prevention.[190]

Chemoprophylaxis

The acyclic nucleoside phosphonate HPMPA (or (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine) known as cidofovir (Visitide, Gilead, Foster City, Calif) has broad-spectrum activity against DNA viruses, including the herpes viruses, papillomavirus, adenovirus, and poxviruses.[191–193] Cidofovir has a pronounced and long-lasting inhibition of viral DNA synthesis allowing for infrequent (weekly or bimonthly) dosing.[194] The drug has been approved by the FDA for treating cytomegalovirus retinitis in acquired immunodeficiency syndrome patients. Cidofovir has been used off-label to treat orthopox infections.

Studies of cidofovir demonstrated improved or prolonged survival in BALB/c mice and mice with severe combined immunodeficiency infected with vaccinia virus, as well as cowpox-infected mouse models, even when treatment was initiated as long as 5 days before and up to 96 hours after infection.[195] The greatest benefit of cidofovir prophylaxis was observed when it was administered within 24 hours before or after exposure.[196–198] Nonhuman primate studies have demonstrated improved survival in monkeypox and smallpox models.[199] In humans, cidofovir has been found effective in the treatment of the poxvirus infection molluscum contagiosum in acquired immunodeficiency syndrome patients. However, treatment of disseminated vaccinia, smallpox, or monkeypox with cidofovir is not FDA approved. Such treatment would be off-label use based on efficacy against these viruses in animal models and anecdotal evidence of efficacy in human poxvirus (molluscum contagiosum) infections.

The animal and human data suggest that cidofovir may be effective in therapy and also in short-term

prophylaxis of smallpox, if given within 5 days of exposure. One dose of intravenous cidofovir may provide potential protection for 7 days.[194] Dose-related nephrotoxicity is the principal complication of cidofovir therapy in humans. Toxicity may be minimized by concomitant intravenous hydration with saline and oral probenecid.[200] The probenecid is generally given orally as a 2-g dose 3 hours before the cidofovir infusion, and again at 2 and 8 hours after infusion. Both the Department of Defense and CDC currently have IND protocols for use of cidofovir in smallpox.

The new Siga drug, ST-246{4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocy cloprop[f]isoindol-2-(1H)-yl-benzamide}, is a potent and specific inhibitor of orthopoxvirus replication. The drug is active against multiple species of orthopoxviruses, including variola virus and cidofovir resistant cowpox variants. This oral drug has been shown to be effective in preventing death in animal models of smallpox infection.[201]

Viral Hemorrhagic Fevers

Countermeasures against the viruses that cause viral hemorrhagic fevers (VHFs) remain a top research priority because of the dearth of licensed vaccines and therapeutic agents to counteract these pathogens. Some success has been achieved with antiviral medications (primarily ribavirin), passive treatment using sera from previously infected donors, and vaccine development. Attempts at immunomodulation with various medications have been less successful. Pathogenesis, prevention, infection control measures, and management of patients with VHF are reviewed in other chapters specifically dedicated to VHF and infection control. This chapter will discuss potential countermeasures to VHFs most likely to be used as biological weapons (Table 21-7).

Vaccination

The only licensed US vaccine for VHFs is the 17D live attenuated yellow fever vaccine. This vaccine has substantially diminished the burden of yellow fever infection worldwide and is well tolerated, although contraindicated in immunosuppressed patients and used with caution in elderly people.[202] The vaccine would probably not be useful for postexposure prophylaxis because of yellow fever's short incubation time (although postexposure use of the vaccine has never been studied).[203] A live attenuated vaccine against Argentine hemorrhagic fever, known as Candid 1, demonstrated efficacy in a field study among 6,500 agricultural workers in Argentina[204]: 22 patients receiving placebo developed Argentine hemorrhagic fever, compared to only 1 patient who received the Candid 1 vaccine. This vaccine is not licensed in the United States.

A number of vaccines developed and licensed in other countries may have efficacy against VHFs. Hantavax (Korea Green Cross Corporation, Yongin-si, Korea) has been licensed in South Korea since 1990. Observational trials in North Korea and China and a randomized-placebo controlled trial in Yugoslavia supported the vaccine's efficacy[205]; however, the humoral immune response, when measured by $PRNT_{80}$ antibodies, was considered protective in only 33.3% of vaccine recipients.[206]

More recent exploration into vaccine candidates for hantaviruses, such as DNA vaccines[207] and vaccinia-vectored constructs,[208] has suggested other potential vaccine options. An inactivated Rift Valley fever vaccine under IND status is used in the Special Immunizations Program at USAMRIID for laboratory workers who may be exposed to the virus.[209] A live attenuated vaccine for Rift Valley fever has also been developed, and is also considered an IND, awaiting further testing. Substantial research has focused on the development of an effective Ebola vaccine. Unfortunately, demonstration of protection in murine models has not translated into successful Ebola vaccines in nonhuman primate models. Three of these unsuccessful vaccines involve (1) Venezuelan equine encephalitis virus replicon particles expressing Ebola virus genes; (2) the vaccinia virus expressing Ebola glycoproteins; and (3) encapsulated, gamma-irradiated Ebola particles in lipid A liposomes.[210] There has also been Ebola vaccine experimentation with some success in nonhuman primate models, involving (*a*) using an adenovirus vector to deliver key glycoproteins, and (*b*) using DNA vaccine technology[211] followed by boosting with an adenovirus vector.[212] Recently, an attenuated recombinant vesicular stomatitis virus vector with either Ebola or Marburg glycoproteins demonstrated protection in nonhuman primate models.[213] Not only did the animals survive the challenge, but they also showed no evidence of Ebola or Marburg virus after challenge, nor evidence of fever or any adverse reaction to vaccination. However, none of the current vaccine candidates will be ready for licensure soon.

Antiviral Agents

Ribavirin. Antiviral medications prescribed to treat VHFs are important primarily after patients have developed symptoms, because there are insufficient data to support their use for postexposure prophylaxis. The medication with the most evidence of efficacy is ribavirin. Ribavirin is a nonimmunosuppressive

nucleoside-analogue with activity against a number of different viruses. The principal mechanism is inhibition of inosine-5'-phosphate (IMP) dehydrogenase, which converts IMP to xanthine monophosphate.[214] Suggestive data exist for using ribavirin to treat the arenaviruses and bunyaviruses.[203] In particular, human studies suggest ribavirin is effective for treating hantavirus associated with hemorrhagic fever with renal syndrome (HFRS)[215] and Lassa fever.[216] It may also be effective for treating Crimean-Congo hemorrhagic fever (CCHF) and the New-World arenaviruses. Data supporting the use of ribavirin for HFRS are derived from a double-blind, placebo-controlled trial[215] demonstrating a reduction in mortality as well as decreased duration of viremia.[217] The largest observational study on CCHF, conducted by Mardani et al, noted that 97 of 139 patients (69.8%) with suspected CCHF receiving oral ribavirin survived, compared to an untreated historical control in which 26 of 48 patients (54%) survived.[218] In another recent study of CCHF by Ergonul et al, eight patients were treated with ribavirin, and all of these patients survived. However, in the same clinical context, 22 patients with CCHF were not treated

and had a mortality rate of 4.5%.[219] Ribavirin also has demonstrated in-vitro activity against CCHF.[220,221]

Ribavirin appears to be effective for treating infection with both Old-World (Lassa fever) and New-World arenaviruses (South American hemorrhagic fever viruses).[222] In Lassa fever, human studies suggest that ribavirin decreases mortality, especially if administered within 7 days of infection (the case fatality rate was reduced from 55% to 5%).[216] Results from nonhuman primate studies also support this finding.[223,224] Ribavirin may also have benefit in Argentine hemorrhagic fever,[225,226] but a large, randomized clinical trial has not been conducted. Ribavirin appears to have benefit in a macaque model for Argentine hemorrhagic fever[227] if therapy is initiated at the onset of symptoms. For animals that were treated at the onset of symptoms, initial improvement was observed in three of the four animals, with one animal dying early in the course of illness. However, the three infected monkeys that initially improved while on ribavirin subsequently developed a central nervous system infection that was fatal in two animals. This study and others suggest that ribavirin, which does not cross the blood–brain barrier,

TABLE 21-7

MEDICAL COUNTERMEASURES FOR VIRAL HEMORRHAGIC FEVERS

Virus	Vaccine	Passive Immunotherapy	Ribavirin as Potential Therapy
Arenaviridae			
Lassa	No	Mixed results	Yes
Guanarito (Venezuelan hemorrhagic fever)	No		Yes
Junin (Argentine hemorrhagic fever)	Yes*	Yes	Yes
Machupo (Bolivian hemorrhagic fever)	No		Yes
Sabia (Brazilian hemorrhagic fever)	No		Yes
Bunyaviridae			
Crimean-Congo hemorrhagic fever	No	Limited data	Yes
Hemorrhagic fever with renal syndrome	Yes†		Yes
Rift Valley fever	Yes‡		No
Filoviridae			
Ebola	No§	Mixed results	No
Marburg	No§	Mixed results	No
Flaviviridae			
Yellow fever	Yes		No
Kyasanur Forest disease	No		No
Omsk hemorrhagic fever	No		No

*Candid 1 live attenuated vaccine for Argentine hemorrhagic fever
†Hantavax (Korea Green Cross Corporation, Yongin-si, Korea) for hemorrhagic fever with renal syndrome from hantaviruses
‡Investigational formalin-inactivated Rift Valley fever vaccine; live attenuated Rift Valley fever vaccine
§Active development program with potential products being tested in nonhuman primate models

may be less useful for infections that have a propensity to infect the central nervous system.[222] An anecdotal report notes recovery from Bolivian hemorrhagic fever after treatment with ribavirin in two patients.[228]

Because of the probable efficacy of ribavirin for some of the VHFs, a consensus statement on the management of these viruses in a biological weapon scenario recommends that ribavirin be started empirically in all cases, until a better identification of the agent is achieved.[203] In addition to the possible benefits in VHF cases, especially when therapy is commenced as close to the onset of symptoms as possible, ribavirin generally has manageable side effects (particularly anemia), making empiric therapy preferable. Ribavirin is not effective against filoviruses or flaviviruses that cause VHFs[222] and should be discontinued if one of these viruses is identified as the causative agent. Although ribavirin is considered teratogenic and is contraindicated in pregnancy, the consensus statement suggests that ribavirin should be used in a biological weapon scenario because the benefits of treatment would likely outweigh the fetal risk.[203] The group recommends clinical observation of exposed patients, with careful observation for fever or other signs and symptoms of infection, rather than using ribavirin for postexposure prophylaxis.[203]

The dose of ribavirin for a contained casualty scenario is as follows: one loading dose of 30 mg/kg (maximum 2 g), followed by 16 mg/kg intravenous (maximum 1 g per dose) every 6 hours for 4 days, followed by 8 mg/kg intravenous (maximum 500 mg per dose) every 8 hours for 6 days.[203] In a mass-casualty situation, oral ribavirin is recommended. No other antiviral medications have been licensed or advocated for widespread use for the treatment of VHFs in a current casualty situation.

Other drugs. Few other options exist for treating VHFs, other than supportive care. Using steroids to treat these viruses is not recommended.[203] Pathogenesis studies with Ebola virus have implicated tissue-factor–induced disseminated intravascular coagulation as a critical component of the fatal outcomes.[229] In an Ebola-infection model, treating rhesus macaques with a factor VIIa/tissue factor inhibitor (recombinant nematode anticoagulation protein c2 or rNAPc2) led to a survival advantage.[230] This compound has not been tested in humans for treating Ebola infection, and tissue factor inhibitors have not been effective in the treatment of septic shock.[231] Other antiviral compounds have been studied for viruses such as CCHF, and in-vitro data suggest that the Mx family of proteins may have antiviral activity against ribonucleic acid viruses, but further study is needed.[232] IMP dehydrogenase inhibitors (similar to ribavirin) have been tested in both in-vitro and animal models against arenaviruses, but these products have not yet been tested in humans.[233]

Other compounds that have demonstrated in-vitro activity against arenaviruses include 3′-fluoro-3′-deoxyadenosine,[234] phenothiazines,[235] and myristic acid compounds.[236] Several antivirals have been tested in a bunyavirus (Punta Toro virus) murine model,[237] suggesting possible compounds for further testing.

Stimulating the immune system is another potential therapeutic modality, but no human studies with this technique have been conducted for any of the VHF viruses. Interferon combinations may be useful, particularly with VHF infections in which the immune response is impaired. However, interferon compounds may be deleterious in some VHF infections, such as Argentine hemorrhagic fever, in which high interferon levels are associated with worse outcomes.[238] Interferons have demonstrated a benefit in bunyavirus murine models,[237] and a slight benefit in a nonhuman primate Ebola virus model (using interferon α-2b).[239]

Passive Immunotherapy

Studies on the benefits of passive immunotherapy for treating VHFs have yielded mixed results.[203] Sera collected from donors after infection with Argentine hemorrhagic fever have been used in the treatment of this disease.[225] However, as with passive immunotherapy for treating other diseases, concerns about the transmission of bloodborne pathogens such as hepatitis C[240] may limit this treatment, or at least necessitate a rigorous screening process. In a cymologous monkey model of Lassa fever infection, treatment with sera from immune monkeys led to a survival advantage when the sera was used alone and combined with ribavirin.[224] However, sera from convalescent patients used to treat Lassa fever did not reduce mortality in patients with a high risk of a fatal outcome.[216] Anecdotal evidence suggests that immunoglobulins and/or transfusions from convalescent patients may improve outcome in human Ebola virus infection.[241,242] Passive treatment with immunoglobulins did not produce a mortality benefit in a macaque model for Ebola virus infection.[239] Substantial supportive data are lacking for using immunoglobulin from survivors for treating CCHF, but a small case series has suggested 100% survival among treated patients.[243] Serum from vaccinated horses has also been suggested as being beneficial for CCHF.[244]

In addition to questions about the safety of donated sera, the impracticality of obtaining large quantities of donated sera from previously infected individuals, with no such population available (particularly in the United States), limits the utility of this treatment. Future technology, such as a means of manufacturing large quantities of monoclonal antibodies, may allow for passive treatment with antibodies to counteract the effects of VHF.

Other Countermeasures

Good infection control practices, particularly the isolation of patients and barrier precautions, are a crucial countermeasure in the efforts to limit the impact of VHFs used as biological weapons. The specific infection control needed for each virus is discussed in chapter 13, Viral Hemorrhagic Fevers. Management measures must also overcome the fear and panic associated with use of a VHF virus such as Ebola.[245]

Modern intensive care unit support will likely improve the outcome for patients infected with VHF viruses, but access to this care may be limited in a mass casualty scenario. For HFRS, the intensive care management is both crucial and challenging; access to dialysis can save lives because the renal failure associated with this infection tends not to be permanent. Fluid management must be carefully followed in HFRS because capillary leak syndrome constitutes one of the primary mechanisms of pathogenesis, and fluid replacement leads to increased pulmonary edema.[246] The effects of various interventions (including blood products such as fresh frozen plasma and fluids) have not been adequately delineated and merit further study.

TOXINS

Botulinum Toxin

Clostridium botulinum is an anaerobic gram-positive bacillus that produces a potent neurotoxin, botulinum toxin. Botulinum toxin blocks the release of neurotransmitters that cause muscle contraction, and may result in muscle weakness, flaccid paralysis, and subsequent respiratory impairment. There are seven immunologically distinct toxin serotypes (A through G) produced by discrete strains of the organism. Botulism is generally acquired from ingestion of food contaminated with botulinum toxin, but may also occur from toxin production by *C botulinum* if present in the intestine or wounds. Botulism is not acquired naturally by aerosolization, and this route of acquisition would suggest a possible bioterrorism event but may also occur from exposure to aerosolized toxin in a research laboratory.[247] Neurologic symptoms after inhalational of botulinum toxin may begin within 24 to 72 hours of the exposure, but may vary with exposure dose.

Vaccination

There are currently no FDA-approved vaccines to prevent botulism. However, an investigational product, the pentavalent botulinum toxoid (PBT) against botulinum toxin serotypes A through E has been used since 1959 for persons at risk for botulism (ie, laboratory workers).[248,249]

Pentavalent Botulinum Toxoid. PBT is available as an investigational product on protocol through the CDC. Derived from formalin-inactivated, partially purified toxin serotypes A, B, C, D, and E, PBT was developed by the Department of Defense and originally manufactured by Parke-Davis Company. Each of the five toxin serotypes was propagated individually in bulk culture and then underwent acid precipitation, filtration, formaldehyde inactivation, and adsorption onto an aluminum phosphate adjuvant. The five individual toxin serotypes were then blended to produce the end product. The Michigan Department of Public Health has been responsible for formulation of recent PBT lots.

PBT has been found to be protective in animal models against intraperitoneal challenge with botulinum toxin serotypes A through E, and protective in nonhuman primates against aerosol challenge to toxin serotype A.[250] From 1945 until 1959, at-risk laboratory workers in the US offensive biological warfare program at Fort Detrick were vaccinated with a bivalent botulinum toxoid (serotypes A and B).[251] There were 50 accidental exposures to botulinum toxins reported from 1945 to 1969 (24 percutaneous, 22 aerosol, and 4 by ingestion), but no cases of laboratory-acquired botulism occurred, possibly attributed in part to protection from the botulinum toxoids. The PBT was initially given as a primary series of three subcutaneous injections (0.5 mL at 0, 2, and 12 weeks) and a booster dose at 12 months. Subsequent booster doses were required yearly, but later required only for a decline in antitoxin titers (antitoxin not present on a 1:16 dilution of serum). Antitoxin titers from vaccination with PBT generally do not occur until 3 to 4 months after initiation of the vaccine (1 month after the third dose), so postexposure vaccination with the PBT is not recommended.

Recent data suggest a declining immunogenicity and potency associated with increasing age of PBT, which was manufactured 30 years ago.[252,253] Antitoxin titers obtained 1 month after booster doses of PBT given between 1999 and 2000 to at-risk USAMRIID laboratory workers were "adequate" (a predetermined antitoxin titer that allowed for deferment of a booster dose) for toxin serotypes A, B, and E in 96%, 73%, and 45% of vaccinees, respectively.[252,253] Adequate titers obtained between 6 and 12 months after a booster dose were noted in only 76%, 29%, and 12% of vaccinees for toxin serotypes A, B, and E, respectively.[252,253] These data suggested declining PBT immunogenicity,

because earlier data (from 1986 to 1990) demonstrated adequate titers to toxin serotypes A and B persisting for 1 year after a booster dose in 96% and 44% of vaccinees, respectively.[254]

The Harris study, conducted from 1998 to 2000, demonstrated that approximately two thirds of vaccinees had a decrease in antitoxin titers by week 24 (6 months).[253,255,256] Studies of the PBT in 1963 demonstrated a decline in antitoxin titers occurring between week 14 and 52 (with most individuals not having measurable antitoxin titers at week 52), suggesting the need for a 6-month dose even with early PBT lots.[257]

Recent potency studies and antitoxin titers in 2005 have demonstrated that PBT may still offer potential protection against toxin serotype A, and to serotype B with lot PBP003. Potency studies demonstrated PBT still protects animals against challenge to toxin serotype C even though the PBT no longer produces adequate neutralizing antibody levels to toxin serotype C for passing potency testing. The PBT no longer provides adequate protection of animals (requires ≥ 50% animal survival postchallenge with lethal dose of toxin) or produces adequate neutralizing antibody levels against toxin serotypes D and E.[253]

Until recently, PBT was given as a primary series of three subcutaneous injections (0.5 mL at 0, 2, and 12 weeks), a booster dose at 12 months, and booster doses thereafter only for declining antitoxin titers.[257] The PBT dosing schedule was changed in 2004 because of the recent decline in immunogenicity and potency, and because of the results of the Harris study. The protocol for PBT lots produced in the 1970s now requires a primary series of four injections (0.5 mL at 0, 2, 12, and 24 weeks). A booster dose is still given at 12 months because antitoxin titers from the 24-week dose declined again by month 12 in the Harris study, and booster doses are now required annually. The CDC's current recommendation for at-risk persons who have received lots of PBT made in the 1970s is to consider personal protective measures as the sole source of protection against all the botulinum toxin serotypes.

Adverse events. PBT has been demonstrated to be safe, with adverse events being mainly local reactions at the injection site. Data from the CDC (passively reported) from over 20,000 vaccinations from 1970 to 2002 showed mild or no reaction associated with 91% of vaccinations, moderate local reactions (edema or induration between 30 and 120 mm) with 7% of vaccinations, and severe local reactions (reaction size greater than 120 mm, marked limitation of arm movement, or marked axillary node tenderness) with less than 1% of vaccinations. Systemic reactions occurred in approximately 5% of vaccinees, and were nondebilitating and reversible (mainly general malaise, chills or fever, itching or hives, and soreness or stiffness of the neck or back).[258]

New vaccine research. Vaccine candidates include formalin-inactivated toxoids (A through F) made in nearly the same way as formalin-inactivated PBT, with the goal of FDA approval.[259,260] However, production of formalin-inactivated toxoids is expensive and relatively time consuming. The production requires partially purified culture supernatants to be treated exhaustively with formaldehyde, performed by a highly trained staff within a dedicated high-containment laboratory space.[261] Furthermore, the resulting PBT is relatively impure, containing only 10% neurotoxoid (90% is irrelevant material). This impurity may be partly responsible for the occurrence of local reactions as well as the need for multiple injections to achieve and sustain protective titers. A bivalent AB botulinum toxoid was developed based on the experience of the PBT that optimized several of the manufacturing issues of the PBT, including a reduction of formaldehyde levels in the final product to potentially reduce local reactinogenicity.[262] Preclinical studies in the guinea pig and mouse models demonstrated that a single dose of 1.0 mL was protective against intraperitoneal challenge with toxin serotypes A and B, and it was associated with neutralizing antibody titers in guinea pigs of 8 IU/mL to toxin serotype A (50 to 100 times higher than generally observed with the PBT) and 1.25 IU/mL to toxin serotype B (10 to 20 times higher than observed with the PBT).

The use of pure and concentrated antigen in recombinant vaccines could offer advantages of increased immunogenicity and decreased reactogenicity (local reactions at the injection site) over formalin-inactivated toxoids.[263] Recombinant techniques use a fragment of the toxin that is immunogenic but is not capable of blocking cholinergic neurotransmitters. Both *Escherichia coli* and yeast expression systems have been used in the production of recombinant fragments, mainly the carboxy-terminal fragment of the heavy chain of the toxin. Vaccine candidates using recombinant fragments of botulinum toxins against botulinum toxin serotypes A, B, C, E, and F were protective in mice.[263–272] A vaccine recombinant candidate for botulinum toxin serotype A was protective in mice challenged intraperitoneally, producing levels of immunity similar to that attained with PBT, but with increased safety and a decreased cost per dose.[261] Phase I trials on the bivalent recombinant vaccine (toxin serotypes A and B) have been completed, with promising preliminary serologic results at 12 months after two doses of vaccine (at 0 and 6 weeks), and phase II trials are being proposed.[253] Recombinant vaccines given by aerosol are also being investigated.[273,274]

A candidate vaccine using a VEE virus replicon vector that involves the insertion of a synthetic carboxy-terminal fragment gene of the heavy chain of toxin serotype A is also being evaluated.[275] This vaccine

induced a strong antibody response in the mouse model and remained protective in mice against intraperitoneal challenge at 12 months.

Postexposure Prophylaxis

Any individuals suspected to have been exposed to botulinum toxin should be carefully monitored for evidence of botulism. Botulinum antitoxin should be administered if a person begins to develop symptoms of botulism. The bivalent botulinum antitoxin (serotypes A and B) is the only FDA-approved antitoxin preparation for adults currently available. The trivalent equine botulinum antitoxin (serotypes A, B, and E) is no longer available at the CDC because of declining antitoxin titers to toxin serotype E in this product. However, botulinum antitoxin for serotype E is available as an investigational product at the CDC (an equine antitoxin) and the California Department of Public Health (a human botulinum toxin immune globulin).

BabyBIG, a human botulism immune globulin derived from pooled plasma of adults immunized with PBT (A through E), was approved by the FDA in October 2003 for the treatment of infants with botulism from toxin serotypes A and B. Because the product is derived from humans, BabyBIG does not carry the high risk of anaphylaxis observed with equine antitoxin products or the risk of lifelong hypersensitivity to equine antigens. BabyBIG may be obtained from the California Department of Health Services (510-231-7600).

Additionally, USAMRIID had developed two equine antitoxin preparations against all toxin serotypes that are available as investigational use drugs for treating botulism: (1) botulism antitoxin, heptavalent, equine, types A, B, C, D, E, F, and G (HE-BAT) and (2) botulism antitoxin, F(ab')$_2$ heptavalent, equine toxin neutralizing activity types A, B, C, D, E, F, and G (Hfab-BAT). These products are "despeciated" equine antitoxin preparations, made by cleaving the F$_c$ fragments from the horse immunoglobulin G molecules, leaving only the F(ab')$_2$ fragments. However, 4% of horse antigens are still present in the preparation, so there is still a risk for hypersensitivity reactions. These investigational products are for use for treatment of botulism, and they would be considered for prophylactic use in asymptomatic persons only in special, high-risk circumstances.

Although passive antibody prophylaxis has been effective in protecting laboratory animals from toxin exposure,[276] the limited availability and short-lived protection of antitoxin preparations make preexposure or postexposure prophylaxis with these agents impractical for large numbers of people. Additionally, the administration of equine antitoxin in asymptomatic persons is not recommended because of the risk of anaphylaxis from the foreign proteins. However, if passive immunotherapy is given, it should be administered within 24 hours of a high-dose aerosol exposure to botulinum toxin.

Staphylococcal Enterotoxin B

Staphylococcal enterotoxins are toxins produced by *Staphylococcus aureus*, referred to as superantigens. Ingestion of staphylococcal enterotoxin B (SEB) is a common cause of food poisoning. However, inhalation of SEB may cause fever with respiratory symptoms within 3 to 12 hours of exposure, which may progress to overt pulmonary edema, acute respiratory disease syndrome, septic shock, and death.[277] The binding of toxin to the major histocompatibility complex stimulates the proliferation of large numbers of T cells, which results in production of cytokines (tumor necrosis factor, interferon-gamma, and interleukin-1) that are thought to mediate many of the toxic effects.

Vaccination

No vaccine against SEB is currently available. However, several candidate vaccines have demonstrated protection against SEB challenge in animal models. These vaccines are based on a correlation between human antibody titers and the inhibition of T-cell response to bacterial superantigens.

New vaccine research is ongoing. A recombinantly attenuated SEB vaccine given by nasal or oral routes, using cholera toxin as a mucosal adjuvant, induced both systemic and mucosal antibodies and provided protection in mice against intraperitoneal and mucosal challenge with wild-type SEB.[278] Subsequently, intramuscular vaccination with recombinantly attenuated SEB using an Alhydrogel (Accurate Chemical & Scientific Corporation, Westbury, NY) adjuvant was found to be protective in rhesus monkeys challenged by aerosols of lethal doses of SEB.[279] All monkeys developed antibody titers, and the release of inflammatory cytokines was not triggered.

A candidate SEB vaccine using a VEE virus replicon as a vector has also been studied.[280] The gene encoding mutagenized SEB was cloned into the VEE replicon plasmid, and the product was then assembled into VEE replicon particles. The vaccine elicited a strong antibody response in animal models and was protective against lethal doses of SEB.

SEB toxoids (formalin-inactivated) incorporated into meningococcal proteosomes or microspheres have been found to be immunogenic and protective against aerosol SEB challenge in nonhuman primates. The proteosome-toxoid given by intratracheal route elicited serum IgG and IgA antibody titers, and a strong IgA

response in bronchial secretions.[281] Vaccination by an intratracheal route with formalinized SEB toxoid-containing microspheres resulted in higher antibody titers in the serum and respiratory tract, a higher survival rate, and a lower illness rate than booster doses given by intramuscular or oral routes. (Microspheres provide controlled release of toxoid, which results in both a primary and an anamnestic secondary antitoxin response and thereby may require fewer doses.)[282] However, enteric symptoms such as vomiting still occurred in many vaccinees with both vaccine candidates.[281–283]

Postexposure Prophylaxis

No postexposure prophylaxis for SEB is available. Although passive immunotherapy can reduce mortality in animal models if given within 4 to 8 hours after inhalation, there are no current clinical trials in humans.

Ricin

Ricin is a protein toxin derived from the beans of the castor plant. Ricin's mechanism of toxicity is by inhibition of protein synthesis, which ultimately results in cell death. Inhalation of ricin as a small-particle aerosol may produce pathological changes within 8 hours, manifested as severe respiratory symptoms associated with fever and followed by acute respiratory failure within 36 to 72 hours. Ingestion of ricin may result in severe gastrointestinal symptoms (nausea, vomiting, cramps, and diarrhea) followed by vascular collapse and death.

Vaccination

No vaccine is currently available, but several vaccine candidates are being studied.[284] Because passive prophylaxis with monoclonal antibodies in animals is protective against ricin challenge, the vaccine candidates are based on induction of a humoral response.[285,286] However, even a single molecule of ricin toxin A-chain (RTA) within the cytoplasm of a cell will completely inhibit protein synthesis,[287] so any ricin toxoid may have the potential toxicity for vascular leak even if it is 1,000-fold less toxic.[288] Therefore, although ricin intoxication in animals can be prevented by vaccination

with a formalinized ricin toxin (toxoid) or a deglycosylated RTA,[289] there is still a concern and potential risk of vascular leak with these vaccine candidates.

The most promising development for a vaccine has been to genetically engineer the RTA subunit to eliminate both its enzymatic activity and its ability to induce vascular leaking. The nontoxic RTA subunit has been demonstrated to induce antibodies in animal models and protect mice against intraperitoneal challenge with large doses of ricin.[284] A pilot clinical trial in humans demonstrated a recombinant RTA vaccine (RiVax) given as three monthly intramuscular injections at doses of 10, 33, or 100 ug (five volunteers at each dose) was safe and elicited ricin-neutralizing antibodies in one of five individuals in the low-dose group, four of five in the intermediate-dose group, and five of five in the high-dose group.[290] Further human trials with this vaccine are not planned due to vaccine instability.

A ricin vaccine candidate (RTA 1-33/44-198) developed at USAMRIID demonstrated high relative stability to thermal denaturation, no detectable cytotoxicity, and immunogenicity in animal studies.[291] The vaccine (given as 3 intramuscular injections at 0, 4, and 8 weeks) was protective in mice against aerosol challenge with ricin at doses between 5 and 10 times the LD_{50}.[291] Additionally, no toxicity was observed in two animal models.[291]

A ricin toxoid vaccine encapsulated in polylactide microspheres or poly(lactide-co-glycolide) microspheres and given intranasally was demonstrated to be protective against aerosolized ricin intoxication in mice. Both systemic and mucosal immune responses were observed, with high titers of antiricin IgG2a at 2 weeks postvaccination and still present and protective in mice 1 year later.[292] Oral vaccination of mice with the ricin toxoid vaccine encapsulated in poly(lactide-co-glycolide) microspheres was also protective against lethal aerosol ricin challenge.[293]

Postexposure Prophylaxis

There is no postexposure prophylaxis for ricin intoxication. Although passive immunoprophylaxis of mice can reduce mortality against intravenous or intraperitoneal ricin challenge if given within a few hours of exposure, passive immunoprophylaxis is not effective against aerosol intoxication.[285,286]

SUMMARY

Although medical countermeasures are effective in preventing disease, the greater challenge is to develop a balanced approach that may provide preexposure and postexposure medical countermeasures to pro-

tect both the military and civilian populations. The military has recognized the benefit of vaccinating troops for protection against exposure from a biological weapons release in a battlefield setting. However,

vaccination of civilians in advance may not be feasible, because of the larger host of potential biological threat agents in a civilian population and the infrequent occurrence of bioterrorism events expected in a civilian population. Risk–benefit assessments must be considered in vaccine recommendations for the civilian and military populations, as well as the logistics of maintaining immunity with vaccine booster doses. Protection of the public from bioterrorism will require the development, production, stockpile maintenance, and distribution of effective medical countermeasures for both prevention and treatment of illness, with careful forethought about the balance of preexposure and postexposure countermeasures. It is likely that the military will be involved with both distribution of medical supplies and management of bioterrorism events within the continental United States, and it is the responsibility that military physicians be properly trained and prepared for managing bioterrorism events.

REFERENCES

1. Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a biological warfare agent. *JAMA* 1999;281:1735–1745.

2. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*. 1997;278:399–411.

3. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related inhalational anthrax—Connecticut, 2001. *MMWR Morb Mortal Wkly Rep*. 2001;50:1049–1051.

4. Watson DW, Cromartie WJ, Bloom WL, Kegeles G, Heckly RJ. Studies on infection with *Bacillus anthracis* III. chemical and immunological properties of the protective antigen in crude extracts of skin lesions of *B anthracis*. *J Infect Dis*. 1947;80:28–40.

5. Boor AK. An antigen prepared in vitro effective for immunization against anthrax. I. Preparation and evaluation of the crude protective antigen. *J Infect Dis*. 1955;97:194–202.

6. Darlow HM, Belton FC, Henderson DW. Use of anthrax antigen to immunize man and monkey. *Lancet*. 1956;2:476–479.

7. Wright GG, Green TW, Kanode RB Jr. Studies on immunity in anthrax. V. Immunizing activity of alum-precipitated protective antigen. *J Immunol*.1954;73:387–391.

8. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *Am J Public Health*. 1962;52:632–645.

9. Puziss M, Manning LC, Lynch JW, Barclay E, Abelow I, Wright GG. Large-scale production of protective antigen of *Bacillus anthracis* in aerobic cultures. *Appl Environ Microbiol*. 1963;11:330–334.

10. Puziss M, Wright GG. Studies on immunity in anthrax. X. Gel-adsorbed protective antigen for immunization of man. *J Bacteriol*. 1963;85:230–236.

11. Centers for Disease Control and Prevention. Adult immunization. Recommendations of the Immunization Practices Advisory Committee. *MMWR Morb Mortal Wkly Rep*. 1984;33(Suppl):1S–68S.

12. Rusnak JM, Kortepeter MG, Hawley RJ, Anderson AO, Boudreau E, Eitzen E. Risk of occupationally acquired illnesses from biological threat agents in unvaccinated laboratory workers. *Biosecur Bioterr*. 2004;2:281–293.

13. Turnbull PC, Broster MG, Carman JA, Manchee RJ, Melling J. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Infect Immun*. 1986;52:356–363.

14. Mahlandt BG, Klein F, Lincoln RE, Haines BW, Jones WI Jr, Friedman RH. Immunologic studies of anthrax. IV. Evaluation of the immunogenicity of three components of anthrax toxin. *J Immunol*. 1966;96:727–733.

15. Ivins BE, Pitt ML, Fellows PF, et al. Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in rhesus macaques. *Vaccine*. 1998;16:1141–1148.

16. Johnson-Winegar A. Comparison of enzyme-linked immunosorbent and indirect hemagglutination assays for determining anthrax antibodies. *J Clin Microbiol*. 1984;20:357–361.

17. Little SF, Ivins BE, Fellows PF, Friedlander AM. Passive protection by polyclonal antibodies against *Bacillus anthracis* infection in guinea pigs. *Infect Immun*. 1997;65:5171–5175.

18. Gladstone GP. Immunity to anthrax: protective antigen present in cell-free filtrates. *Brit J Exp Pathol*. 1946;27:294–418.

19. Turnbull PCB. Anthrax vaccines: past, present and future. *Vaccine*. 1991;9:533–539.

20. Ward MK, McGann VG, Hogge AL Jr, Huff ML, Kanode RG Jr, Roberts EO. Studies on anthrax infections in immunized guinea pigs. *J Infect Dis*. 1965;115:59–67.

21. Friedlander AM, Pittman PR, Parker GW. Anthrax vaccine: evidence for safety and efficacy against inhalational anthrax. *JAMA*. 1999;282:2104–2106.

22. Advisory Committee on Immunization Practices. Use of anthrax vaccine in the United States. *MMWR Recomm Rep*. 2000;49(RR-15):1–23.

23. Henderson DW, Peacock S, Belton FC. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. *J Hyg*. 1956;54:28–36.

24. Friedlander AM, Welkos SL, Pitt ML, et al. Postexposure prophylaxis against experimental inhalation anthrax. *J Infect Dis*. 1993;167:1239–1242.

25. Vietri NJ, Purcell BK, Lawler JV, et al. Short-course postexposure antibiotic prophylaxis combined with vaccination protects against experimental inhalational anthrax infection. *Proc Natl Acad Sci U S A*. 2006;103:7813–7816.

26. Centers for Disease Control and Prevention. *Application and Report on Manufacture of Anthrax Protective Antigen, Aluminum Hydroxide Adsorbed (DBS–IND 180). Observational Study*. Atlanta, Ga: CDC; 1970.

27. Chen RT, Rastogi SC, Mullen JR, et al. The vaccine adverse event reporting system (VAERS). *Vaccine.* 1994;12:542–50.

28. Pittman PR, Gibbs PH, Cannon TL, Friedlander AM. Anthrax vaccine: short-term safety experience in humans. *Vaccine*. 2002;20:972–978.

29. Pittman PR, Kim-Ahn G, Pifat DY, et al. Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. *Vaccine.* 2002;20:1412–1420.

30. US Army Medical Command. *Clinical Guidelines for Managing Adverse Events After Vaccination*. Falls Church, Va: USA MEDCOM; 2004.

31. Shlyakhov EN, Rubinstein E. Human live anthrax vaccine in the former USSR. *Vaccine.* 1994;12:727–730.

32. Shlyakhov E, Rubinstein E, Novikov I. Anthrax post-vaccinal cell-mediated immunity in humans: kinetics pattern. *Vaccine*.1997;15:631–636.

33. Brachman PS, Friedlander AM. Anthrax. In: Plotkin SA, Orenstein WA, eds. *Vaccines*. 3rd ed. Philadelphia, Pa: WB Saunders, 1999: 629–637.

34. Mikszta JA, Sullivan VJ, Dean C, et al. Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. *J Infect Dis*. 2005;191:278–288.

35. Opal SM, Artenstein AW, Cristofaro PA, et al. Inter-alpha-inhibitor proteins are endogenous furin inhibitors and provide protection against experimental anthrax intoxication. *Infect Immun*. 2005;73:5101–5105.

36. Bell DM, Kozarsky PE, Stephens DS. Clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. *Emerg Infect Dis*. 2002;8:222–225.

37. Centers for Disease Control and Prevention. Occupational health guidelines for remediation workers at *Bacillus anthracis*-contaminated sites—United States, 2001–2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:786–789.

38. Meselson M, Guillemin J, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science.* 1994;266:1202–1208.

39. Shepard CW, Soriano-Gabarro M, Zell ER, et al. Antimicrobial postexposure prophylaxis for anthrax: adverse events and adherence. *Emerg Infect Dis*. 2002;8:1124–1132.

40. Centers for Disease Control and Prevention. Update: adverse events associated with anthrax among postal employees—New Jersey, New York City, and the District of Columbia metropolitan area, 2001. *MMWR Morb Mortal Wkly Rep*. 2001;50:1051–1054.

41. Centers for Disease Control and Prevention. Use of anthrax vaccine in response to terrorism: supplemental recommendations of the advisory committee on immunization practices. *MMWR Morb Mortal Wkly Rep*. 2002;51:1024–1026.

42. Pittman PR, Kim-Ahn G, Pifat DY, et al. Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. *Vaccine.* 2002;20:1412–1420.

43. Centers for Disease Control and Prevention. Suspected cutaneous anthrax in a laboratory worker—Texas, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:279–281.

44. Centers for Disease Control and Prevention. Notice to readers: evaluation of postexposure antibiotic prophylaxis to prevent anthrax. *MMWR Morb Mortal Wkly Rep*. 2002;51:59.

45. Centers for Disease Control and Prevention. Notice to readers: interim guidelines for investigation of and response to *Bacillus anthracis* exposures. *MMWR Morb Mortal Wkly Rep*. 2001;50:987–990.

46. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon; medical and public health management. *JAMA*. 2001;285:2763–2773.

47. Burke DS. Immunization against tularemia: analysis of the effectiveness of live *Francisella tularensis* vaccine in the prevention of laboratory-acquired tularemia. *J Infect Dis.* 1977;135:55–60.

48. Eigelsbach HT, Downs CM. Prophylactic effectiveness of live and killed tularemia vaccines. I. Production of vaccine and evaluation in the white mouse and guinea pig. *J Immunol.* 1961;87:415–425.

49. Overholt EL, Tigertt WD, Kadull PJ, et al. An analysis of forty-two cases of laboratory-acquired tularemia. Treatment with broad spectrum antibiotics. *Am J Med.* 1961;30:785–806.

50. Tarnvik A. Nature of protective immunity to *Francisella tularensis*. *Rev Infect Dis.* 1989;11:440–451.

51. Sjostedt A, Sandstrom G. Tarnvik A. Humoral and cell-mediated immunity in mice to a 17-kilodalton lipoprotein of *Francisella tularensis* expressed by *Salmonella typhimurium*. *Infect Immun*. 1992;60:2855–2862.

52. Anthony LS, Kongshavn PA. Experimental murine tularemia caused by *Francisella tularensis*, live vaccine strain: a model of acquired cellular resistance. *Microb Pathog*. 1987;2:3–14.

53. Buchanan TM, Brooks GF, Brachman PS. The tularemia skin test. 325 skin tests in 210 persons: serologic correlation and review of the literature. *Ann Intern Med*. 1971;74:336–343.

54. Eigelsbach HT, Hunter DH, Janssen WA, Dangerfield HG, Rabinowitz SG. Murine model for study of cell-mediated immunity: protection against death from fully virulent *Francisella tularensis* infection. *Infect Immun*. 1975;12:999–1005.

55. Kostiala AA, McGregor DD, Logie PS. Tularemia in the rat. I. The cellular basis on host resistance to infection. *Immunology.* 1975;28:855–869.

56. Tarnvik A, Lofgren ML, Lofgren G, Sandstrom G, Wolf-Watz H. Long-lasting cell-mediated immunity induced by a live *Francisella tularensis* vaccine. *J Clin Microbiol*. 1985;22:527–530.

57. Koskela P, Herva E. Cell-mediated and humoral immunity induced by a live *Francisella tularensis* vaccine. *Infect Immun*. 1982;36:983–989.

58. Tarnvik A, Sandstrom G, Lofgren S. Time of lymphocyte response after onset of tularemia and after tularemia vaccination. *J Clin Microbiol*. 1979;10:854–860.

59. Tarnvik A, Lofgren S. Stimulation of human lymphocytes by a vaccine strain of *Francisella tularensis. Infect Immun*. 1975;12:951–957.

60. Hornick RB, Eigelsbach HT. Aerogenic immunization of man with live Tularemia vaccine. *Bacteriol Rev*. 1966;30:532–538.

61. Massey ED, Mangiafico JA. Microagglutination test for detecting and measuring serum agglutinins of *Francisella tularensis. Appl Microbiol*. 1974;27:25–27.

62. Sandstrom GA, Tarnvik A, Wolf-Watz H, Lofgren S. Antigen from *Francisella tularensis*: nonidentity between determinants participating in cell-mediated and humoral reactions. *Infect Immun*. 1984;45:101–106.

63. Saslaw S, Eigelsbach HT, Wilson GR, Prior JA, Carhart S. Tularemia vaccine study. I. Intracutaneous challenge. *Arch Intern Med*. 1961;107:698–701.

64. Conlan JW. Vaccines against *Francisella tularensis*—past, present, and future. *Expert Rev Vaccines*. 2004;3:307–314.

65. Sawyer WD, Dangerfield HG, Hogge AL, Crozier D. Antibiotic prophylaxis and therapy of airborne tularemia. *Bacteriol Rev*. 1966;30:542–550.

66. McCrumb FR Jr, Snyder MJ, Woodward TE. Studies on human infection with *Pasteurella tularensis*. Comparison of streptomycin and chloramphenicol in the prophylaxis of clinical disease. *Trans Assoc Am Physicians*. 1957;70:74–80.

67. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA*. 2000;283:2281–2290.

68. 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.

69. Titball RW, Williamson ED. Vaccination against bubonic and pneumonic plague. *Vaccine*. 2001;19:4175–4184.

70. Russell P, Eley SM, Hibbs SE, Manchee RJ, Stagg AJ, Titball RW. A comparison of Plague vaccine, USP and EV76 vaccine induced protection against *Yersinia pestis* in a murine model. *Vaccine*. 1995;13:1551–1556.

71. Gage KL, Dennis DT, Tsai TF. Prevention of plague: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep*. 1996;45:1–15.

72. Cavanaugh DC, Elisberg BL, Llwellyn CH, et al. Imminization. V. Indirect evidence for the efficacy of plague vaccine. *J Infect Dis*. 1974;129:S37–S40.

73. Cavanaugh DC, Dangerfield HG, Hunter DH, et al. Some observations on the current plague outbreak in the Republic of Vietnam. *Am J Pub Health*. 1968;58:742–752.

74. Meyer KF. Effectiveness of live or killed plague vaccine in man. *Bull World Health Organ*. 1970;42:653–656.

75. Food and Drug Administration. Biological products; bacterial vaccines and toxoids; implementation of efficacy review. *Federal Register*. 1985;50:51002–51117.

76. Meyer KF, Cavanaugh DC, Bartelloni PJ, Marshall JD Jr. Plague immunization. I. Past and present trends. *J Infect Dis*. 1974;129(suppl):S13–S18.

77. Anderson GW Jr, Heath DG, Bolt CR, Welkos SL, Friedlander AM. Short- and long-term efficacy of single-dose subunit vaccines against *Yersinia pestis* in mice. *Am J Trop Med Hyg*. 1998;58:793–799.

78. Heath DG, Anderson GW Jr, Mauro JM, et al. Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine. *Vaccine*. 1998;16:1131–1137.

79. Russell P, Eley SM, Green M, et al. Efficacy of doxycycline or ciprofloxacin against experimental *Yersinia pestis* infection. *J Antimicrob Chemother*. 1998;41:301–305.

80. Russell P, Eley SM, Bell DL, Manchee RJ, Titball RW. Doxycycline or ciprofloxacin prophylaxis and therapy against experimental *Yersinia pestis* infection in mice. *J Antimicrob Chemother.* 1996;37:769–774.

81. Bonacorsi SP, Scavizzi MR, Guiyoule A, Amouroux JH, Carniel E. Assessment of a fluoroquinolone, three beta-lactams, two aminoglycosides and a cycline in treatment of murine *Yersinia pestis* infection. *Antimicrob Agents Chemother*. 1994;38:481–486.

82. White ME, Gordon D, Poland JD, Barnes AM. Recommendations for the control of *Yersinia pestis* infections. Recommendations from the CDC. *Infect Control*. 1980;1:324–329.

83. Prevention of plague: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 1996;45(RR-14):1–15.

84. McGovern TW, Friedlander AM. Plague. In: Sidell FR, Takafuji ET, Franz DR, eds. *Medical Aspects of Chemical and Biological Warfare*. In: Zajtchuk R, Bellamy RF, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of the Surgeon General, Borden Institute; 1997: Chap 23.

85. Currie BJ, Fisher DA, Howard DM, et al. Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. *Clin Infect Dis*. 2000;31:981–986.

86. Srinivasan A, Kraus CN, DeShazer D, et al. Glanders in a military research microbiologist. *N Engl J Med*. 2001;345:256–258.

87. Howe C, Miller WR. Human glanders: report of six cases. *Ann Int Med*. 1947;26:92–115.

88. Centers for Disease Control and Prevention. Laboratory exposure to *Burkholderia pseudomallei*—Los Angeles, California, 2003. *MWWR Morb Mortal Wkly Rep*. 2004;53:988–990.

89. Green RN, Tuffnell PG. Laboratory acquired melioidosis. *Am J Med*. 1968;44:599–605.

90. Schlech WF III, Turchik JB, Westlake RE Jr, Klein GC, Band JD, Weaver RE. Laboratory-acquired infection with *Pseudomonas pseudomallei* (melioidosis). *N Engl J Med*. 1981;305:1133–1135.

91. Batmanov VP, Ilyukhin VI, Lozovaya NA, Andropova NV. Urgent prophylaxis of experimental melioidosis in white rats [Abstract no. 61]. In: *Programs and Abstracts Book of the 2004 American Society for Microbiology Biodefense Research Meeting, Baltimore, MD*. Washington, DC: ASM; 2004: 31.

92. Russell P, Eley SM, Ellis J, et al. Comparison of efficacy of ciprofloxacin and doxycycline against experimental melioidosis and glanders. *J Antimicrob Chemother*. 2000;45:813–818.

93. Manzeniuk IN, Dorokhin VV, Svetoch EA. The efficacy of antibacterial preparations against *Pseudomonas mallei* in in-vitro and in-vivo experiments. *Antibiot Khimioter*. 1994;39:26–30.

94. Kenny DJ, Russell P, Rogers D, Eley SM, Titball RW. In vitro susceptibilities of *Burkholderia mallei* in comparison to those of other pathogenic *Burkholderia* spp. *Antimicrob Agents Chemother*. 1999;43:2773–2775.

95. Winton MD, Everett ED, Dolan SA. Activities of five new fluoroquinolones against *Pseudomonas pseudomallei*. *Antimicrob Agents Chemother*. 1988;32:928–929.

96. Leelarasamee A, Bovornkitti S. Melioidosis: review and update. *Rev Infect Dis.* 1989;11:413–425.

97. Chaowagul W, Suputtamongkul Y, Smith MD, White NJ. Oral fluoroquinolones for maintenance treatment of melioidosis. *Trans R Soc Trop Med Hyg*. 1997;91:599–601.

98. Lumbiganon P, Sookpranee T. Ciprofloxacin therapy for localized melioidosis. *Pediatr Infect Dis J*. 1992;11:418–419.

99. Centers for Disease Control and Prevention. Laboratory exposure to *Burkholderia pseudomallei*—Los Angeles, California, 2003. *MMWR Morb Mortal Wkly Rep*. 2004;53:988–990.

100. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*. 1997;278:399–411.

101. Noviello S, Gallo R, Kelly M, et al. Laboratory-acquired brucellosis. *Emerg Infect Dis*. 2004;10:1848–1850.

102. Luzzi GA, Brindle R, Sockett PN, Solera J, Klenerman P, Warrell DA. Brucellosis: imported and laboratory-acquired cases, and an overview of treatment trials. *Trans R Soc Trop Med Hyg*. 1993;87:138–41.

103. Joint FAO/WHO Expert Committee on Brucellosis. *Sixth Report: Joint FAO/WHO Expert Committee on Brucellosis*. Geneva, Switzerland: World Health Organization; 1986. Technical Report Series 740.

104. Fiori PL, Mastrandrea S, Rappelli P, Cappuccinelli P. *Brucella abortus* infection acquired in microbiology laboratories. *J Clin Microbiol*. 2000;38:2005–2006.

105. Robichaud S, Libman M, Behr M, Rubin E. Prevention of laboratory-acquired brucellosis. *Clin Infect Dis*. 2004;38: e119–e122.

106. Akova M, Uzun O, Akalin HE, Hayran M, Unal S, Gur D. Quinolones in treatment of human brucellosis: comparative trial of ofloxacin-rifampin virus doxycycline-rifampin. *Antimicrob Agents Chemother*. 1993;37:1831–1834.

107. McLean DR, Russell N, Khan MY. Neurobrucellosis: clinical and therapeutic features. *Clin Infect Dis*. 1992;15:582–590.

108. Lang R, Rubinstein E. Quinolones for the treatment of brucellosis. *J Antimicrob Chemother*. 1992;29:357–360.

109. Baca OG, Paretsky D. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. *Microbiol Rev*. 1983;47:127–149.

110. Kishimoto RA, Stockman RW, Redmond CL. Q fever: diagnosis, therapy, and immunoprophylaxis. *Mil Med*. 1979;144:183–187.

111. Benenson AS, Tigertt WD. Studies on Q fever in man. *Trans Assoc Am Physicians*. 1956;69:98–104.

112. Ormsbee RA, Bell EJ, Lackman DB, Tallent G. The influence of phase on the protective potency of Q fever vaccine. *J Immunol*. 1964; 404–412.

113. Marmion BP, Ormsbee RA, Kyrkou M, et al. Vaccine prophylaxis of abattoir-associated Q fever: eight years' experience in Australian abattoirs. *Epidemiol Infect*. 1990;104:275–287.

114. Shapiro RA, Siskind V, Schofield FD, Stallman N, Worswick DA, Marmion BP. A randomized, controlled, double-blind, cross-over, clinical trial of Q fever vaccine in selected Queensland abattoirs. *Epidemiol Infect*. 1990;104:267–273.

115. Krutitskaya L, Tokarevich N, Zhebrun A, et al. Autoimmune component in individuals during immune response to inactivated combined vaccine against Q fever. *Acta Virol*. 1996;40:173–177.

116. Izzo AA, Marmion BP, Worswick DA. Markers of cell-mediated immunity after vaccination with an inactivated, whole-cell Q fever vaccine. *J Infect Dis*. 1988;157:781–789.

117. Stoker MG. Q fever down the drain. *Brit Med J*. 1957;1:425–427.

118. Benenson AS. *A Q Fever Vaccine: Efficacy and Present Status*. Washington, DC: Walter Reed Army Institute of Research; 1959. Med. Science Publication 6:47060.

119. Lackman DB, Bell EJ, Bell JF, Pickens EG. Intradermal sensitivity testing in man with a purified vaccine for Q fever. *AmJ Public Health*. 1962;52:87–93.

120. Kishimoto RA, Gonder JC, Johnson JW, Reynolds JA, Larson EW. Evaluation of a killed phase I *Coxiella burnetii* vaccine in cynomolgus monkeys (*Macaca fascicularis*). *Lab Anim Sci*. 1981;31:48–51.

121. Behymer DE, Biberstein EL, Riemann HP, et al. Q fever (*Coxiella burnetii*) investigations in dairy cattle. Challenge of immunity after vaccination. *Am J Vet Res*.1976;37:631–34.

122. Johnson JE, Kadull PJ. Laboratory-acquired Q fever. A report of fifty cases. *Am J Med*. 1966;41:391–403.

123. Rusnak JM, Kortepeter MG, Aldis J, Boudreau E. Experience in the medical management of potential laboratory exposures to agents of bioterrorism on the basis of risk assessment at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). *J Occup Environ Med*. 2004;46:801–811.

124. Izzo AA, Marmion BP, Hackstadt T. Analysis of the cells involved in the lymphoproliferative response to *Coxiella burnetii* antigens. *Clin Exp Immunol*. 1991;85:98–108.

125. Luoto L, Bell JF, Casey M, Lackman DB. Q fever vaccination of human volunteers. I. The serological and skin-test response following subcutaneous injections. *Am J Hyg*. 1963;78:1–15.

126. Annual reports for BB-IND 610, Q-fever vaccine, inactivated, NDBR 105, submitted to the Food and Drug Administration, 1972–2002.

127. Johnson JW, McLeod CG, Stookey JL, Higbee GA, Pedersen CE Jr. Lesions in guinea pigs infected with *Coxiella burnetii* strain M-44. *J Infect Dis*. 1977;135:995–998.

128. Madariaga MG, Rezai K, Trenholme GM, Weinstein RA. Q fever: a biological weapon in your backyard. *Lancet*. 2003;3:709–721.

129. Marmion BP. Development of Q-fever vaccines, 1937 to 1967. *Med J Aust*. 1967;2:1074–1078.

130. Zhang G, Samuel JE. Vaccines against *Coxiella* infection. *Expert Rev Vaccines*. 2004;3:577–584.

131. Williams JC, Hoover TA, Waag DM, Banerjee-Bhatnagar N, Bolt CR, Scott GH. Antigenic structure of *Coxiella burnetii*. A comparison of lipopolysaccharide and protein antigens as vaccines against Q fever. *Ann N Y Acad Sci*. 1990;590:370–380.

132. Zhang YX, Zhi N, Yu SR, Li QJ, Yu GQ, Zhang X. Protective immunity induced by 67 K outer membrane protein of phase I *Coxiella burnetii* in mice and guinea pigs. *Acta Virol*. 1994;38:327–332.

133. Lennette EH, Koprowski H. Human infection with Venezuelan equine encephalomyelitis virus. *JAMA*. 1943;123:1088–1095.

134. Sutton LS, Brooke CC. Venezuelan equine encephalomyelitis due to vaccination in man. *JAMA*. 1954;155:1473–1478.

135. McKinney RW, Berge TO, Sawyer WD, Tigertt WD, Crozier D. Use of an attenuated strain of Venezuelan equine encephalomyelitis virus for immunization in man. *Am J Trop Med Hyg*. 1963;12:597–603.

136. Dynport Vaccine Company (Frederick, Md). IND stockpile assessment, Venezuelan equine encephalomyelitis VEE-TC-83 summary reports. August 2001.

137. Pittman PR, Makuch RS, Mangiafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine*.1996;14:337–343.

138. Ryder E, Ryder S. Human Venezuelan equine encephalitis virus infection and diabetes in Zulia State, Venezuela. *J Med Virol*. 1983;11:327–332.

139. Gorelkin L, Jahrling PB. Pancreatic involvement by Venezuelan equine encephalomyelitis virus in the hamster. *AmJ Pathol*. 1974;75:349–362.

140. Rayfield EJ, Gorelkin L, Curnow RT, Jarhling PB. Virus-induced pancreatic disease by Venezuelan encephalitis virus. Alterations in glucose tolerance and insulin resistance. *Diabetes*. 1976;25:623–631.

141. Bowen GS, Rayfield EJ, Monath TP, Kemp GE. Studies of glucose metabolism in rhesus monkeys after Venezuelan equine encephalitis virus infection. *J Med Virol*. 1980;6:227–234.

142. Casamassima AC, Hess LW, Marty A. TC-83 Venezuelan equine encephalitis vaccine exposure during pregnancy. *Teratology*. 1987;36:287–289.

143. London WT, Levitt NH, Kent SG, Wong VG, Sever JL. Congenital cerebral and ocular malformations induced in rhesus monkeys by Venezuelan equine encephalitisvirus. *Teratology*. 1977;16:285–296.

144. Wenger F. Venezuelan equine encephalitis. *Teratology*. 1977;16:359–362.

145. Cole FE Jr, May SW, Eddy GA. Inactivated Venezuelan equine encephalomyelitis vaccine prepared from attenuated (TC-83 strain) virus. *Appl Microbiol*. 1974;27:150–153.

146. Jahrling PB, Stephenson EH. Protective efficacies of live attenuated and formaldehyde-inactivated Venezuelan equine encephalitis virus vaccines against aerosol challenge in hamsters. *J Clin Microbiol*. 1984;19:429–431.

147. Monath TP, Cropp CB, Short WF, et al. Recombinant vaccinia-Venezuelan equine encephalomyelitis (VEE) vaccine protects nonhuman primates against parenteral and intranasal challenge with virulent VEE virus. *Vaccine Res*. 1992;1:55–68.

148. Pratt WD, Gibbs P, Pitt ML, Schmaljohn AL. Use of telemetry to assess vaccine-induced protection against parenteral and aerosol infections of Venezuelan equine encephalitis virus in non-human primates. *Vaccine*. 1998;16:1056–1064.

149. Hart MK, Pratt W, Panelo F, Tammariello R, Dertzbaugh M. Venezuelan equine encephalitis virus vaccines induce mucosal IgA responses and protection from airborne infection in BALB/c, but not C3H/HeN mice. *Vaccine*. 1997;15:363–369.

150. Bartelloni PJ, McKinney RW, Calia FM, Ramsburg HH, Cole FE Jr. Inactivated western equine encephalomyelitis vaccine propagated in chick embryo cell culture. Clinical and serological evaluation in man. *Am J Trop Med Hyg*. 1971;20:146–149.

151. Pedersen CE Jr. Preparation and testing of vaccine prepared from the envelopes of Venezuelan, eastern, and western equine encephalomyelitis viruses. *J Clin Microbiol*. 1976;3:113–118.

152. Cole FE Jr, McKinney RW. Use of hamsters for potency assay of Eastern and Western equine encephalitis vaccines. *Appl Microbiol*. 1969;17:927–928.

153. Barber TL, Walton TE, Lewis KJ. Efficacy of trivalent inactivated encephalomyelitis virus vaccine in horses. *Am J Vet Res*. 1978;39:621–625.

154. Pittman PR. Personal communication. Chief, Division of Medicine, USAMRIID, Fort Detrick. July 9, 2007.

155. Bartelloni PJ, McKinney RW, Duffy TP, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture. II. Clinical and serologic responses in man. *Am J Trop Med Hyg*. 1970;19:123–126.

156. Maire LF III, McKinney RW, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture. I. Production and testing. *Am J Trop Med Hyg*. 1970;19:119–122.

157. Sorrentino JV, Berman S, Lowenthal JP, Cutchins E. The immunologic response of the guinea pig to Eastern equine encephalomyelitis vaccines. *Am J Trop Med Hyg*. 1968;17:619–624.

158. Mack TM. Smallpox in Europe, 1950–1971. *J Infect Dis*. 1972;125:161–169.

159. Mack TM, Thomas DB, Ali A, Muzaffar Khan M. Epidemiology of smallpox in West Pakistan. I. Acquired immunity and the distribution of disease. *Am J Epidemiol*. 1972;95:157–168.

160. Dixon CW. *Smallpox*. London, England: J & A Churchill Ltd; 1962.

161. Wharton M, Strikas RA, Harpaz R, et al. Recommendations for using smallpox vaccine in a pre-event vaccination program. Supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep*. 2003;52(RR-7):1–16.

162. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. *Clin Infect Dis*. 2003;37:251–271.

163. Grabenstein JD, Winkenwerder W Jr. US military smallpox vaccination program experience. *JAMA*. 2003;289:3278–3282.

164. Cono J, Casey CG, Bell DM; Centers for Disease Control and Prevention. Smallpox vaccination and adverse reactions. Guidance for clinicians. *MMWR Recomm Rep*. 2003;52(RR-4):1–28.

165. Fillmore GL, Ward TP, Bower KS, et al. Ocular complications in the Department of Defense Smallpox Vaccination Program. *Ophthalmology*. 2004;111:2086–2093.

166. Ruben FL, Lane JM. Ocular vaccinia: an epidemiologic analysis of 348 cases. *Arch Ophthalmol*. 1970;84:45–48.

167. Fulginiti VA, Winograd LA, Jackson M, Ellis P. Therapy of experimental vaccinal keratitis. Effect of idoxuridine and VIG. *Arch Ophthalmol*. 1965;74:539–544.

168. Lane JM, Ruben FL, Neff JM, Millar, JD. Complication of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis*. 1970;122:303–309.

169. Kempe CH. Studies on smallpox and complications of smallpox vaccination. *Pediatrics*. 1960;26:176–189.

170. Vellozzi C, Lane JM, Averhoff F, et al. Generalized vaccinia, progressive vaccinia, and eczema vaccinatum are rare following smallpox (vaccinia) vaccination: United States surveillance, 2003. *Clin Infect Dis*. 2005;41:689–697.

171. Smee DF, Bailey KW, Wong MH, Wandersee MK, Sidwell RW. Topical cidofovir is more effective than is parenteral therapy for treatment of progressive vaccinia in immunocompromised mice. *J Infect Dis*. 2004;190:1132–1139.

172. de Vries E. Post-vaccinial perivenous encephalitis. Amsterdam, The Netherlands: Elsevier; 1960.

173. Neff JM, Lane JM, Pert JH, Moore R, Millar JD, Henderson DA. Complications of smallpox vaccination I. National survey in the United States, 1963. *New Engl J Med*. 1967;276:125–132.

174. Lane JM, Ruben FL, Abrutyn E, Millar JD. Deaths attributable to smallpox vaccination, 1959 to 1966, and 1968. *JAMA*. 1970;212:441–444.

175. Sejvar JJ, Labutta RJ, Chapman LE, Grabenstein JD, Iskander J, Lane JM. Neurologic adverse events associated with smallpox vaccination in the United States, 2002–2004. *JAMA*. 2005;294:2744–2750.

176. Chen RT, Lane JM. Myocarditis: the unexpected return of smallpox vaccine adverse events. *Lancet*. 2003;362:1345–1346.

177. Thorpe LE, Mostashari F, Karpati AM, et al. Mass smallpox vaccination and cardiac deaths, New York City, 1947. *Emerg Infect Dis*. 2004;10:917-920.

178. Centers for Disease Control and Prevention. Cardiac adverse events following smallpox vaccination—United States, 2003. *MMWR Morb Mortal Wkly Rep*. 2003;52:248–250.

179. Centers for Disease Control and Prevention. Supplemental recommendations on adverse events following smallpox vaccine in the pre-event vaccination program: recommendations of the Advisory Committee on Immunization Practices. *MMWR Morb Mortal Wkly Rep*. 2003;52:282–284.

180. Centers for Disease Control and Prevention. Update: cardiac-related events during the civilian smallpox vaccination program—United States, 2003. *MMWR Morb Mortal Wkly Rep*. 2003;52:292–296.

181. Monath TP, Caldwell JR, Mundt W, et al. ACAM2000 clonal Vero cell culture vaccinia virus (New York City Board of Health strain)—a second-generation smallpox vaccine for biodefense. *Int J Infect Dis.* 2004;8(suppl 2):S31–S44.

182. Mayr A. Smallpox vaccination and bioterrorism with pox viruses. *Comp Immunol Microbiol Infect Dis.* 2003;26:423–430.

183. Ober BT, Bruhl P, Schmidt M, et al. Immunogenicity and safety of defective vaccinia virus lister: comparison with modified vaccinia virus Ankara. *J Virol.* 2002;76:7713–7723.

184. Kempe CH, Bowles C, Meiklejohn G, et al. The use of vaccinia hyperimmune gamma-globulin in the prophylaxis of smallpox. *Bull World Health Organ.* 1961;25:41–48.

185. Marennikova SS. The use of hyperimmune antivaccinia gamma-globulin for the prevention and treatment of smallpox. *Bull World Health Organ.* 1962;27:325–330.

186. Hopkins RJ, Lane JM. Clinical efficacy of intramuscular vaccinia immune globulin: a literature review. *Clin Infect Dis.* 2004;39:819–826.

187. Breman JG, Henderson DA. Diagnosis and management of smallpox. *N Eng J Med.* 2002;346:1300–1308.

188. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization, 1988. Available at: http://www.who.int/eme/disease/smallpox/Smallpoxeradication.html. Accessed April 5, 2002.

189. Hopkins RJ, Kramer WG, Blackwelder WC, et al. Safety and pharmacokinetic evaluation of intravenous vaccinia immune globulin in healthy volunteers. *Clin Infect Dis.* 2004;39:759–766.

190. Updated interim CDC guidance for use of smallpox vaccine, cidofovir, and vaccinia immune globulin (VIG) for prevention and treatment in the setting of an outbreak of monkeypox infections. Available at: http://www.cdc.gov/ncidod/monkeypox/treatmentguidelines.htm. Accessed July 5, 2007.

191. DeClerq E, Neyts J. Therapeutic potential of nucleoside/nucleoside analogues against poxvirus infections. *Rev Med Virol.* 2004;14:289–300.

192. DeClercq E, Holy A, Rosenberg I, Sakuma T, Balzarini J, Maudgal PC. A novel selective broad-spectrum anti-DNA virus agent. *Nature.* 1986;323:464–467.

193. DeClerq E. Cidofovir in the treatment of poxvirus infections. *Antiviral Res.* 2002;55:1–13.

194. Neyts J, Snoeck R, Balzarini, DeClercq E. Particular characteristics of the anti-human cytomegalovirus activity of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) in vitro. *Antiviral Res.* 1991;16:41–52.

195. Quenelle DC, Collins DJ, Kern ER. Efficacy of multiple- or single-dose cidofovir against vaccinia and cowpox virus infections in mice. *Antimicrob Agents Chemother.* 2003;47:3275–3280.

196. Bray M, Martinez M, Smee DF, Kefauver D, Thompson E, Huggins JW. Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. *J Infect Dis.* 2000;181:10–19.

197. Smee DE, Bailey KW, Wong MH, Sidwell RW. Effects of cidofovir on the pathogenesis of a lethal vaccinia virus respiratory infection in mice. *Antiviral Res.* 2001;52:55–62.

198. Smee DF, Bailey KW, Sidwell RW. Treatment of lethal vaccinia virus respiratory infections in mice with cidofovir. *Antivir Chem Chemother.* 2001;12:71–76.

199. LeDuc JW, Damon I, Relman DA, Huggins J, Jahrling PB. Smallpox research activities: U.S. interagency collaboration, 2001. *Emerg Infect Dis.* 2002;8:743–745.

200. Lalezari JP, Kuppermann BD. Clinical experience with cidofovir in the treatment of cytomegalovirus retinitis. *J Acquir Immune Defic Syndr Hum Retroviol.* 1997;14(suppl 1):S27–S31.

201. Yang G, Pevear DC, Davies MH, et al. An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge. *J Virol.* 2005;79:13139–13149.

202. Monath TP, Cetron MS. Prevention of yellow fever in persons traveling to the tropics. *Clin Infect Dis.* 2002;34:1369–1378.

203. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA.* 2002;287:2391–2405.

204. Maiztegui JI, McKee KT Jr, Barrera Oro JG, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis.* 1998;177:277–283.

205. Kruger DH, Ulrich R, Lundkvist AA. Hantavirus infections and their prevention. *Microbes Infect.* 2001;3:1129–1144.

206. Sohn YM, Rho HO, Park MS, Kim JS, Summers PL. Primary humoral immune response to formalin inactivated hemorrhagic fever with renal syndrome vaccine (Hantavax): consideration of active immunization in South Korea. *Yonsei Med J.* 2001;42:278–284.

207. Hooper JW, Custer DM, Thompson E, Schmaljohn CS. DNA vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralizing antibody response in rhesus monkeys. *J Virol.* 2001;75:8469–8477.

208. McClain DJ, Summers PL, Harrison SA, Schmaljohn AL, Schmaljohn CS. Clinical evaluation of a vaccinia-vectored Hantaan virus vaccine. *J Med Virol.* 2000;60:77–85.

209. Pittman PR, Liu CT, Cannon TL, et al. Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. *Vaccine.* 1999;18:181–189.

210. Geisbert TW, Pushko P, Anderson K, Smith J, Davis KJ, Jahrling PB. Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg Infect Dis.* 2002;8:503–507.

211. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature.* 2003;424:681–684.

212. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature.* 2000;408:605–609.

213. Jones SM, Feldman H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med.* 2005:11:786–790.

214. Streeter DG, Witkowski JT, Khare GP, et al. Mechanism of action of 1- -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proc Natl Acad Sci U S A.* 1973;70:1174–1178.

215. Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis.* 1991;164:1119–1127.

216. McCormick JB, King IJ, Webb PA, et al. Lassa fever. Effective therapy with ribavirin. *N Engl J Med.* 1986;314:20–26.

217. Yang ZQ, Zhang TM, Zhang MV, et al. Interruption study of viremia of patients with hemorrhagic fever with renal syndrome in the febrile phase. *Chin Med J (Engl).* 1991;104:149–153.

218. Mardani M, Jahromi MK, Naieni KH, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis.* 2003;36:1613–1618.

219. Ergonul O, Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. *Clin Infect Dis.* 2004;39:284–287.

220. Paragas J, Whitehouse CA, Endy TP, Bray M. A simple assay for determining antiviral activity against Crimean-Congo hemorrhagic fever virus. *Antiviral Res.* 2004;62:21–25.

221.	Watts DM, Ussery MA, Nash D, Peters CJ. Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. *Am J Trop Med Hyg*. 1989;41:581–585.

222.	Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis*. 1989;11(suppl 4):S750–S761.

223.	Jahrling PB, Hesse RA, Eddy GA, Johnson KM, Callis RT, Stephen EL. Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *J Infect Dis*. 1980;141:580–589.

224.	Jahrling PB, Peters CJ, Stephen EL. Enhanced treatment of Lassa fever by immune plasma combined with ribavirin in cynomolgus monkeys. *J Infect Dis*. 1984;149:420–427.

225.	Enria DA, Maiztegui JI. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral Res*. 1994;23:23–31.

226.	Enria DA, Briggiler AM, Levis S, Vallejos D, Maiztegui JI, Canonico PG. Tolerance and antiviral effect of ribavirin in patients with Argentine hemorrhagic fever. *Antiviral Res*. 1987;7:353–359.

227.	McKee KT Jr, Huggins JW, Trahan CJ, Mahlandt BG. Ribavirin prophylaxis and therapy for experimental Argentine hemorrhagic fever. *Antimicrob Agents Chemother*. 1988;32:1304–1309.

228.	Kilgore PE, Ksiazek TG, Rollin PE, et al. Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis*. 1997;24:718–722.

229.	Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis*. 2003;188:1618–1629.

230.	Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet*. 2003;362:1953–1958.

231.	Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA*. 2003;290:238–247.

232.	Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145–160.

233.	Charrel RN, de Lamballerie X. Arenaviruses other than Lassa virus. *Antiviral Res*. 2003;57:89–100.

234.	Smee DF, Morris JL, Barnard DL, Van Aerschot A. Selective inhibition of arthropod-borne and arenaviruses in vitro by 3′-fluoro-3′-deoxyadenosine. *Antivir Res*. 1992;18:151–162.

235.	Candurra NA, Maskin L, Damonte EB. Inhibition of arenavirus multiplication in vitro by phenotiazines. *Antivir Res*. 1996;31:149–158.

236.	Cordo SM, Candurra NA, Damonte EB. Myristic acid analogs are inhibitors of Junin virus replication. *Microb Infect*. 1999;1:609–614.

237.	Sidwell RW, Smee DF. Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control. *Antivir Res*. 2003;57:101–111.

238.	Levis SC, Saavedra MC, Ceccoli C, et al. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. *J Interferon Res*. 1985;5:383–389.

239.	Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *J Infect Dis*. 1999;179(suppl 1):S224–S234.

240.	Saavedra MC, Briggiler AM, Enria D, Riera L, Ambrosio AM. Prevalence of hepatitis C antibodies in plasma donors for the treatment of Argentine hemorrhagic fever. *Medicina (B Aires)*. 1997;57:287–293.

241. Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis*. 1999;179(suppl 1):S18–S23.

242. Emond RT, Evans B, Bowen ET, Lloyd G. A case of Ebola virus infection. *Br Med J*. 1977;2:541–544.

243. Vassilenko SM, Vassilev TL, Bozadjiev LG, Bineva IL, Kazarov GZ. Specific intravenous immunoglobulin for Crimean-Congo haemorrhagic fever. *Lancet*. 1990;335:791–792.

244. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol*. 1979;15:307–417.

245. Bray M. Defense against filoviruses used as biological weapons. *Antiviral Res*. 2003;57:53–60.

246. Peters CJ. California encephalitis, hantavirus pulmonary syndrome, and bunyavirid hemorrhagic fevers. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas and Bennett's Principles and Practices of Infectious Diseases*. 5th ed. Philadelphia, Pa: Churchill Livingstone; 2000: 1849–1855.

247. Holzer E. Botulism caused by inhalation. *Med Klin*. 1962;41:1735–1740.

248. US Army, Office of the Surgeon General. *Evaluation of Safety and Immunogenicity of Pentavalent Botulinum Toxoid (A-E) Administered to Healthy Volunteers, Log A-9241*. Washington, DC: OTSG; February 2001.

249. Cardella MA, Wright GG. *Specifications for Manufacture of Botulism Toxoids, Adsorbed, Pentavalent, Types ABCDE*. Fort Detrick, Md: US Army Biological Laboratories, Medical Investigation Division; January 1964. Technical Study 46.

250. Brown JE, Parker GW, Pitt LM, et al. Protective efficacy of monkey pentavalent botulinum toxoid vaccine on an abbreviated immunization schedule [abstract]. ASM International Conference on Molecular Genetics and Pathogenesis of the Clostridia. Tuscon, Arizona, 1994.

251. Rusnak JM, Kortepeter MG, Hawley RJ, Anderson AO, Boudreau E, Eitzen E. Risk of occupationally acquired illnesses from biological threat agents in unvaccinated laboratory workers. *Biosecur Bioterr*. 2004;2:281–293.

252. Rusnak JM, Smith L, Boudreau E, et al. Decreased immunogenicity of botulinum pentavalent toxoid to toxins B and E. In: *Programs and Abstracts of the Sixth Annual Conference on Vaccine Research*. Arlington, Va: Annual Conference on Vaccine Research; 2003: Abstract S10.

253. Smith LA, Rusnak JM. Botulinum neurotoxin vaccines: past, present, and future. *Crit Rev Immun*. In press.

254. Siegel LS. Human immune response to botulinum pentavalent (ABCDE) toxoid determined by a neutralization test and by an enzyme-linked immunosorbent assay. *J Clin Microbiol*. 1988,26:2351–2356.

255. US Department of Defense Chemical and Biological Defense Information Analysis Center. *Evaluation of Safety and Immunogenicity of Pentavalent Botulinum Development of Safe and Effective Products to Exposure to Biological Chemical Warfare Agents*. Gunpowder, Md: CBIAC; 2001.

256. US Department of Defense Chemical and Biological Defense Information Analysis Center. *Evaluation of Safety and Immunogenicity of Pentavalent Botulinum Toxoid (A-E) Administered to Healthy Volunteers–Continuation of Study for Determination of Booster Vaccination Interval*. Gunpowder, Md: CBIAC; 2002.

257. Fiock MA, Cardella MA, Gearinger NF. Studies on immunity to toxins of *Clostridium botulinum*. IX. Immunologic response of man to purified ABCDE botulinum toxiod. *J Immunol*. 1963;90:697–702.

258. Centers for Disease Control and Prevention. *Pentavalent (ABCDE) Botulinum Toxoid*. Informational brochure. Atlanta, Ga: US Department of Health and Human Services; 2000.

259. Edelman R, Wasserman SS, Bodison SA, Perry JG, O'Donnoghue M, DeTolla LJ. Phase II safety and immunogenicity study of type F botulinum toxoid in adult volunteers. *Vaccine*. 2003;21:4335–4347.

260. Torii Y, Tokumaru Y, Kawaguchi S, et al. Production and immunogenic efficacy of botulinum tetravalent (A,B,E,F) toxoid. *Vaccine*. 2002;20:2556–2561.

261. Middlebrook JL. Protection strategies against botulinum toxin. *Adv Exp Med Biol*. 1995;383:93–98.

262. Tong X, Ford P, Johnson V, et al. *Bivalent AB botulinum toxoid (BBT)* [Abstract]. 43rd Interagency Botulism Research Coordinating Committee Meeting. Rockville, Md: Nov 14-17, 2006.

263. Holley JL, Elmore M, Mauchline M, Minton N, Titball RW. Cloning, expression and evaluation of a recombinant subunit vaccine against *Clostridium botulinum* type F toxin. *Vaccine*. 2000;19:288–297.

264. Clayton MA, Clayton JM, Brown DR, Middlebrook JL. Protective vaccination with a recombinant fragment of *Clostridium botulinum* neurotoxin serotype A expressed from a synthetic gene in *Escherichia coli*. *Infect Immun*. 1995;63:2738–2742.

265. Byrne MP, Smith TJ, Montgomery VA, Smith LA. Purification, potency, and efficacy of the botulinum neurotoxin type A binding domain from *Pichia pastoris* as a recombinant vaccine candidate. *Infect Immun*. 1998;66:4817–4822.

266. Clayton J, Middlebrook JL. Vaccination of mice with DNA encoding a large fragment of botulinum neurotoxin serotype A. *Vaccine*. 2000;18:1855–1862.

267. Shyu RH, Shaio MF, Tang SS, et al. DNA vaccination using the fragment C of botulinum neurotoxin type A provided protective immunity in mice. *J Biomed Sci*. 2000;7:51–57.

268. Lee JS, Pushko P, Parker MD, Dertzbaugh MT, Smith LA, Smith JF. Candidate vaccine against botulinum neurotoxin serotype A derived from a Venezuelan equine encephalitis virus vector system. *Infect Immun*. 2001;69:5709–5715.

269. Potter KJ, Bevins MA, Vassilieva EV, et al. Production and purification of the heavy-chain fragment C of botulinum neurotoxin, serotype B, expressed in the methylotrophic yeast *Picia pastoris*. *Protein Expr Purif*. 1998;13:357–365.

270. Kiyatkin N, Maksymowych AB, Simpson LL. Induction of an immune response by oral administration of recombinant botulinum toxin. *Infect Immun*. 1997;65:4586–4591.

271. Foynes S, Holley JL, Garmory HS, Titball RW, Fairweather NF. Vaccination against type F botulinum toxin using attenuated *Salmonella enterica var Typhimurium* strains expressing the BoNT/F H$_c$ fragment. *Vaccine*. 2003;21:1052–1059.

272. Smith LA, Jensen MJ, Montgomery VA, Brown DR, Ahmed SA, Smith TJ. Roads from vaccines to therapies. *Mov Disord*. 2004;19(suppl 8):S48–S52.

273. Park JB, Simpson LL. Progress toward development of an inhalation vaccine against botulinum toxin. *Expert Rev Vac*. 2004;3:477–487.

274. Park JB, Simpson LL. Inhalational poisoning by botulinum toxin and inhalation vaccination with its heavy-chain component. *Infect Immun*. 2003;71:1147–1154.

275. Lee JS, Pushko P, Parker MD, Dertzbaugh MT, Smith LA, Smith JF. Candidate vaccine against botulinum neurotoxin serotype A derived from a Venezuelan equine encephalitis virus vector system. *Infect Immun*. 2001;69:5709–5715.

276. Gelzleichter TR, Myers MA, Menton RG, Niemuth NA, Matthews MC, Langford MJ. Protection against botulinum toxins provided by passive immunization with botulinum human immune globulin: evaluation using an inhalation model. *J Appl Toxicol*. 1999;19(suppl 1):S35–S38.

277. Rusnak JM, Kortepeter M, Ulrich RG, Poli M, Boudreau E. Laboratory exposures to staphylococcal enterotoxin B. *Emerg Infect Dis*. 2004;10:1544–1549.

278. 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;69:2031–2036.

279. Boles JW, Pitt ML, LeClaire RD, 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;108:51–59.

280. Lee JS, Dyas BK, Nystrom SS, Lind CM, Smith JF, Ulrich RG. Immune protection against staphylococcal enterotoxin-induced toxic shock by vaccination with a Venezuelan equine encephalitis virus replicon. *J Infect Dis*. 2002;185:1192–1196.

281. Lowell GH, Colleton C, Frost D, 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;64:4686–4693.

282. Tseng J, Komisar JL, Trout RN, et al. Humoral immunity to aerosolized staphylococcal enterotoxin B (SEB), a superantigen, in monkeys vaccinated with SEB toxoid-containing microspheres. *Infect Immun*. 1995;63:2880–2885.

283. Eldridge JH, Staas JK, Chen D, Marx PA, Tice TR, Gilley RM. New advances in vaccine delivery systems. *Semin Hematol*. 1993;30(4 suppl 4):16–24.

284. Doan LG. Ricin: mechanism of toxicity, clinical manifestations, and vaccine development. A review. *J Toxicol Clin Toxicol*. 2004;42:201–208.

285. Lemley PV, Wright DC. Mice are actively immunized after passive monoclonal antibody prophylaxis and ricin toxin challenge. *Immunology*. 1992;76:511–513.

286. Lemley PV, Amanatides P, Wright DC. Identification and characterization of a monoclonal antibody that neutralizes ricin toxicity in vitro and in vivo. *Hybridoma*. 1994;13:417–421.

287. Eiklid K, Olsnes S, Pihl A. Entry of lethal doses of abrin, ricin and modeccin into the cytosol of HeLa cells. *Exp Cell Res*. 1980;126:321–326.

288. Thorpe PE, Detre SL, Foxwell BM, et al. Modification of the carbohydrate in ricin with metaperiodate-cyanoborohydride mixtures. Effects on toxicity and in vivo distribution. *Eur J Biochem*. 1985;147:197–206.

289. Hewetson JF, Rivera VR, Lemley PV, Pitt ML, Creasia DA, Thompson WL. A formalinized toxoid for protection of mice with inhaled ricin. *Vac Res*. 1995;4:179–185.

290. Vitetta ES, Smallshaw JE, Coleman E, et al. A pilot clinical trial of a recombinant ricin vaccine in normal humans. *PNAS*. 2006;103:2268–2273.

291. Olson MA, Carra JH, Roxas-Duncan V, Wannemacher RW, Smith LA, Millard CB. Finding a new vaccine in the ricin protein fold. *Protein Engineering, Design, and Selection*. 2004;17:391–397.

292. Yan C, Rill WL, Malli R, et al. Intranasal stimulation of long-lasting immunity against aerosol ricin challenge with ricin toxoid vaccine encapsulated in polymeric microspheres. *Vaccine*. 1996;14:1031–1038.

293. Kende M, Yan C, Hewetson J, Frick MA, Rill WL, Tammariello R. Oral immunization of mice with ricin toxoid vaccine encapsulated in polymeric microspheres against aerosol challenge. *Vaccine*. 2002;20:1681–1691.