Chapter 27

MEDICAL COUNTERMEASURES

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INTRODUCTION

Countermeasures against bioterrorism, intended to minimize morbidity and mortality and 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 first three countermeasures are nonmedical and are discussed in other chapters. This chapter covers medical countermeasures, which include interventions such as active immunoprophylaxis (ie, vaccines), passive immunoprophylaxis (immunoglobulins and antitoxins), and chemoprophylaxis (antimicrobial medications). Medical countermeasures may be initiated before an exposure (if individuals can be identified as being at high risk for exposure), after a confirmed exposure event, or after the onset of symptoms in infected individuals.

Because medical countermeasures may be associated with adverse events, the recommendation for their use must be weighed against the risk of exposure and disease. Vaccines, including both investigational vaccines and those approved by the US Food and Drug Administration (FDA), are available for some bioterrorism agents (Table 27-1). In the event of a bioterrorism 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 bioterrorism agent, particularly with vaccinations that require multiple primary (or priming) doses to achieve immunity or repeated booster doses to maintain it. In these cases, chemoprophylaxis—and, in some cases, immunoprophylaxis—after identifying an exposure or infection may be effective in preventing disease or death (Table 27-2). Any effective plan for countering bioterrorism should address the logistics of maintaining adequate supplies of drugs and vaccines as well as personnel coordinating and dispensing needed supplies to the affected site.

FDA-approved vaccines against anthrax and smallpox are available; however, for many potential bioterrorism agents, only investigational vaccines that were developed and manufactured more than 30 years ago are available. 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 they can be obtained under investigational new drug (IND) protocols at limited sites in the United States, these vaccines are in extremely limited supply and some are declining in immunogenicity with age.

Under the FDA Animal Rule instituted in 2002, approval of vaccines, antimicrobials, and other drugs can now be based on demonstration of efficacy in animal models alone if efficacy studies in humans would be unsafe or unethical. This rule provides an opportunity to develop many new and improved vaccines and other medications, with the ultimate goal of FDA licensure. However, drug development generally is a long process. In vaccine development, for example, generally 3 to 5 years is required to identify a potential vaccine candidate and conduct animal studies to test for vaccine immunogenicity and efficacy, followed by 5 years of clinical trials for FDA approval and licensure. FDA vaccine approval then takes from 7 to 10 years, and under the FDA Animal Rule, additional time must be devoted to animal studies to identify correlates of protection. Thus, 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 that can be found in many soil environments worldwide. It occurs in a vegetative state and in a spore state; the spore state, which can remain viable for decades, is the infectious form.[1–3] Ruminants acquire spores by ingesting contaminated soil while grazing. Humans can become infected through skin contact, ingestion, or inhalation of *B anthracis* spores from infected animals or animal products.[3] Anthrax is not transmissible from person to person. Cutaneous anthrax is the most common naturally occurring form of anthrax, and gastrointestinal anthrax is the least common form. Inhalational anthrax, which occurs as a result of exposure to aerosolized spores, is considered the form of disease most likely to result from an act of bioterrorism. Meningitis can occur, as secondary seeding from bacteremia, with any form of anthrax.[4] Because of its virulence, ease of preparation, the potential to aerosolize spores, and the stability and prolonged survival of the spore stage, *B anthracis* is an ideal agent for bioterrorism.[4,5]

TABLE 27-1

VACCINES, VACCINE DOSAGE SCHEDULES, AND CORRELATES OF POSTVACCINATION PROTECTION

Disease	Vaccine (Dose and Route)	Type of Vaccine	Primary Series	Booster Doses	Immunogenicity Criteria
		BACTERIA			
Anthrax	AVA (BioThrax) (0.5 mL IM)	Sterile, acellular filtrate	Months 0, 1, & 6	Months 12 & 18, annually	3 weeks after 3rd dose
Tularemia	NDBR 101[*] (15 punctures, 1 drop [0.06 mL] PC)	Live attenuated	Day 0	None	Take reaction[†] by day 7 after vaccination; day 28 microagglutination titer ≥4-fold rise from prevaccination baseline
Q fever	NDBR 105[*‡§] (0.5 mL SC)	Inactivated	Day 0	None	3–5 weeks after vaccination
		VIRUSES			
VEE	TC-83 NDBR 102[*] (0.5 mL SC)	Live attenuated	Day 0	None; boost with C-84 per titer	$PRNT_{80}$ titer ≥1:20
	C-84 TSI-GSD 205[*] (0.5 mL SC)	Inactivated	None[¥]	Initial responders to TC-83 and past recipients of C-84: single boost of C-84 Initial nonresponders to TC-83: boost with C-84	$PRNT_{80}$ titer ≥1:20
WEE	TSI-GSD 210[*§] (0.5 mL SC)	Inactivated	Days 0, 7, & 28	Mandatory boost: month 6; then as needed per titer	$PRNT_{80}$ titer ≥1:40
EEE	TSI-GSD 104[*] (0.5 mL SC)	Inactivated	Days 0 & 28	Mandatory boost: month 6; before month 6 and after: as needed per titer (0.1 mL ID)	$PRNT_{80}$ titer ≥1:40
Smallpox	ACAM2000 (15 punctures PC)	Cell culture–based live vaccinia virus	Day 0	Every 1, 3, or 10 years[¶]	Take reaction[**] after primary vaccination
Yellow fever	YF-Vax (0.5 mL SC)	Live attenuated	Day 0	Every 10 years	1 month after vaccination
RVF	TSI-GSD 200[*††] (1 mL SC)	Inactivated	Days 0, 7, & 28	Initial responders[‡‡]: mandatory boost at month 6; then as needed per titer Initial nonresponders[‡‡]: boost within 90 days of low titer	$PRNT_{80}$ titer ≥1:40

(**Table 27-1** *continues*)

Table 27-1 continued

Notes: Vaccines listed are those available in the United States and used (under current or recent protocols) in the Special Immunizations Program at the US Army Medical Research Institute of Infectious Diseases. Vaccines are available elsewhere for Argentine hemorrhagic fever (Candid #1, available in Argentina, which may have cross-protection for Bolivian hemorrhagic fever); Omsk hemorrhagic fever (for which cross-protection is provided from the tickborne encephalitis vaccine FSME-IMMUN); Kyasanur Forest disease (for which a vaccine is available in India); Crimean–Congo hemorrhagic fever (for which a vaccine is available in Bulgaria); and hemorrhagic fever with renal syndrome (Hantavax, which is available in South Korea).

*Investigational product.

†The take reaction is an erythematous papule, vesicle, and/or eschar, with or without induration, at the vaccination site. Compared with smallpox vaccination (using ACAM2000), tularemia vaccination (using NDBR 101) results in a smaller take reaction with less induration.

‡Prevaccination skin test is required before administration of NDBR 105.

§Q fever and WEE vaccines are not currently administered in the SIP.

¥Used only as booster (if needed per titer) after vaccination with TC-83.

¶Booster doses of the smallpox vaccine are recommended every 3–10 years, depending on risk; for example, laboratory researchers working with variola virus (only at CDC) may receive yearly boosters.

**The take reaction after smallpox vaccination (using ACAM2000) is a clear vesicle or pustule, approximately 1 cm in diameter.

††A live attenuated RVF MP12 vaccine has recently undergone phase 1 and 2 clinical trials.

‡‡Titer is assessed 28 days after 3rd primary dose of RVF vaccine. Initial responders ($PRNT_{80}$ titer ≥1:40 after 3rd dose) receive a boost at 6 months with titer assessed 28 days later. For responders to 6-month booster, titer is assessed annually with subsequent booster doses as needed. For initial nonresponders ($PRNT_{80}$ titer <1:40), booster is given within 90 days and titer is assessed 28 days later. For a nonresponse, boost/titer may be repeated up to 4 times before declaring individual a nonresponder. (The month 6 booster does not apply to initial nonresponders.)

AVA: anthrax vaccine adsorbed; EEE: eastern equine encephalitis; ID: intradermal; IM: intramuscular; PC: percutaneous (scarification); $PRNT_{80}$: 80% plaque reduction neutralization titer; RVF: Rift Valley fever; SC: subcutaneous; TSI-GSD: The Salk Institute-Government Services Division; VEE: Venezuelan equine encephalitis; WEE: western equine encephalitis; YF-Vax: yellow fever vaccine

Data sources: (1) Dembek Z, ed. *USAMRIID's Medical Management of Biological Casualties Handbook*. 7th ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (2) Emergent BioSolutions. *BioThrax* (Anthrax Vaccine Adsorbed) [package insert]. Lansing, MI: Emergent BioDefense Operations Lansing LLC; 2015. (3) Rusnak J, ed. *Occupational Health Manual for Laboratory Exposures to Select (BSL-3 & BSL-4) and Other Biological Agents*. 3rd ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (4) 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 Occ Env Med*. 2004;46:801–811. (5) Sanofi Pasteur Biologics. *ACAM2000*. Cambridge, MA: Sanofi Pasteur Biologics; 2009. (6) Rotz LD, Dotson DA, Damon IK, Becher JA; Advisory Committee on Immunization Practices. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Recomm Rep*. 2001;50(RR-10):1–25. (7) Monath TP, Cetron MS. Prevention of yellow fever in persons traveling to the tropics. *Clin Infect Dis*. 2002;34:1369–1378. (8) 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.

Vaccination

History of the Anthrax Vaccine. In 1947, a factor isolated from the edema fluid of cutaneous *B anthracis* lesions was found to successfully vaccinate animals.[6] This factor, identified as the protective antigen (PA), was subsequently recovered by incubating *B anthracis* in special culture medium.[7,8] This work 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*.[9]

This early version of the anthrax vaccine was found to protect small laboratory animals and nonhuman primates (NHPs) from inhalational anthrax.[9] The vaccine also demonstrated protection against cutaneous anthrax infections in employees working in textile mills processing raw imported goat hair. In particular, only three cases of cutaneous anthrax occurred in 379 vaccinated employees, whereas 18 cases of cutaneous anthrax and all five cases of inhalational anthrax occurred in the 754 unvaccinated employees. Based on these results, the vaccine efficacy for anthrax was estimated to be 92.5%. The vaccine failures were found in one 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 (and was infected at 13 months), and a third person who was within a week of the fourth (month 6) vaccine dose, when titers are known to be lower.[10] Vaccine breakthroughs were uncommon; the few documented cases of cutaneous anthrax occurred in individuals who had not completed the primary series or who were within days of a scheduled primary or booster dose.[10,11]

Anthrax Vaccine Adsorbed (BioThrax). The current FDA-approved anthrax vaccine adsorbed (AVA; see Table 27-1) was derived through improvements of the early alum-precipitated anthrax vaccine, specifically:

- using a *B anthracis* strain that produced a higher fraction of PA;
- growing the culture under microaerophilic instead of aerobic conditions; and
- substituting an aluminum hydroxide adjuvant in place of the aluminum potassium salt adjuvant.[12,13]

TABLE 27-2

TREATMENT AND POSTEXPOSURE PROPHYLAXIS FOR BACTERIAL DISEASES

Disease	Preferred/Recommended Antimicrobials[*]	Vaccine	Passive Immunotherapy or Antitoxin
Anthrax (inhalational)	***Treatment*** *Meningitis not ruled out*: Initial IV treatment for ≥14 days (or until clinically stable) of ≥3 antibiotics: ciprofloxacin (400 mg IV every 8 h) AND meropenem (2 g IV every 8 h) AND linezolid (600 mg IV every 12 h) Followed by oral treatment as described for PEP, for a total of ≥60 days of antibiotic treatment *Meningitis ruled out*: Initial IV treatment for ≥14 days (or until clinically stable) of ≥2 antibiotics: ciprofloxacin (400 mg IV every 8 h) AND clindamycin (900 mg IV every 8 h) OR linezolid (600 mg IV every 12 h) Followed by oral treatment as described for PEP, for a total of ≥60 days of antibiotic treatment	***Treatment*** Not recommended	***Treatment*** Raxibacumab (40 mg/kg IV) infused over 2 h, 15 min, within 1 h after premedication with diphenhydramine, in combination with antibiotics OR AIGIV (7 vials [420 units] IV), infused at 0.5 mL/min (first 30 min), 1 mL/min incremental infusion rate if tolerated (every 30 min), max infusion rate of 2 mL/min
	PEP[†] 60 days of ciprofloxacin (500 mg PO every 12 h) OR doxycycline (100 mg PO every 12 h)	***PEP*** AVA[‡] (up to 3 doses SC)	***PEP***[§] Raxibacumab (40 mg/kg IV), infused over 2 h, 15 min, within 1 h after premedication with diphenhydramine
Tularemia	***Treatment*** 10 days of streptomycin (1 g IM every 12 h) OR gentamicin (5 mg/kg IM or IV daily)	***Treatment*** Not recommended	No passive immunotherapy available
	PEP[†] 14 days of doxycycline (100 mg PO every 12 h) OR ciprofloxacin (500 mg PO every 12 h)	***PEP*** Not recommended	
Plague	***Treatment*** ≥10 days of streptomycin (1 g IM every 12 h) OR gentamicin (5 mg/kg IM or IV daily)	No vaccine available	No passive immunotherapy available
	PEP[†] 7 days of doxycycline (100 mg PO every 12 h) OR ciprofloxacin (500 mg PO every 12 h)		
Glanders or melioidosis	***Treatment*** *Intensive therapy, no complications*[¥]: 10–14 days of ceftazidime (50 mg/kg [up to 2 g] IV every 8 h) *Intensive therapy, with complications*[¥]: 10–14 days of meropenem (25 mg/kg [up to 1 g] IV every 8 h) *Eradication therapy*[¶]: ≥12 weeks of TMP-SMZ (PO) OR amoxicillin–clavulanic acid (PO)	No vaccine available	No passive immunotherapy available
	PEP[**] 21 days of TMP-SMZ (PO) OR amoxicillin–clavulanic acid (PO)		

(**Table 27-2** *continues*)

Table 27-2 continued

Brucellosis	**Treatment** *Uncomplicated*: 6 weeks of doxycycline (100 mg PO every 12 h) AND 2–3 weeks of streptomycin (15 mg/kg IM daily) *Complicated*: ≥12 weeks of triple-antibiotic therapy (see text and sources cited therein) **PEP**[††] 21 days of doxycycline (100 mg PO every 12 h) AND rifampin (450–600 mg PO daily)	No vaccine available	No passive immunotherapy available
Q Fever	**Treatment** *Acute*: 14 days of doxycycline (100 mg PO every 12 h) *Chronic*: Prolonged treatment with doxycycline (100 mg PO every 12 h) AND hydroxychloroquine (200 mg PO every 8 h) (see text and sources cited therein) **PEP** Not recommended; monitor for ≥21 days (see text and sources cited therein)	**Treatment** Not recommended **PEP** Not recommended	No passive immunotherapy available

[*]Antimicrobials listed are the first-line therapies or those most often recommended. For alternative antimicrobials, see text and sources cited therein. Dosages are for adults (men and nonpregnant women); for children and for pregnant women, see text and references cited therein.

[†]The antibiotic regimen described for PEP may also be used for treatment in a mass casualty situation where parenteral antibiotics are not available. In the case of plague, treatment in a mass casualty situation would be extended to 10 days.

[‡]Individuals who were not vaccinated before exposure should receive three doses of AVA as PEP; those who previously received one or two doses of AVA should receive two doses of AVA as PEP, and those who received three or more doses of AVA preexposure should receive one dose of AVA as PEP.

[§]Raxibacumab may be used for PEP in high-risk spore exposure cases.

[¥]Initial IV antibiotic therapy for glanders or melioidosis should be continued for a minimum of 10 days and until the patient's condition improves; the intensive IV therapy may need to be extended to ≥4 weeks. For severe neurologic, cutaneous, bone, joint, or prostate infections, TMP-SMZ could be added to the regimen, administered as described for PEP, or see Lipsitz et al (see data source reference 9).

[¶]In the eradication phase of therapy, the dosage of TMP-SMZ depends on weight: for adult >60 kg, give 160 mg/800 mg tablets (2 tablets every 12 h); for adult 40–60 kg, give 80 mg/400 mg tablets (3 tablets every 12 hours); for adult <40 kg, give 160 mg/800 mg tablets (1 tablet every 12 hours) or 80 mg/400 mg tablets (2 tablets every 12 hours). The dosage of amoxicillin–clavulanic acid (which can be given to those who are intolerant of TMP-SMZ or those with strains that are resistant to TMP-SMZ) also depends on weight: for adult ≥60 kg, give 500 mg/125 mg tablets (3 tablets every 8 hours); for adult <60 kg, give 500 mg/125 mg tablets (2 tablets every 8 hours).

[**]This recommendation is based on animal studies and PEP used for possibly exposed laboratory workers; efficacy in preventing disease among exposed humans has not been evaluated. The dosage of TMP-SMZ and amoxicillin–clavulanic acid depends on weight, as described in the previous note.

[††]For high-risk exposures.

AIGIV: Anthrax Immune Globulin Intravenous; AVA: anthrax vaccine adsorbed; IM: intramuscular; IV: intravenous; PEP: postexposure prophylaxis; PO: by mouth; SC: subcutaneous; TMP-SMZ: trimethoprim and sulfamethoxazole, or co-trimoxazole

Data sources: (1) Hendricks KA, Wright ME, Shadomy SV, et al. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. *Emerg Infect Dis*. 2014;20(2). (2) Wright JG, Quinn CP, Shadomy S, Messonnier N; Centers for Disease Control and Prevention. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep*. 2010;59(RR-6):1–30. (3) Dembek Z, ed. *USAMRIID's Medical Management of Biological Casualties Handbook*. 7th ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (4) Emergent BioSolutions. BioThrax (Anthrax Vaccine Adsorbed) [package insert]. Lansing, MI: Emergent BioDefense Operations Lansing LLC; 2015. (5) GlaxoSmithKline. *Raxibacumab*. Research Triangle Park, NC: GlaxoSmithKline; 2014. (6) Rusnak J, ed. *Occupational Health Manual for Laboratory Exposures to Select (BSL-3 & BSL-4) and Other Biological Agents*. 3rd ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (7) Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA*. 2001;285:2763–2773. (8) Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. *JAMA*. 2000;283:2281–2290. (9) Lipsitz R, Garges S, Aurigemma R, et al. Workshop on treatment of and postexposure prophylaxis for *Burkholderia pseudomallei* and *B mallei* infection, 2010. *Emerg Infect Dis*. 2012;18:e2. (10) Ariza J, Bosilkovski M, Cascio A, et al. Perspectives for

Table 27-2 continued

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Originally produced by the Michigan Department of Public Health, AVA is manufactured by Emergent BioDefense Operations Lansing LLC (Lansing, MI) and marketed under the name BioThrax. It is licensed for preexposure use (and postexposure use, see below) in adults aged 18 to 65.[14,15]

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 the relative absence of other toxin components, such as lethal factor or edema factor.[12,16] 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.[16] Currently, AVA is given as an intramuscular injection (in the lower two-thirds of the deltoid muscle) of 0.5 mL at months 0, 1, and 6 (the primary series) with boosters at months 12 and 18 followed by yearly boosters as long as the individual remains at risk for anthrax infection.[15]

Both the earlier alum-precipitated vaccine and AVA have demonstrated efficacy against aerosol challenge in animal models.[8,9,13,17–23] In their summary of several NHP studies, Friedlander et al[24] noted that, of 55 monkeys given two doses of AVA, 52 (95%) survived lethal aerosol challenge without antibiotics. More recently, the Centers for Disease Control and Prevention (CDC) showed that three doses of AVA, administered intramuscularly at months 0, 1, and 6, protected rhesus macaques against inhalational anthrax for up to 4 years.[23]

Evidence suggests that both humoral and cellular immune responses against PA are critical to protection against disease after exposure.[12,17,18] 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.[19] In the CDC study, survival of macaques was correlated with cellular and humoral immune responses measured during and after administration of the first three doses of the primary series.[23] After three doses of AVA, 83% to 100% of human vaccinees seroconvert.[25–27] A missed dose of AVA does not necessitate restarting the primary series as recent evidence

has demonstrated that the immune response among vaccinees whose month 6 dose is delayed by as much as 7 years is noninferior to that of individuals receiving this dose on schedule.[28] However, the correlation between protection against anthrax infection and a specific antibody titer in humans is not yet clear.[17]

Vaccine Adverse Events. Adverse reactions to AVA are rarely severe when they occur. Adverse reactions in 6,985 persons who received a total of 16,435 doses of AVA, administered subcutaneously according to the original dosing schedule (at weeks 0, 2, and 4 and months 6, 12, and 18 followed by annual boosters), were primarily local reactions (edema or induration). These reactions were severe (>12 cm) in fewer than 1% of vaccinations, moderate (3–12 cm) in 3% of vaccinations, and mild (<3 cm) in 20% of vaccinations. Systemic reactions were uncommon, occurring in fewer than 0.06% of vaccinees, and included fever, chills, body aches, or nausea.[29] After the distribution of around 2 million doses of vaccine, according to the original dosing schedule and route, to more than 500,000 military personnel, data from the Vaccine Adverse Event Reporting System (VAERS) from 1998 to 2001 showed approximately 1,841 reports describing 3,991 adverse events following AVA vaccination. The most frequently reported events were injection site inflammation (752), "flu-like symptoms" (254), systemic rash (251), malaise/fatigue (236), arthralgia (229), and headache (196). Only 96 events (2%) were serious; of those, only 19 were deemed possibly, probably, or certainly related to the receipt of AVA, including anaphylaxis reported in two cases. Three additional cases of anaphylactic-like reactions were reported, but were not deemed serious.[30]

With intramuscular injections administered according to the current dosing schedule (which extends the interval between the first and second primary doses, such that doses are given at months 0, 1, 6, 12, and 18 followed by annual boosters), the incidence of injection site (but not systemic) reactions has been reduced compared with the original AVA dosing schedule and route, with immune responses that are, by month 7, noninferior to those elicited using the original route and schedule.[27,31,32]

Women are more likely than men to experience adverse reactions, particularly certain local reactions, after AVA administration. 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, female vaccinees were more likely than male vaccinees to have injection site reactions, including edema and lymphadenopathy, after subcutaneous injections of AVA.[33] A recent phase 4 clinical trial comparing the subcutaneous and intramuscular routes of administration found that female vaccinees are more likely than male vaccinees to experience injection site reactions. Although this was true regardless of the route of administration, adverse reactions in vaccinees of both genders were reduced with intramuscular administration. For example, erythema occurred in 34% (intramuscular injection of AVA) vs 76% (subcutaneous injection of AVA) of female vaccinees and in 24% vs 48% of male vaccinees; induration occurred in 14% (intramuscular) vs 43% (subcutaneous) of female vaccinees and 10% vs 24% of male vaccinees; and edema occurred in 19% vs 36% of female vaccinees and in 14% vs 27% of male vaccinees.[27] Other factors also appear to predict reactogenicity; in particular, obese women are more likely than those of normal weight to experience local reactions, at least with subcutaneous administration.[34,35] Race may also be associated with reactogenicity.[35]

A 2002 report by the Institute of Medicine's Committee to Assess the Safety and Efficacy of the Anthrax Vaccine found that immediate, short-term adverse effects occur after AVA administration at rates similar to those associated with other licensed vaccines. Rare but serious problems have been reported, but this is true of other licensed vaccines as well. No evidence suggests that AVA causes long-term health problems; however, as with all vaccines, data regarding potential long-term effects are limited.[35a]

Protocols for managing adverse events associated with AVA administration 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.[36]

In persons who have experienced an anaphylactic reaction to the vaccine or any of the vaccine components, subsequent anthrax vaccine doses are contraindicated. AVA is also contraindicated in persons with a history of anthrax infection because of previous observations of an increase in severe adverse events.[37] The vaccine may be given in pregnancy only if the benefit outweighs the risk.

Other Anthrax Vaccines. Another PA-based anthrax vaccine, anthrax vaccine precipitated (AVP), is made by alum precipitation of a cell-free culture filtrate of a derivative of the attenuated *B anthracis* Sterne strain.

This vaccine, which is currently licensed in the United Kingdom, is administered as a primary series of four vaccinations at weeks 0, 3, 6, and 32 followed by annual boosters.[21,38,39]

A live attenuated anthrax vaccine (LAAV), which is produced in Russia, is licensed for use in humans in Georgia and Azerbaijan; it is unclear whether the vaccine is licensed elsewhere, such as other former Soviet Union republics or China. LAAV 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.[40–42]

Vaccine Research. Although AVA and AVP are safe and effective, the lengthy dosing regimens for these vaccines are onerous, even with the recent dose reduction for AVA,[43] and do not lend themselves to use for rapid prophylactic protection of military personnel deploying to high-risk regions. Ongoing research to improve the current vaccines includes efforts to enhance their efficacy by combining them with alternative adjuvants[44] and by extending the intervals between some doses.[28,39]

The ability to prepare purified components of anthrax toxin by recombinant technology has presented the possibility of new anthrax vaccines. For example, a phase 1 clinical trial has found that an anthrax vaccine using recombinant *Escherichia coli*–derived *B anthracis* PA was safe and well tolerated and elicited a robust humoral and cellular response after two doses.[45] Other new PA-based vaccine candidates combine PA with other components of *B anthracis*, such as formaldehyde-inactivated spores, or use alternative delivery systems, such as intranasal or transdermal routes. DNA vaccines, in which immunogen-encoding genetic material is introduced into a host cell, may provide longer-lasting immunity. Such vaccines have only been explored in animal models.[43,46]

Passive Immunotherapy

The passive administration of polyclonal or monoclonal antibodies directed against PA or other *B anthracis* components is receiving attention as potential postexposure prophylaxis (PEP) or treatment. The recombinant, fully humanized monoclonal antibody raxibacumab and the polyclonal antiserum anthrax immune globulin intravenous (human) (AIGIV; marketed as Anthrasil by Emergent BioSolutions, Lansing, MI), which is derived from the plasma of AVA-vaccinated individuals, both have shown promise in animal studies of efficacy and appear to be safe and well tolerated in humans.[3] This approach to neutralizing anthrax

toxins may be especially effective when used in combination with antibiotic treatment or vaccination.[46–48] Recently, raxibacumab obtained FDA approval for the treatment—combined with antibiotics—of adults and children with inhalational anthrax. Raxibacumab may also be used as PEP for possible aerosol exposure to *B anthracis* when other options are not available or appropriate.[49,50] In March 2015, AIGIV received FDA approval for the treatment of inhalational anthrax, in combination with appropriate antibacterial drugs. MDX-1303 (marketed as Valortim by PharmAthene, Annapolis, MD), is another fully human monoclonal antibody being developed for therapeutic and PEP uses.

Antibiotic Agents

Antibiotics are effective against only the vegetative form of *B anthracis*, not the spore form. In the NHP model of inhalational anthrax, spores have survived in lung tissue for months (with 15%–20% spore survival at 42 days, 2% at 50 days, <1% at 75 days, and trace spores present at 100 days) in a dormant state.[37,51,52] The 1979 outbreak of inhalational anthrax in humans after an accidental release of spores from a Soviet biological weapons production facility (the Sverdlovsk outbreak) further supports the notion that lethal spores can persist in lung tissue after the initial exposure because cases of human anthrax developed as late as 43 days after the release.[53] For this reason, a 60-day course of antibiotics is recommended both for the treatment of inhalational anthrax and as prophylaxis after inhalational exposure (but before symptom onset) in vaccinated and unvaccinated individuals. Prolonged spore survival has not been observed for other routes of exposure.

Ciprofloxacin, doxycycline, levofloxacin, and penicillin G procaine have been FDA approved for treatment of inhalational anthrax and for PEP.[16,37,51,54–56] Ciprofloxacin, doxycycline, and penicillin have reduced the incidence or progression of disease in NHPs after aerosol exposure to *B anthracis*.[37,51,55,57] In macaques exposed to 240,000 to 560,000 anthrax spores (8 median lethal doses), 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. 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 on day 58 postexposure, and one monkey treated with ciprofloxacin died on day 36 postexposure.[51] These deaths were attributed to the germination of spores that had persisted in lung tissue after inhalational exposure.

Among human patients with inhalational anthrax between 1900 and 2005, Holty et al[58] found that mortality was significantly lower for those who received (*a*) multidrug antibiotic regimens, (*b*) treatment (with antibiotics or anthrax antiserum) during the prodromal phase of the illness, or (*c*) pleural fluid drainage. Compared with historical cases, patients who were treated for inhalational anthrax during the fall 2001 bioterrorism incident at the Brentwood Post Office and Senate office building in the United States were more likely to have had therapy initiated during the prodromal phase of the disease, to have received several antibiotics, or to have had pleural fluid drainage. These patients were also less likely to die (45% vs 92%).

Adverse events associated with the prolonged, 60-day, antibiotic prophylaxis regimen have had a significant impact on compliance. Overall compliance was reported to be around 44% among the 10,000 persons at six eastern US sites in the 2001 incident for whom the regimen (using ciprofloxacin, doxycycline, or amoxicillin) was recommended.[59] At least one adverse event was reported by 45% and 77% (at day 10 and day 30, respectively) of the individuals receiving PEP most recently with ciprofloxacin. Among those receiving PEP most recently with doxycycline, 49% (day 10) and 71% (day 30) reported experiencing at least one adverse event. Adverse events at day 30 for ciprofloxacin and doxycycline were primarily gastrointestinal symptoms, including nausea, vomiting, diarrhea, abdominal pain, or heartburn (42% and 49% for ciprofloxacin and doxycycline, respectively); fainting, dizziness, light-headedness, or seizures (23% and 18%); rash, hives, or itchy skin (14% and 14%); and joint problems (25% and 16%). Among the 2,631 individuals who took at least one dose of an antibiotic as PEP but stopped taking the drug before completing the full 60-day course, reasons cited for early discontinuation included adverse events (43%), fear of long-term side effects from PEP (7%), and a perception of having a low risk for anthrax (25%).[59] Other adverse events that can occur with quinolones but were not reported in this survey include headache, tremors, restlessness, confusion, and Achilles tendon rupture.

Because of the long-term persistence of spore forms of *B anthracis* in lung tissue after an inhalational exposure, antibiotic prophylaxis combined with vaccination would provide more prolonged protection than postexposure antibiotic prophylaxis alone.[3,37,52] Several studies in rabbits and NHPs have demonstrated that PEP that combines antimicrobial treatment with two or three doses of AVA is protective.[51,60] However, postexposure vaccination without concomitant antimicrobial treatment will not prevent disease from inhalational anthrax.

Some strains of *B anthracis* have shown resistance to certain broad-spectrum antibiotics, such as penicillin, trimethoprim combined with sulfamethoxazole (TMP-SMZ, also called co-trimoxazole), and cefuroxime. Because *B anthracis* strains could be engineered to be resistant to multiple antibiotics, including the current first-line treatments, more selective antibiotic drugs (eg, triclosan derivatives and oligochlorophens) and drug targets (eg, the bacterial cell division protein FtsZ) are being studied.[61–63]

Postevent Countermeasures: Current Options

Treatment. The recommended treatment for inhalational anthrax—and for other forms of anthrax with systemic involvement—varies somewhat depending on whether meningitis has been ruled out. According to the CDC's guidelines,[3] if meningitis has not been ruled out, patients with inhalational anthrax (adults including pregnant women and children)—whether vaccinated or not—should be treated initially with a combination of at least three antimicrobial drugs—all with good central nervous system (CNS) penetration—administered intravenously. This treatment should be continued for at least 2 to 3 weeks or until the patient is clinically stable, whichever is longer.[3,64,65] The drug combination should include at least one bactericidal agent—although Bradley et al[64] recommend two bactericidal agents for children—and at least one protein synthesis inhibitor (see Table 27-2). The preferred bactericidal agents for adults and children are ciprofloxacin (with levofloxacin or moxifloxacin as alternatives) and meropenem (with imipenem and doripenem as alternatives); for penicillin-susceptible strains, penicillin G or ampicillin can serve as the second bactericidal agent.[3,64,65] For pregnant women, ciprofloxacin is the preferred bactericidal agent.[65] The preferred protein synthesis inhibitor for adults (including pregnant women) and children is linezolid (with clindamycin, rifampin, or chloramphenicol as alternatives).[3,64,65] For pregnant women, at least one antibiotic in the combination should be able to cross the placenta (eg, ciprofloxacin, levofloxacin, amoxicillin, or penicillin).[65]

If meningitis has been ruled out, the CDC's guidelines indicate that the initial intravenous treatment for patients of all age groups (including pregnant women) should consist of a combination of at least two antimicrobial drugs, administered intravenously, for at least 2 weeks or until the patient is stable. In this case, CNS penetration is not crucial, but again, at least one agent should be bactericidal and at least one should be a protein synthesis inhibitor. Ciprofloxacin remains the first-choice bactericidal agent for adults (including

pregnant women) and children, though penicillin G or ampicillin could be used if the strain is susceptible. Alternative bactericidal agents include meropenem, levofloxacin, imipenem, and vancomycin[3,64] for adults. Hendricks et al[3] and Meaney-Delman et al[65] additionally include moxifloxacin and doripenem as alternative bactericidal agents. Clindamycin or linezolid are the first-choice protein synthesis inhibitors for patients of all ages in whom meningitis has been ruled out,[3,64,65] though Bradley et al[64] indicate that clindamycin is preferred over linezolid for children. Alternative protein synthesis inhibitors include doxycycline and rifampin.[3,64,65] For pregnant women, at least one antibiotic in the combination should be able to cross the placenta.[65]

The CDC's guidelines recommend adding AIGIV or raxibacumab, when available, to combination antibiotic therapy for adults (including pregnant women) and children with inhalational anthrax or other forms of anthrax with systemic involvement.[3,64,65]

For adults, whether or not meningitis has been ruled out, intravenous combination therapy should be followed by oral administration of a single antibiotic, as described below for PEP, such that antibiotic treatment continues for a total of at least 60 days.[3,64,65] For children, Bradley et al[64] recommend follow-up therapy that is essentially the same as that for adults, except that a combination of two antibiotics—one bactericidal agent and one protein synthesis inhibitor—should be used for children who are slower to recover or who, at the end of the initial intravenous treatment, continue to show signs of infection. Ciprofloxacin (with levofloxacin as an alternative) is the preferred bactericidal agent unless the strain is susceptible to penicillins, in which case, amoxicillin (or penicillin VK) would be preferred. Clindamycin is the preferred protein synthesis inhibitor, with doxycycline or linezolid as alternatives.[64]

Postexposure Prophylaxis. Any individual with known or suspected exposure (of greater than negligible risk) to aerosolized *B anthracis*, whether vaccinated or not, should receive antibiotic prophylaxis starting as soon as possible and continuing until *B anthracis* exposure has been excluded (see Table 27-2). If exposure is confirmed or cannot be excluded, PEP should continue for at least 60 days (to clear germinating spores). Prophylaxis should be initiated without delay for the greatest chance of success, but the specific drugs chosen should be subsequently modified if necessary based on the results of strain sensitivity testing.[3,4,14,64–66]

First-line drugs for PEP (prior to symptom onset) for adults (including pregnant women) and children are ciprofloxacin or doxycycline, administered

orally[3,64,65]; ciprofloxacin is preferred over doxycycline for pregnant women.[65] Alternatives, if first-line drugs are contraindicated, not tolerated, or unavailable, include levofloxacin, moxifloxacin, and clindamycin. Amoxicillin and penicillin VK are also acceptable alternatives if the *B anthracis* strain is susceptible to penicillins.[3,4,14,66] Although permanent dental staining has been associated with use of tetracyclines in young children, Bradley et al[64] suggest that doxycycline may be less likely than older tetracyclines to have this effect and argue that such risks are outweighed by the benefits of its use in the event of possible exposure to anthrax. Similarly, Bradley et al[64] suggest that the potential risk of cartilage toxicity from ciprofloxacin is outweighed by the benefits of its use as PEP in this context. If the strain of *B anthracis* involved is found to be susceptible to penicillins, amoxicillin would be the first choice for children.[64]

As of November 2015, AVA is licensed by FDA for PEP—when used in conjunction with recommended antibiotics—in adults aged 18 to 65 years who have been exposed to aerosolized spores.[15] The recommended BioThrax PEP vaccination schedule for those not previously vaccinated is 0.5 mL subcutaneously at weeks 0 (diagnosis), 2, and 4. Individuals who received one or two doses of AVA before exposure should receive two doses of AVA (at weeks 0 and 2). Those who received three or more doses of AVA before exposure should receive a single booster as soon as possible after exposure.[3,15] The CDC additionally recommends the use of AVA and antimicrobial therapy in individuals of all ages, including children and pregnant women, after an aerosol exposure,[3,4,64,65] although Bradley et al[64] recommend delaying administration of AVA for newborns until 6 weeks of age.

Tularemia

Francisella tularensis is a highly infectious, aerobic, non-spore-forming, gram-negative coccobacillus responsible for serious illness and occasionally death.[67,68] 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 arthropods, (*c*) ingestion of contaminated food or water, or (*d*) inhalation of aerosolized agent from infected animal secretions.[67] Person-to-person transmission of tularemia—although theoretically possible and reported at least once[69]—is considered rare and unlikely.[67]

Most patients with naturally occurring tularemia present with the ulceroglandular form of the disease (generally from intradermal exposure), and up to about one-quarter of patients have typhoidal tularemia (usually resulting from inhalation of infectious aerosols but occasionally from other exposure routes).[14,66,70] Other presentations of tularemia include glandular, oculoglandular, oropharyngeal, and pneumonic (from inhalation or from hematogenous spread from other sites).[66] Pneumonic tularemia and typhoidal tularemia with pulmonary symptoms are the most lethal forms of the disease, yet antibiotics have greatly reduced mortality from all forms of tularemia.[66,70] Disease severity varies by subspecies (or biovar); in particular, two subspecies—*F tularensis* subspecies *tularensis* (type A) and *F tularensis* subspecies *holarctica* (type B)—cause the majority of human disease.[71] Outbreaks of tularemia—particularly inhalational tularemia—in nonendemic areas should alert officials to the possibility of a bioterrorism event.

Vaccination

Investigational Live Tularemia Vaccine. No licensed vaccine protecting against tularemia is available. 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.[72–74] A sample of the Soviet live *F tularensis* subspecies *holarctica* vaccine (known as strain 15), which the Soviet Union used to vaccinate millions of persons during epidemics of type B tularemia beginning in the 1930s, was made available to Fort Detrick in 1956.[71,73] Both a gray-variant and a blue-variant colony were cultivated from this vaccine (colonies appeared blue when illuminated with oblique light under a dissecting microscope). The blue-variant colony proved to be both more virulent and more immunogenic than the gray-variant colony. To improve protection against the virulent *F tularensis* subspecies *tularensis* SCHU S4 strain, the blue-variant colony was passaged through white mice. These passages 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.

During the 27 years of the US offensive biological warfare program at Fort Detrick, tularemia was the most common laboratory-acquired infection. Most of the 161 cases were acquired from aerosol exposures.[11] Beginning in 1959, the live attenuated tularemia vaccine—prepared from, and known as, LVS—was administered to the program's at-risk laboratory personnel until the program closed in 1969 (Figure 27-1).[72] After vaccination using LVS was instituted, the incidence of typhoidal/pneumonic tularemia decreased from 5.7 to 0.27 cases per 1,000 at-risk employee-years. Although
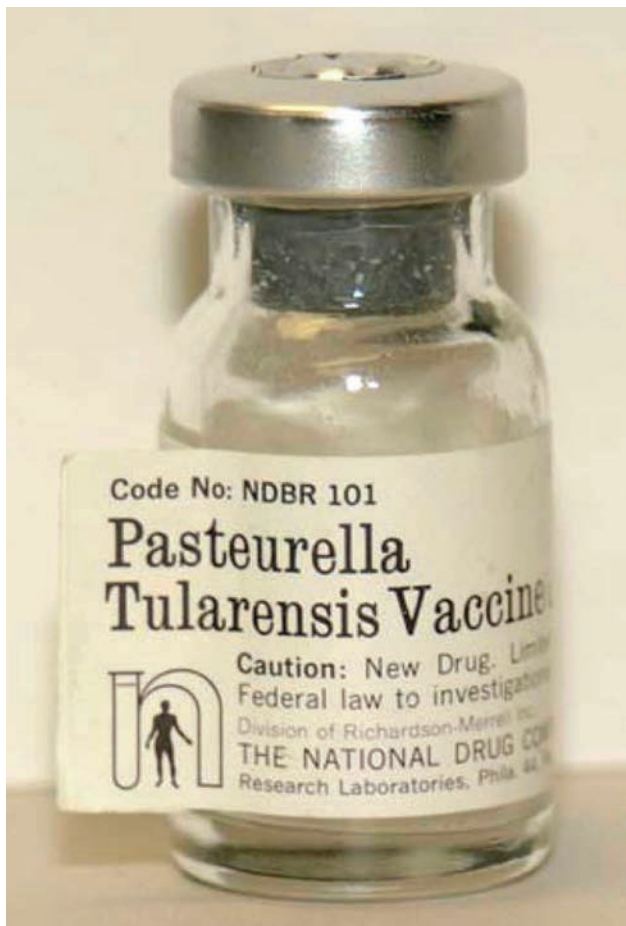
Figure 27-1. Live attenuated NDBR 101 tularemia vaccine. Vaccination of at-risk laboratory workers, beginning in 1959, resulted in a decreased incidence of typhoidal tularemia from 5.7 to 0.27 cases per 1,000 at-risk employee-years, and ameliorated symptoms from ulceroglandular tularemia. The vaccine is administered by scarification with 15 to 30 pricks on the forearm, using a bifurcated needle.

no decrease in ulceroglandular tularemia was noted, the vaccine did ameliorate symptoms from ulceroglandular tularemia, and, unlike those who were unvaccinated before the start of the vaccination program, vaccinated persons did not require hospitalization.[11] The occurrence of ulceroglandular tularemia in vaccinated persons was consistent with the observation that, although natural disease confers immunity to subsequent infections of typhoidal/pneumonic tularemia, it fails to protect against ulceroglandular tularemia. In 1961, commercial production of LVS was initiated by the National Drug Company (Swiftwater, PA), under contract to the US Army Medical Research and Materiel Command. This vaccine, designated NDBR 101, continues to be given to at-risk laboratory workers at USAMRIID under an IND protocol (see Table 27-1).

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 reaction, 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 is generally seen in vaccinia vaccinations. Although a take is related to immunity, its exact correlation has not yet been determined (Figure 27-2).

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.[75,76] Cell-mediated immune responses occur within 1 to 4 weeks after naturally occurring infection or after LVS vaccination and reportedly last 1 to 3 decades.[75,77–85] 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 those in the unvaccinated control group.[86] A similar experience was observed in studies of the inactivated Foshay tularemia vaccine,

Figure 27-2. "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.

in which antibodies were induced by the vaccine but were not protective against tularemia.[72,74] Although nearly all vaccinees develop a humoral response, with microagglutination titers appearing between 2 and 4 weeks postvaccination,[75,82,87] a correlation could not be demonstrated between antibody titers and the magnitude of lymphocyte proliferative responses.[76,84,88,89] 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.[88]

NDBR 101 has not been licensed in the United States because of drawbacks, including the following:

- the vaccine's uncertain history;
- its unclear mechanism of attenuation (and therefore risk of reversion to virulence); and
- the inconsistency across lots in the proportion of blue and gray colonies present.[71,90,91]

In experimental aerosol exposures of human volunteers, this vaccine protected only 71% to 83% of individuals from inhalational tularemia.[92,93] Because of the short incubation period of tularemia, postexposure use of this vaccine is not recommended. NDBR 101 is recommended for laboratory personnel working with *F tularensis*.[67]

The local skin lesion after vaccination (the 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 forms a scab lasting approximately 2 to 3 weeks. Local axillary lymphadenopathy is reported in 20% to 36% of vaccinees. 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 was not determined to be vaccine related. The main contraindications of the vaccine are prior tularemia infection, immunodeficiency, liver disease, and pregnancy.

Vaccine Research

Research is ongoing to develop a new LVS tularemia vaccine (using NDBR 101 as starting material) as well as subunit vaccines against tularemia.[94] Improvements to the LVS vaccine have included efforts to produce LVS under current good manufacturing practice conditions. Subunit vaccines have shown some promise with newly developed adjuvants, such as immune stimulating complexes and CpGs.[91] Live attenuated mutant strains of *F tularensis* derived from LVS and SCHU S4 are also being studied for their attenuation and protection against lethal *F tularensis* challenge.[71]

Antibiotic Agents

The early initiation and adequate duration of therapy are key to the successful treatment of patients with tularemia.[95] Streptomycin, the traditional treatment choice, has proven to be highly efficacious with a low risk of relapse, based on documented cases.[96] However, this aminoglycoside carries the risk of side effects, such as vestibular toxicity and nephrotoxicity, and is often unavailable. Gentamicin, which has also proven efficacious in documented cases, appears to be an acceptable substitute. Fluoroquinolones, such as ciprofloxacin, have shown promise as therapy based on evidence in mice and from use in a human outbreak.[70,95] Fluoroquinolones are highly active against *F tularensis* types A and B in vitro, and evidence from animal studies as well as human cases suggest that ciprofloxacin, levofloxacin, and moxifloxacin are likely to be effective.[95,97] Ciprofloxacin, in particular, has generally had high efficacy with few side effects in adults and children.[67,96] Treatment with 15 days of tetracycline (2 g daily, beginning within 48 hours of symptom onset) was effective in human volunteers exposed to an aerosol challenge of *F tularensis*. Reducing the treatment duration to 10 days or reducing the dose to 1 g daily increased the incidence of relapse.[98] However, tetracyclines may be associated with a greater risk of treatment failure or relapse.[67]

In humans challenged intradermally with an inoculation of *F tularensis*, 5 days of streptomycin, which is bactericidal at concentrations achieved in humans,[95] successfully prevented tularemia.[99] However, neither chloramphenicol nor tetracycline given in a 5-day course was effective as PEP.[99] Tetracycline—given as a 1-g dose twice daily for 14 days, starting within 24 hours of exposure—prevented the development of tularemia in eight humans exposed to aerosols of 25,000 *F tularensis* SCHU S4 spores. 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 caused by considerable fluctuations in tissue levels of the antibiotic, as demonstrated in monkeys given once daily tetracycline, in which the antibiotic ameliorated symptoms but did not prevent tularemia.[98] *F tularensis* is an intracellular pathogen that is cleared slowly from host cells, even in the presence of

bacteriostatic antibiotics. Even in high concentrations, tetracyclines and chloramphenicol merely suppress multiplication of the organisms,[94] which may explain the need for a somewhat prolonged 14-day course of these bacteriostatic antibiotics.

Ongoing research seeks to find:

- treatments that are safer, especially for children and pregnant women;
- therapeutic agents more effective in preventing relapse; and
- alternative treatments for antibiotic-resistant strains.[97]

Postevent Countermeasures: Current Options

Treatment. According to the consensus-based recommendations developed by the Working Group on Civilian Biodefense, the first-line therapy in a contained casualty situation (in which a modest number of individuals require treatment) is 10 days of parenteral streptomycin, with gentamicin as an acceptable substitute, for nonpregnant adults and children. Alternatives include 15 to 21 days of doxycycline or chloramphenicol or 10 to 14 days of ciprofloxacin, although treatment with tetracyclines or chloramphenicol may be more likely to result in treatment failure or relapse. For pregnant women, gentamicin (for 10 days) is preferred over streptomycin; if aminoglycosides cannot be used, alternatives include doxycycline (14–21 days) or ciprofloxacin (10 days) if the risks of their use during pregnancy are outweighed by the benefits of treating tularemia.[67]

In a mass casualty setting, in which logistics and supply limitations may preclude the use of parenteral antibiotics, treatment recommendations are identical to those described below for PEP. Treatment should begin immediately after symptom onset and continue for at least 14 days. The choice of antimicrobial should be modified based on the results of strain susceptibility testing and clinical response. Antibiotics initially administered by the intravenous route may be administered orally once the patient's condition improves.[14,66,67,70,95]

Postexposure Prophylaxis. PEP for asymptomatic individuals who have been exposed to *F tularensis* is most effective when initiated within 24 hours of exposure and continued for at least 14 days. First-line antibiotics for PEP, for adults (including pregnant women) and children, are oral doxycycline or ciprofloxacin (see Table 27-2).[67,70,95] Doxycycline and ciprofloxacin both have the potential to cause adverse effects in the fetus and young child. For an asymptomatic potentially exposed pregnant woman

or child, the risk of disease must be weighed against the potential toxicity of the antibiotics. A patient at low risk of exposure could be instructed to closely monitor body temperature for 14 days, with treatment initiated if symptoms appear.[66,67,96]

Plague

Plague is an acute bacterial disease caused by *Yersinia pestis*, a nonmotile, gram-negative bacillus. Naturally occurring disease in humans is generally acquired when the bites of infected fleas result in lymphatic and blood infections (bubonic and septicemic plague, respectively). Pneumonic plague, the most deadly form of the disease, may be acquired by inhaling droplets emitted from an infected person, inhaling aerosols from infected animal tissues, inhaling *Y pestis* as an aerosolized weapon, or as a result of secondary hematogenous seeding from bubonic or septicemic plague.[100,101] Given the high mortality and person-to-person transmissibility associated with pneumonic plague, *Y pestis* is a candidate for use as a biological warfare or terrorism agent to cause pneumonic plague.

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 and other formalin-killed plague vaccines demonstrated efficacy in the prevention or amelioration of bubonic plague based on retrospective, indirect evidence in vaccinated military troops, evidence did not support its efficacy in preventing pneumonic plague.[101–109] The vaccine's efficacy against aerosolized plague was demonstrated to be poor in animal models, and several individuals developed pneumonic plague despite vaccination.[103–109] Furthermore, these vaccines caused significant adverse reactions and required frequent boosting.[101]

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.[104] EV76 has been licensed for human use in the former Soviet Union and China for decades and has apparently caused no vaccine-related deaths, though adverse reactions are significant.[110] For safety reasons, EV76 is not used for humans in most countries.

Vaccine Research. Because of safety issues with live vaccines, recent efforts have focused on the development of a subunit vaccine using virulence factors

from the surface of the plague bacterium to induce immunity.[103,111] Two virulence factors—identified as the fraction 1 (F1) capsular antigen and the virulence (V) antigen—induced immunity and provided protection against plague in animal models. At USAMRIID, a new plague vaccine was developed by fusing the F1 capsular antigen with the V antigen to produce the recombinant F1-V vaccine. (A similar recombinant subunit vaccine formulation mixes the two antigens [F1+V].) In mice and rabbits, evidence indicates that the F1-V vaccine candidate is protective against both pneumonic and bubonic plague. In NHPs, it provided better protection than either the F1 antigen or the V antigen alone during aerosol challenge experiments.[111–113] Ongoing approaches for improving F1-V–type vaccine candidates include genetic modification of antigens and the use of different adjuvants. Phase 1 and 2 clinical trials exploring subunit plague vaccines have been recently completed. Other researchers are exploring the use of bacterial, viral, and plant live carrier platforms.[111]

Antibiotic Agents. In general, studies are lacking on the relative effectiveness of various antibiotics in the treatment or PEP of pneumonic plague in humans. Streptomycin has traditionally been the preferred treatment for plague and has been effective when initiated promptly. Gentamicin, which is more widely available than streptomycin, has also been used successfully but is not currently FDA approved to treat plague.[100] In particular, a randomized clinical trial conducted in Tanzania found that both gentamicin and doxycycline were highly effective in the treatment of all forms of plague.[114] In murine models of pneumonic plague, doxycycline and tetracycline have not consistently performed as well as other antibiotics.[100,115,116] However, the weight of experimental and anecdotal evidence for the effectiveness of doxycycline led the Working Group on Civilian Biodefense to recommend the use of the tetracycline class of antibiotics to treat plague when aminoglycosides cannot be used.[100] Fluoroquinolones also have been used successfully to treat severe cases of plague.[100] Recently, the FDA approved the fluoroquinolones levofloxacin and moxifloxacin for the treatment (and PEP) of plague based on studies demonstrating the efficacy of these antibiotics in NHPs.[117,118]

PEP with ciprofloxacin for 5 days was highly effective in mice when initiated within 24 hours after aerosol exposure to *Y pestis*, but not when initiated 48 hours after exposure.[115] Doxycycline was relatively ineffective as prophylaxis in a mouse model, even if initiated within 24 hours after aerosol exposure with mean inhibitory concentrations (MICs) ranging from 1 to 4 mg/L.[115,116] The effectiveness of doxycycline, a bacteriostatic drug, generally requires antibiotic levels to be four times the MIC. Two lines of evidence suggest that the treatment failure may be related, in part, to increased metabolism of doxycycline in mice. First, tetracycline has been used successfully in humans to treat or prevent pneumonic plague.[100,119,120] Second, doxycycline stabilized the bacterial loads in the spleens of mice infected with *Y pestis* strains with lower MICs (≤1 mg/L).[121]

Postevent Countermeasures: Current Options

Treatment. The prompt initiation of treatment (within 18–24 hours of symptom onset) is crucial, especially for primary pneumonic plague. According to consensus-based recommendations developed by the Working Group on Civilian Biodefense,[100] the first-line antibiotic for treatment of plague in adult men, nonpregnant women, and children in a contained casualty situation is parenteral streptomycin or gentamicin (although gentamicin is not FDA approved for this use); alternatives include doxycycline, ciprofloxacin, levofloxacin, moxifloxacin (in adults), or chloramphenicol. For pregnant women, gentamicin is the preferred choice, with doxycycline and ciprofloxacin as alternatives. Treatment should be continued for at least 10 days. Antibiotics initially administered intravenously can be administered orally pending improvement in the patient's condition.[14,66,100]

In a mass casualty setting, in which parenteral administration of antibiotics may not be feasible, oral doxycycline or ciprofloxacin are the preferred choices, as described below for PEP, except that treatment duration should be 10 days.[100]

Postexposure Prophylaxis. Asymptomatic individuals exposed to aerosolized *Y pestis*—as well as persons who have had unprotected face-to-face contact (within 2 meters) with patients with pneumonic plague or those potentially exposed to aerosolized *Y pestis*—should receive PEP, beginning as soon as possible and continuing for 7 days after the last known or suspected *Y pestis* exposure or until exposure has been excluded. Individuals with cough or fever within an area in which cases of pneumonic plague are known or suspected to be occurring should also be given PEP. The first-line antibiotics for PEP in adults (including pregnant women) and children are doxycycline or ciprofloxacin; chloramphenicol is an alternative, but this drug carries the risk of causing aplastic anemia. Moxifloxacin (in adults) or levofloxacin may also be appropriate (see Table 27-2).[14,66,100,105,115,116,122,123] Antibiotic sensitivity testing should be performed to assess for resistant strains. For an asymptomatic potentially exposed pregnant woman or child, the risk of disease must be weighed against the potential toxicity of the antibiotics.[66,100]

Glanders and Melioidosis

Glanders and melioidosis are zoonotic diseases caused by the gram-negative bacteria, *Burkholderia mallei* and *B pseudomallei*, respectively.[124–126] Equids serve as the natural reservoir for *B mallei*, which is generally restricted to parts of the Middle East, Asia, and South America.[127] Glanders in humans is not common and has typically been associated with contact with equids or laboratory exposure. 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.[11,14,66,125,128]

B pseudomallei is a natural saprophyte that can be isolated from soil, stagnant waters, rice paddies, and market produce primarily in endemic areas, such as Southeast Asia and northern Australia. However, it has been found in many tropical and subtropical regions.[124,129,130] Infection in humans is generally acquired through soil contamination of skin abrasions, but it may also be acquired by ingesting or inhaling the organism. Although symptoms of *B pseudomallei* infection are variable, the pulmonary form of melioidosis is the most common and may occur as a primary pneumonia or from secondary hematogenous seeding.[14,66,124,129]

Both *B mallei* and *B pseudomallei* have been studied in the past as potential biowarfare agents. The recent increase in biodefense concerns has renewed research interest in these organisms because of their potentially high mortality, availability, resistance to many antibiotics, and inhalational infectivity.[130–132]

Vaccination

No vaccines are available for preventing glanders or melioidosis in humans or animals. Efforts to develop vaccines are made more challenging by the propensity for both of these pathogens to develop into chronic or recurring disease.[130,131] Among the more promising lines of research are vaccines using live attenuated bacteria (which are more immunogenic in animal models) and recombinant subunit vaccines (which are less immunogenic but appear to be safer).[130,131]

Antibiotic Agents

No FDA-approved therapy or PEP exists for glanders or melioidosis. Treatment and PEP are complicated by the tendency for many strains of both *B mallei* and *B pseudomallei* to be resistant to a variety of antibiotics.[133–135] For glanders, effective treatment and PEP strategies are especially uncertain because of the rarity of the disease in humans.

Most strains of both *B mallei* and *B pseudomallei* have generally been susceptible to ceftazidime, meropenem, imipenem, ciprofloxacin, and tetracyclines. *B mallei* is also generally sensitive to rifampin and aminoglycosides, to which most isolates of *B pseudomallei* are resistant.[133–135] Resistance of *B pseudomallei* to TMP-SMZ is rare in Australia[135]; in Thailand, however, the percentage of *B pseudomallei* isolates that are resistant to TMP-SMZ may be increasing.[136]

Because of the potential for latent or recurrent *B pseudomallei* infection, which can occur several decades after exposure, treatment of melioidosis is biphasic. The first phase consists of short-term, intensive, parenterally administered antibiotics; in the second phase, antibiotics are administered orally as long-term eradication therapy.[134,137] In human cases of melioidosis, intravenous ceftazidime—with or without TMP-SMZ—has been effective during the initial intensive phase of treatment. For example, a randomized trial found a significant reduction in mortality among patients with severe melioidosis who were treated during the intensive phase with intravenous ceftazidime alone compared with those who received the conventional treatment of the time—a combination of chloramphenicol, doxycycline, and TMP-SMZ.[138] Amoxicillin/clavulanic acid (or co-amoxiclav), imipenem, meropenem, and cefoperazone–sulbactam have also been effective.[134] In particular, Cheng et al[139] found that patients with severe melioidosis who were treated with meropenem during the intensive phase had outcomes similar to those treated with ceftazidime. However, imipenem has been associated with a higher frequency of CNS adverse effects and problems for patients with impaired renal function, and co-amoxiclav may be more likely than ceftazidime to result in treatment failure.[140]

In the eradication phase of melioidosis treatment, a combination of TMP-SMZ plus doxycycline has been used successfully, as has co-amoxiclav alone.[134,141] However, a recent trial comparing combination antibiotic regimens during the eradication phase found that TMP-SMZ alone was noninferior to TMP-SMZ plus doxycycline, a combination that has been commonly recommended in Thailand. Excluding doxycycline may reduce adverse reactions and improve adherence by patients.[141] An adequate duration of the eradication phase of treatment is crucial for preventing relapse. Limmathurotsakul et al[142] have found that the most significant risk factors for relapse are choice and duration of oral antimicrobial therapy. Among patients treated during the eradication phase with an appropriate oral antibiotic regimen, such as TMP-SMZ plus doxycycline, a 12- to 16-week treatment duration reduced the risk of relapse by 90% compared with a treatment lasting no longer than 8 weeks.

Treatment of patients with glanders is based largely on experience with treating melioidosis as well as the results of animal studies. In addition to antibiotic treatment, surgical drainage of abscesses may be required for some patients.[128,134]

Most evidence on the efficacy of antibiotics as PEP for melioidosis comes from laboratory exposures. For example, among 17 laboratory workers who manipulated cultures of *B pseudomallei*, 13 individuals experienced high-risk exposure to *B pseudomallei* from sniffing 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). Beginning 0 to 4 days after the exposure, 16 of the exposed workers were treated with a 3-week course of TMP-SMZ, and 1 was treated with a 3-week course of doxycycline. None of the 17 individuals developed symptoms consistent with melioidosis for 5 months after exposure.[133] However, this response may reflect the low risk of laboratory-acquired illness from the organism as opposed to the effectiveness of antibiotic prophylaxis.[143]

Animal studies are also informative for the use of antibiotics as PEP for both *B pseudomallei* and *B mallei*. In mice, TMP-SMZ initiated 6 hours after exposure to aerosolized *B pseudomallei* or *B mallei* effectively prevented acute melioidosis and acute glanders. However, these mice nevertheless succumbed to melioidosis or glanders after relapse or immunosuppression, indicating that chronic infections had been established. In rats, PEP with 10 days of quinolones or TMP-SMZ, initiated within 3 hours of subcutaneous exposure to 105 organisms of *B pseudomallei*, was completely effective in preventing disease (verified by necropsy after animals were sacrificed at 2 months postexposure).[144]

Administration of either doxycycline or ciprofloxacin (twice daily for 5 or 10 days) protected mice from disease if started 48 hours before or immediately after intraperitoneal challenge with *B pseudomallei*, though relapses occurred in a few animals within 5 weeks of discontinuation of the antibiotics. However, when the initiation of antibiotic prophylaxis was delayed to 24 hours after exposure, the treatment provided minimal protection, resulting in only a delay of infection, which occurred at least 5 weeks after discontinuation of the antibiotic.[127]

Doxycycline or ciprofloxacin (twice daily for 5 days), initiated 48 hours before or immediately after intraperitoneal challenge with *B mallei*, had a protective effect in hamsters. But the effect was temporary in some animals, with disease occurring after discontinuation of the antibiotic. Relapses were associated with both ciprofloxacin and doxycycline beginning at day 18

and day 28, respectively, after challenge. Necropsies of fatalities revealed splenomegaly with splenic abscesses from which *B mallei* could be isolated; necropsies of surviving animals revealed splenomegaly with an occasional abscess. Delay of ciprofloxacin or doxycycline prophylaxis initiation to 24 hours after exposure merely delayed disease, with relapses occurring in hamsters within 4 weeks of the challenge.[127]

The differences in results among animal models may be related—in part—to differential susceptibility among species to melioidosis and glanders. In particular, hamsters are highly susceptible to infection from *B mallei*; the protective effect of chemoprophylaxis in humans may be greater.

Postevent Countermeasures: Current Options

Treatment. According to consensus recommendations developed at the 2010 US Department of Health and Human Services Burkholderia Workshop,[140] patients with suspected or confirmed glanders or melioidosis should receive intensive therapy with intravenous antibiotics for 10 to 14 days, and until the patient's condition improves, followed by prolonged oral eradication therapy for a minimum of 12 weeks to minimize the risk of relapse. The initial intravenous treatment should be extended to greater than or equal to 4 weeks for severe disease or lack of improvement. To reduce the likelihood of relapse, the duration of oral eradication therapy should depend on disease severity and the response to treatment.[140] These consensus recommendations do not provide separate guidelines for children or pregnant women for the intensive phase of treatment. However, based on 24 years of pediatric melioidosis treatment at the Royal Darwin Hospital in the Northern Territory, Australia, McLeod et al[145] similarly recommend a minimum of 14 days of intensive intravenous therapy followed by at least 12 weeks of eradication therapy for children aged 16 years or younger with disseminated disease. For pediatric patients with localized cutaneous melioidosis, McLeod et al indicate that 12 weeks of oral antibiotic therapy (without the initial intensive parenteral treatment phase) is generally sufficient.

For intensive intravenous therapy in adults and children, ceftazidime is adequate in most cases without complications. Meropenem is an acceptable alternative and may be preferable in cases with complications, such as neuromelioidosis or persistent bacteremia, or when the patient must be admitted to an intensive care unit. Patients whose condition worsens while taking ceftazidime should be switched to meropenem. TMP-SMZ (administered intravenously, orally, or via

nasogastric tube, using the dosing for eradication therapy) may be added to this regimen for patients with severe neurologic, cutaneous, bone, joint, or prostate infections.[140,145] The optimal intravenous therapy for pregnant women is not clear; however, Wuthiekanun and Peacock[146] indicate that intravenous co-amoxiclav is used to treat pregnant women (and children) in Thailand during the intensive phase of therapy.

For eradication therapy in nonpregnant adults and in children, TMP-SMZ is the first-line antibiotic. However, potential side effects include mild allergic reactions, Stevens–Johnson syndrome, bone marrow suppression, renal failure, and liver damage. In addition, TMP-SMZ may result in adverse pregnancy outcomes. For pregnant women, patients who cannot tolerate TMP-SMZ, and cases in which the organism is resistant to TMP-SMZ, co-amoxiclav (at an amoxicillin to clavulanic acid ratio of 4:1) is an alternative eradication-phase antibiotic, but it may be associated with a greater risk of relapse.[140,145] McLeod et al[145] indicate that co-amoxiclav is also used as an alternative to TMP-SMZ in pediatric patients with melioidosis in Thailand.

Postexposure Prophylaxis. Current recommendations for PEP after suspected *B mallei* or *B pseudomallei* exposure are based largely on animal studies and in vitro work. Ideally, PEP should be initiated promptly after a known or suspected exposure and continued for a total duration of 21 days. The first-line agent for adults and children is TMP-SMZ. For cases in which the organism is resistant to TMP-SMZ or the patient cannot tolerate this antibiotic, co-amoxiclav is the second-line choice. However, although these recommendations are appropriate for small-scale (eg, laboratory) exposures, it is not clear whether the provision of PEP to all individuals potentially exposed in a large exposure event would be feasible or advisable.[140]

Because of the potential for delayed-onset disease and relapse, monitoring (including serologic testing) should continue for at least 6 months after cessation of antibiotic PEP in exposed individuals; infected individuals may require lifelong monitoring following treatment.[11,14,66,147] Seroconversion may be indicative of relapse. If relapse is suspected, treatment (as described above) should be initiated. Antibiotic regimens should be adjusted based on results of sensitivity testing.[14,66]

Brucellosis

Brucellosis, a common zoonotic disease with a global distribution, is caused by infection with one of several *Brucella* spp, including *B abortus*, *B melitensis*, and *B suis*. These intracellular, nonspore-forming, gram-negative coccobacilli can cause severe disease in humans; mortality is low, but chronic, debilitating illness can result.[54,148–150] Infection is transmitted to humans by direct contact with infected animals or their carcasses, ingestion of unpasteurized milk or milk products, and via laboratory exposure.[148] Person-to-person transmission of brucellosis has been documented, but is rare.[54,151] *Brucella* are highly infectious by aerosol and remain one of the most common causes of laboratory-acquired exposure,[11,152] with an infective dose of only 10 to 100 organisms.[54] In untreated survivors, chronic illness can last for years. Infection with *Brucella* spp during pregnancy, if untreated, can cause spontaneous abortion or intrauterine fetal death.[14,66] *Brucella* spp are potential agents of bioterrorism because of their widespread availability, the ease with which they can be aerosolized, their stability in the environment, and their ability to induce chronic disease.[149]

Vaccination

Live vaccines licensed for use in animals have eliminated brucellosis in most domestic animal herds in the United States, but no licensed human vaccine exists. Ongoing research is evaluating the following:

- live, attenuated vaccine candidates, in some cases encapsulated within microspheres for slow release;
- subunit vaccines;
- vaccines based on recombinant proteins;
- vectored vaccines; and
- DNA vaccines.[150]

Antibiotic Agents

No approved chemoprophylaxis exists for brucellosis, whether as treatment or as PEP. Although few studies have compared monotherapy with combination therapy in treating brucellosis, the existing evidence suggests that monotherapy is more likely to result in relapse and treatment failure.[153–155] An adequate duration of therapy is also crucial to the effective treatment of brucellosis.

In a meta-analysis of randomized controlled trials comparing various treatment regimens for brucellosis, Skalsky et al[155] found that treatment consisting of doxycycline combined with rifampin was more likely to fail (generally from relapse) than was a regimen of doxycycline plus streptomycin. Triple-antibiotic therapy with doxycycline, rifampin, and an aminoglycoside was even less likely to result in treatment failure, whereas use of a quinolone plus rifampin was among the least effective of the regimens compared.

These authors concluded that the preferred treatment should consist of two or three antibiotics, including an aminoglycoside.

In a review, Franco, Mulder, and Smits[156] noted that relapse rates with the doxycycline–rifampin regimen ranged from 16% to 40% (depending—in part—on duration of treatment), whereas the relapse rates for doxycycline–streptomycin and rifampin–minocycline were 5.3% and 2%, respectively. Monotherapy has resulted in a combined treatment failure and relapse rate as high as 50%. In a more recent meta-analysis, Solís García del Pozo and Solera[153] examined clinical trials using various antimicrobial combinations in the treatment of human brucellosis. With relapse, therapeutic failure, and adverse effect rates as the primary outcome variables, they found that the doxycycline–streptomycin combination outperformed a combination of doxycycline and rifampin. For example, across 15 studies and a total of 700 patients with brucellosis, 6 to 8 weeks of treatment with doxycycline–rifampin resulted in treatment failure or relapse in 15.2% to 16.6% of patients (in trials employing this combination for only 4 weeks, treatment failure or relapse occurred in 26.5% of 83 patients). In contrast, in 11 studies evaluating more than 700 patients, doxycycline (45 days) plus streptomycin (15–21 days) resulted in treatment failure or relapse in 6.7%–7.6% of patients. Rates of serious side effects were similar (around 1%) for both of these combinations. The doxycycline–gentamicin combination appeared to be equivalent to doxycycline–streptomycin.[153]

Evidence regarding effective PEP regimens comes largely from laboratory exposures. One study reported prophylaxis using the doxycycline–rifampin combination administered to nine asymptomatic laboratory workers who seroconverted after exposure to *B abortus* serotype 1 atypical strain (a strain with low virulence).[157] These individuals subsequently developed symptoms of fever, headache, and chills that lasted a few days. In contrast, three persons who did not receive prophylaxis had symptoms of fever, headache, and chills for 2 to 3 weeks as well as 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. Five of these individuals were given PEP for 3 weeks (four individuals received doxycycline twice daily plus rifampin once daily, and one pregnant laboratory worker received TMP-SMZ twice daily). One individual declined prophylaxis and subsequently developed brucellosis (confirmed by culture). The five individuals who received PEP remained healthy and did not seroconvert.[158] In late 2007, the CDC became aware of 916 laboratory workers in 254 laboratories with potential exposure to RB51, an attenuated vaccine strain of *B abortus* used to vaccinate cattle, during a laboratory preparedness proficiency test. PEP was recommended for the 679 individuals characterized as having had high-risk exposures and was also offered to the 237 laboratory workers with low-risk exposures. No cases of brucellosis were reported, but the number of individuals who actually received PEP has not been documented.[159]

Postevent Countermeasures: Current Options

Treatment. For uncomplicated brucellosis in adults, a combination of oral doxycycline (for 6 weeks) and intramuscular streptomycin (for 2–3 weeks) is recommended as the "gold standard" treatment in the position paper that resulted from a 2006 consensus meeting (the Ioannina recommendations).[154] Parenteral gentamicin (for 7 days) is an acceptable substitute for streptomycin. Six weeks of oral doxycycline plus oral rifampin is an alternative first-line regimen because the convenience of (and therefore, presumably, better adherence to) an entirely oral therapy is likely to overcome the drawbacks of this combination.[154] The optimal treatment for pregnant women with brucellosis has not been sufficiently studied. TMP-SMZ and/or rifampin could be considered, with risks to the fetus of antimicrobial treatment balanced against the risk of spontaneous abortion resulting from the disease (and the risk of relapse in the case of monotherapy with rifampin).[154,160,161]

In adult patients with serious complications, such as neurobrucellosis or Brucella endocarditis, the optimal antibiotic combination and treatment duration are not clear. In general, however, the duration of treatment should extend to at least 3 months. The World Health Organization (WHO) recommends the addition of either TMP-SMZ or rifampin—both of which cross the blood–brain barrier—to the doxycycline–streptomycin combination for the treatment of neurobrucellosis. Because rifampin and TMP-SMZ penetrate cell membranes, the WHO also recommends the addition of one of these antibiotics to the combination therapy for Brucella endocarditis.[160,162]

The WHO[160] recommends that children aged 8 years and older receive the same antibiotics as adults for the same duration. For younger children, the

WHO indicates that satisfactory results have been achieved using a combination of TMP-SMZ for 6 weeks and parenteral streptomycin (for 3 weeks) or gentamicin (for 7–10 days). Alternatives include TMP-SMZ–rifampin for 6 weeks or rifampin with an aminoglycoside.[160] More recent guidelines[163] developed for the treatment of children in Saudi Arabia—where brucellosis is endemic—recommend 6 weeks of treatment with a combination of rifampin and TMP-SMZ or 6 weeks of rifampin and 7 days of gentamicin for children younger than 8 years old. For more severe disease in young children, these authors recommend rifampin, TMP-SMZ, and ciprofloxacin for 3 to 9 months with gentamicin added for the first 14 days. They recommend avoidance of doxycycline in young children because of the potential for dental staining. For older children (≥8 years), they recommend doxycycline and rifampin for 6 weeks or doxycycline for 6 weeks and either streptomycin (14 days) or gentamicin (7 days). For more severe disease in older children, they suggest using doxycycline–TMP-SMZ–rifampin for 3 to 9 months with gentamicin added during the first 14 days.[163]

Postexposure Prophylaxis. For asymptomatic individuals who have had a high-risk exposure (such as exposure to laboratory aerosols or biowarfare exposure) to Brucella isolates, the CDC[164] recommends a combination of doxycycline and rifampin for 3 weeks (see Table 27-2). If that combination cannot be used, TMP-SMZ could be offered.[14,66,160,164] For an asymptomatic pregnant woman with a high-risk exposure, PEP should be considered in consultation with an obstetrician, weighing the risk of disease against the potential toxicity of the antibiotics.[164]

Q Fever

Q fever is a zoonotic disease caused by a rickettsia, *Coxiella burnetii*, a gram-negative, obligately intracellular coccobaccillus with a global distribution.[165] *C burnetii* is environmentally stable and remains viable in the soil and other substrates for weeks or potentially longer.[166] Humans typically acquire *C burnetii* infection by inhaling aerosols contaminated with the organisms (generally from the excreta of infected animals).[167] Less common routes of transmission include the consumption of unpasteurized dairy products and transmission via tick bites.[168,169] Person-to-person transmission has been reported only rarely.[170,171] Cases of Q fever among US military personnel in Iraq have been linked to tick bites and helicopter-generated aerosols.[172,173] Q fever manifests in an acute form—which may be asymptomatic—as well as a rare but potentially more serious chronic form (most often presenting as

endocarditis) that can occur weeks, months, or years after the initial acute infection.[14,66,165,167,174–177] Long-term sequelae—notably, chronic fatigue and cardiovascular disease—often occur after acute infection.[175]

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, 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.[178]

No FDA-approved vaccine is available for vaccination against Q fever in the United States. However, one vaccine (Q-Vax) is approved in Australia and a similar IND vaccine (NDBR 105) has been used in at-risk researchers at Fort Detrick since 1965.

Q-Vax. Currently licensed in Australia, Q-Vax (CSL Ltd, Parkville, Victoria, Australia) has been demonstrated to be safe and effective for preventing Q fever. Q-Vax is a formalin-inactivated, highly purified *C burnetii* whole-cell vaccine derived from the Henzerling strain, phase I antigenic state.[179,180] More than 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 after vaccination (before vaccine-induced immunity) versus 97 cases in unvaccinated persons (among approximately 2,200 unvaccinated individuals, but the exact number is not known).[179] In another study, among 2,555 vaccinated abattoir workers, only two cases of Q fever were diagnosed between 1985 and 1990, with both cases occurring within a few days of vaccination (before immunity developed).[181] Nearly 49,000 individuals (primarily abattoir workers and farmers) were vaccinated between 2001 and 2004 during a national Q fever vaccination campaign in Australia. Compared with Q fever notification rates in 2001 and 2002, those in 2005 and 2006 declined by more than 50% to the lowest levels on record.[182] A recent meta-analysis of four studies assessing the effectiveness of Q-Vax in a total of 4,956 subjects found that, after excluding patients who developed symptoms of Q fever within 15 days after vaccination, the vaccine's effectiveness was 100% (with those cases included, the effectiveness was 98%).[183]

The main adverse event noted with this vaccine was the risk of severe necrosis (which resulted in sterile abscesses) at the vaccine site in vaccinees with prior

exposure to Q fever.[179,184] Therefore, a skin test using 0.02 mg of the vaccine is required before vaccination. Because of the risk of vaccine-site necrosis, vaccination against Q fever is contraindicated in persons with previous exposure to *C burnetii* as denoted by a positive skin test, which is defined as either (*a*) erythema of at least 30 mm or induration of at least 20 mm at day 1 or later after the skin test or (*b*) erythema and induration of at least 5 mm on day 7 after the test. Persons with a positive skin test are considered to be naturally immune and do not require vaccination. The exclusion from vaccination of individuals with a positive skin test has eliminated sterile abscesses (Figure 27-3).[185,186]

NDBR 105 Q Fever Vaccine. The NDBR 105 (IND 610) Q fever vaccine is an inactivated, lyophilized vaccine whose preparation is similar to that of Q-Vax. The vaccine originates from chick fibroblast cultures derived from specific pathogen-free eggs infected with the phase I Henzerling strain. NDBR 105 has been effective in animal studies.[185,187,188] The vaccine also prevented further cases of Q fever in at-risk laboratory workers in the final 4 years (1965–1969) of Fort Detrick's offensive biological warfare program, compared to an average of three cases per year before the vaccine was available.[11,189] In the 45 years of the biodefense research program at USAMRIID, only one case of Q fever (mild febrile illness with serologic confirmation)—attributed to a high-dose exposure from a breach in the filter of a biosafety cabinet—has occurred among vaccinated laboratory workers. However, the vaccine may have ameliorated disease symptoms in this case.[190]

As with Q-Vax, a skin test is required before vaccination to identify persons with prior exposure to *C burnetii*. For NDBR 105, skin testing is performed by
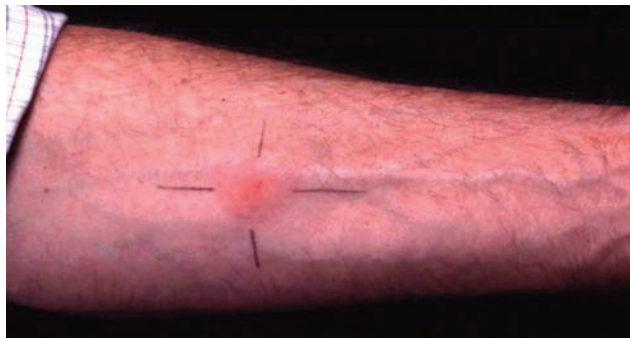
Figure 27-3. Positive Q fever skin test. Skin testing, performed by injecting 0.1 mL of skin test antigen intradermally in the forearm, is required before vaccination against Q fever to identify persons with prior exposure. Vaccination is contraindicated in individuals with a positive skin test because they are at risk for severe necrosis at the vaccine site. Photograph: Courtesy of Herbert Thompson, MD, MPH.

injecting 0.1 mL of skin-test antigen (a 1:1500 dilution of the vaccine with sterile water) intradermally into the forearm.

The vaccine is given only once, both because it is presumed to result in lifelong immunity and because of the potential for serious local reactions in individuals with prior exposure via disease or vaccination. The vaccine is administered by injecting 0.5 mL subcutaneously in the upper outer aspect of the arm (see Table 27-1). Protection against Q fever is primarily cell-mediated immunity. Markers to determine vaccine immunity to NDBR 105 have been studied (ie, cell-mediated immunity studies, skin testing, and pre- and postimmunization antibody studies), but reliable markers have not yet been identified for NDBR 105. After vaccination with the similar Q-Vax, skin-test seroconversion occurred in only 31 of 52 persons (60%), but lymphoproliferative responses to *C burnetii* antigens persisted for at least 5 years in 85% to 95% of vaccinated persons.[179,186,191–193] Vaccine breakthroughs have been rare in vaccinated persons.

Adverse events from NDBR 105, which were reported by 72 (17%) of 420 skin-test–negative vaccinees, comprised mainly local reactions, including erythema, induration, or a 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 were characterized by headache, chills, malaise, fatigue, myalgia, and arthralgia.[194]

NDBR 105 is available only at USAMRIID on an investigational basis, although it is on hold (as of November 2015) because lot release data for the skin test antigen are unavailable.

Other Vaccines. Several studies are underway to explore new techniques for vaccine development, including research focusing on Th1 peptides from the major immunodominant proteins.[195,196]

Antibiotic Agents

Antibiotics are known to be effective for the treatment of Q fever, but the recommended treatment varies with the form (acute vs chronic) and severity of disease. Acute Q fever often resolves without treatment within 2 to 3 weeks.[197,198] Doxycycline, which is considered the most effective antibiotic to treat Q fever, reduces elevated body temperature within 2 to 3 days from the start of treatment; in untreated patients, fever resolves in 12.5 days (on average). Other antibiotics, including macrolides, TMP-SMZ, quinolones, and rifampin, can also be helpful, yet typically less so than doxycycline. Some

doxycycline-resistant isolates of *C burnetii* have been reported, but such resistance does not appear to be common.[198]

Among 438 patients with Q fever during an outbreak in the Netherlands, doxycycline and moxifloxacin were the first and second most commonly prescribed initial antibiotics, respectively. However, several other antibiotics were also prescribed, including potentially effective alternatives (eg, low-dose doxycycline, TMP-SMZ, ciprofloxacin, clarithromycin, and cefuroxim) as well as beta-lactam antibiotics and azithromycin, which are considered ineffective against Q fever. Patients who were treated initially with beta-lactams or azithromycin were at greatest risk of hospitalization after at least 2 days of treatment. Those receiving doxycycline at the recommended dosage (200 mg/day) had the lowest risk of hospitalization.[176]

Doxycycline has also been the most effective antibiotic to treat chronic Q fever, particularly when combined with hydroxychloroquine, which increases the bactericidal activity of the treatment. Although treatment must be continued for 18 to 24 months, the use of doxycycline alone required treatment for up to 5 years.[198] Lifelong follow-up, and sometimes lifelong treatment, may be required.[175]

Q fever infection during pregnancy, particularly during the first trimester, can result in obstetric complications as well as a greater risk of chronic Q fever for the mother, with spontaneous abortions of future pregnancies more likely.[168,175,199] Carcopino et al[199] compared maternal and fetal outcomes for 53 women who were diagnosed with Q fever during pregnancy, including 16 women who received long-term (≥5 weeks) treatment with TMP-SMZ and 37 who did not. Among the women who did not receive long-term TMP-SMZ, 81% experienced obstetric complications, including spontaneous abortion, intrauterine growth retardation, intrauterine fetal death, and premature delivery. They found that long-term TMP-SMZ during pregnancy protected against chronic Q fever in the mother, placental infection by *C burnetii*, and obstetric complications.

Postevent Countermeasures: Current Options

Treatment. According to recommendations from the CDC and the Q Fever Working Group, nonpregnant adults and older children (≥8 years old) with symptomatic acute Q fever should be treated with doxycycline. Ideally, treatment should be initiated within the first 3 days of symptom onset and continued for 14 days. Alternative antibiotics include moxifloxacin, clarithromycin, TMP-SMZ, or rifampin.

Asymptomatic individuals and those whose symptoms have resolved without treatment generally should not receive antibiotic treatment, with the possible exception of individuals who are at high risk of developing chronic Q fever.[197]

For young children (<8 years old) with mild or uncomplicated illness, doxycycline should be administered for 5 days (which should not result in dental staining). If the patient remains febrile after this short course of doxycycline—or if the healthcare provider decides not to administer doxycycline at all—TMP-SMZ should be administered for 14 days.[197]

For pregnant women with acute Q fever, a longer (≥5 weeks) course of TMP-SMZ may be effective in reducing the risk of intrauterine fetal death, conversion to chronic Q fever in the mother, and adverse outcomes in future pregnancies. Treatment should not continue beyond 32 weeks' gestation because of the risk of hyperbilirubinemia. Concomitant use of folic acid may prevent antifolate effects of TMP-SMZ. However, data on the safety of Q fever treatment during pregnancy are limited; consultation with an infectious disease expert is recommended.[197]

After an acute infection, healthy patients with no risk factors for the development of chronic Q fever should be regularly evaluated for clinical and serologic signs of illness for at least 6 months after diagnosis, as described by Anderson et al.[197] Persons with risk factors for development of chronic disease should be serologically and clinically monitored more frequently and for a longer duration (at 3, 6, 12, 18, and 24 months after diagnosis of acute infection or, for pregnant women, after delivery). All patients who have recovered from an acute Q fever infection should be advised to seek immediate medical attention if symptoms of chronic Q fever reoccur at any time throughout their lives; this vigilance is particularly important for those with valvular defects or vascular abnormalities.[197]

Treatment of chronic Q fever typically involves a long course of doxycycline combined with hydroxychloroquine. A discussion of the appropriate duration of treatment, contraindications, and recommendations for the treatment of pregnant women and young children is beyond the scope of this chapter. However, these topics are discussed in depth by Anderson et al.[197]

Postexposure Prophylaxis. Limited data are available on the effectiveness of PEP for Q fever. The CDC and the Q Fever Working Group do not recommend PEP after potential exposure to *C burnetii*. Serologic and clinical (fever) monitoring is recommended for at least 3 weeks after exposure. At the first sign of fever, treatment should be initiated.[197]

VIRAL DISEASES

Vaccination is the mainstay of medical countermeasures against viral agents of bioterrorism. FDA-approved vaccines (eg, smallpox and yellow fever vaccines) and investigational vaccines (eg, vaccines against Rift Valley fever virus [RVFV] and Venezuelan, Eastern, and Western equine encephalitis viruses [VEEV, EEEV, and WEEV]) 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.

Encephalitic New World Alphaviruses

VEEV, WEEV, and EEEV are lipid-enveloped RNA viruses of the genus Alphavirus (family *Togaviridae*). These viruses, found in regions of North, Central, and South America, can cause severe neurologic disease in humans and equids, which typically are infected via the bite of an infected mosquito.[200,201] Infections may also be acquired via respiratory exposure to aerosolized virus, as may occur in a laboratory setting or a bioterrorism event. The VEEV complex consists of at least 13 subtypes and varieties, including epidemic/epizootic viruses, which are pathogenic to humans and equids, and enzootic viruses that are generally avirulent in equids but, in some cases, pathogenic to humans.[202–205] Humans with VEEV infections typically present with nonspecific febrile illness. However, in up to 14% of patients, VEEV causes neurologic disease.[202,203] WEEV infections typically are either asymptomatic or cause mild, nonspecific symptoms; a minority of patients experience encephalitis or encephalomyelitis. Of the three New World encephalitic alphaviruses, EEEV is the most likely to cause severe disease or death.[203] Among the survivors of encephalitis, up to 30% may experience neurologic sequelae.[14,66,202,203] Young children tend to develop more severe illness, such as encephalitis, compared with adults and older children.[200,202]

Vaccination

Vaccines are licensed for use in equids, but the only vaccines available for humans against VEE, WEE, and EEE are investigational (see Table 27-1).[201] Laboratory-acquired infections with VEEV in particular became problematic soon after discovery of the agent in 1938[206] and remain a concern.[11] 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 against both EEE and

WEE are also available on an IND basis at USAMRIID. These vaccines, which have demonstrated efficacy in animal models, have been used in at-risk laboratory workers at the institute for more than 50 years in the case of TC-83. However, the live attenuated vaccine, TC-83, has high reactogenicity, and the inactivated vaccines have lower immunogenicity. Also, because of their investigational status and limited supply, use of these vaccines in a bioterrorism event would be extremely limited.

Venezuelan Equine Encephalitis TC-83 Vaccine. Live attenuated VEE TC-83 vaccine (IND 142, NDBR 102) was manufactured at the National Drug Company (Swiftwater, PA) in 1965 using serial propagation of the Trinidad strain (subtype IAB) of VEEV 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 VEEV was used as seed stock to propagate in the 81st passage in fetal guinea pig heart cells.[207] The TC-83 designation refers to the number of passages in cell culture. The seed stock (81-2-4), which was provided by Fort Detrick, was diluted 1:100. 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 1:400 with modified Earle's medium and 0.5% human serum albumin, and 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. 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 single 0.5 mL subcutaneous injection (approximately 10^4 plaque-forming units per dose) in the deltoid area of the arm.

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 dose of the vaccine (containing approximately 10^6 virions) administered intraperitoneally. All animals survived. Additional rabbit, suckling mouse, mouse virulence, and monkey neurovirulence testing were conducted. The vaccine was protective against both subcutaneous and aerosol challenge with VEEV in mice and hamsters. In a monkey model of aerosol exposure, protection was inconsistent. Periodic postrelease potency analyses have shown that infectivity for all lots has declined by one to two logs from the original data in the IND 142 submitted in 1965.[208]

At-risk laboratory workers at Fort Detrick have received the TC-83 vaccine since 1963. Administration of this vaccine to more than 6,000 individuals in initial evaluations demonstrated its excellent immunogenicity.[209] In a study of 624 vaccinees, Pittman and colleagues[210] found that 513 (82%) responded to one dose of TC-83 with an 80% plaque reduction neutralization titer ($PRNT_{80}$) of at least 1:20. However, because the vaccine is derived from epizootic strains of VEEV, it may not protect against enzootic strains and may not adequately protect against distantly related VEEV subtype IAB variants.[190]

The severity and frequency of adverse events from the VEE TC-83 vaccine vary with the vaccine lot. Among 624 vaccinees, for example, 134 (21.5%) reported self-limited reactions, primarily systemic reactions such as malaise (reported by 90 vaccinees), headache (68), fever (65), chills (50), and myalgia (43). In some vaccinees, these symptoms were severe enough to require bedrest, but in all cases symptoms resolved without permanent effects.[210] No person-to-person transmission of VEE has been documented after vaccination with TC-83. Local reactions are rarely seen.

Some evidence has hinted at an association between glucose metabolism or insulin release and either infection with VEE or inoculation with the VEE TC-83 vaccine. In most studies in humans and in animal models, results have been inconclusive or negative.[211–214] However, out of an abundance of caution, 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.[215] This kind of event has been reproduced in many animal models. Rhesus monkey fetuses were inoculated with VEE vaccine virus by the direct intracerebral route at approximately gestational day 100. 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.[216] VEE vaccine virus, which is teratogenic for NHPs, must be considered a potential teratogen of humans. The wild type VEE virus is known to cause fetal malformations, abortions, and stillbirths.[217]

Venezuelan Equine Encephalitis C-84 Vaccine. The VEE C-84 formalin-inactivated vaccine (IND 914, TSI-GSD 205) was developed in part because of the high rate of adverse reactions in humans vaccinated with TC-83. C-84, which is made from the TC-83 production seed, 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 is freeze dried. The VEE C-84 vaccine contains neomycin and streptomycin at a concentration of 50 µg/mL, sodium bisulfite, chicken eggs, and formalin.

In animal models, the VEE C-84 vaccine's efficacy, particularly in protecting against aerosol challenge, has been inconsistent.[218–222] However, it has successfully been used to boost human vaccinees who have previously received the VEE TC-83 vaccine.[210] Therefore, although the C-84 vaccine is not used for primary vaccination against VEE, it has been used in at-risk laboratory workers at Fort Detrick as a booster for those individuals who have received the VEE TC-83 vaccine and have either (*a*) an inadequate initial response with a $PRNT_{80}$ of no more than 1:20, or (*b*) an adequate initial response to VEE TC-83 but $PRNT_{80}$ levels that subsequently drop below 1:20.

Adverse events tend to be minor. Among 128 individuals who received C-84 as a booster, only minor local reactions occurred in 6.3% of vaccinees.[210] From 2002 to 2006 at USAMRIID, 8% to 33% of individuals receiving C-84 as a booster through the Special Immunizations Program (SIP) 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 symptoms resolved without sequelae.

The vaccine is administered as a 0.5 mL subcutaneous injection above the triceps area. The current protocol used in the SIP allows for a maximum of four doses per year if postvaccination titers are not adequate.

Western Equine Encephalitis Vaccine. The inactivated WEE vaccine (IND 2013, TSI-GSD 210) is a lyophilized product originating from the supernatant harvested from primary chicken fibroblast cell cultures.[223] The vaccine was prepared from pathogen-free eggs infected with the attenuated CM4884 strain of WEEV. 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 National Drug Company originally manufactured the inactivated WEE vaccine. The current product, lot 3-1-92, was manufactured at the Salk Institute, Government Services Division (Swiftwater, PA) in 1992. 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 that the vaccine protected mice against intracerebral challenge with WEEV[224] and protected hamsters against intraperitoneal challenge.[225] The inactivated WEE vaccine protected 17 of 17 horses against intradermal challenge 12 months after vaccination, even in the absence of detectable WEE protective neutralizing antibodies.[226] Human subjects who were administered the 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. 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 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.[223]

The inactivated WEE vaccine has been administered to at-risk personnel at Fort Detrick since the 1970s. Pittman and colleagues evaluated the vaccine for its immunogenicity and safety in 363 at-risk workers enrolled in evaluation trials at USAMRIID between 1987 and 1997. All volunteers received subcutaneous injections 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 administered 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. Of 115 initial nonresponders, 76 (66%) 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 (unpublished data).

Passive collection was used to record local and systemic adverse events from the inactivated WEE vaccine from 1987 to 1997. Of the 363 vaccinees who received three initial injections, only 5 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 SIP 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, it is not advisable to vaccinate pregnant women.

According to the current SIP protocol, the primary series of the WEE vaccine is given subcutaneously at days 0, 7, and 28; a mandatory booster is given at month 6, with subsequent booster doses (up to four in a 12-month period) administered if and when the $PRNT_{80}$ titer falls below 1:40.

Eastern Equine Encephalitis Vaccine. The Salk Institute manufactured the formalin-inactivated EEE vaccine (TSI-GSD 104) in 1989.[227] The seed for the EEE vaccine was passaged twice in adult mice, twice in guinea pigs, and nine times in embryonated eggs.[228] The final EEE vaccine was derived from supernatant fluids bearing virus accumulated from three successive passages in primary chick embryo fibroblast cell cultures prepared from pathogen-free eggs infected with the attenuated PI-6 strain of virus. The supernatant was harvested and filtered and the virus inactivated with formalin. The product was then lyophilized for storage at −20°C.

Animal studies have demonstrated that the EEE vaccine is 95% protective against intracerebral challenge with EEEV in guinea pigs, with survival correlating to serum neutralizing antibody titers.[229] Vaccination of horses was also protective against intradermal challenge at 12 months postvaccination, even with an absence of detectable neutralizing antibody titers in 16 of the 17 animals.[226]

The vaccine has been given to at-risk laboratory workers at Fort Detrick for more than 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 the EEE vaccine resulted in an adequate titer in 66% of the initial nonresponders. Adverse events from the EEE vaccine, which occurred in approximately 20% of these individuals, consisted 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 vaccination site have also been reported (unpublished data).

The EEE vaccine contains 50 µg/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. If the titer falls 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.

Vaccine Research. The live attenuated VEE vaccine candidate V3526 was scheduled to replace the 50-year-old VEE TC-83 IND vaccine. This VEE vaccine candidate, a recombinant vaccine derived from the Trinidad donkey strain of VEEV, had improved activity against VEE enzootic strains. In phase 1 clinical trials, the vaccine elicited strong immune responses. However, because of high rates of severe neurologic adverse events in these trials, further development of this product was halted. These high rates were unexpected with V3526 because it demonstrated less reactogenicity in NHP studies than the VEE TC-83 product. Recently, research in mice has suggested that a formalin-inactivated V3526 vaccine could replace C-84.[230]

Another line of research has explored the use of live chimeric Sindbis virus (an Old World alphavirus that is among the least pathogenic alphaviruses in humans) engineered to express structural proteins of VEEV, EEEV, or WEEV. Studies in animal models suggest that this approach has promise for all three New World alphaviruses. Other approaches include DNA vaccines expressing proteins of the TC-83 and Trinidad donkey strains of VEEV, viral-vectored vaccines, and nonreplicating virus-like particles.[200,201] In a recent phase 1 clinical trial, a DNA vaccine against VEEV was well tolerated, with VEEV-neutralizing antibodies detected in 100% of subjects receiving the vaccine via intramuscular electroporation and in 63% to 88% of those receiving the vaccine via intradermal electroporation.[230a]

Many of the existing New World encephalitic alphavirus vaccines have been under IND status for more than 30 years. For several reasons, including funding shortfalls, these products have never been transitioned from development to licensure.

Passive Immunotherapy

Hyperimmune serum has protected animals from lethal challenge with VEEV, WEEV, and EEEV. This line of work has progressed toward safer approaches using humanized murine monoclonal antibodies. Administration of humanized murine monoclonal antibodies against a VEEV envelope protein protected 75% to 100% of mice challenged with lethal doses of VEEV if the antibodies were given within 24 hours after exposure; delaying administration to 48 hours postexposure greatly reduced the efficacy. Similar results have been found in animal models with the administration of human antibodies or human-like (macaque) antibody fragments.[200]

Antiviral Agents

Research on antiviral compounds effective against the encephalitic New World alphaviruses remains at an early stage. Although approaches using interferons (IFNs) and toll-like receptors have shown some promise in animal models, they must be administered before and after exposure.[200] Some evidence suggests that carbodine (carbocyclic cytosine) may have potential as an antiviral agent to treat VEE postexposure.[231] Recently, Chung et al[232] reported their discovery and characterization of a novel anti-VEEV and anti-WEEV compound, the quinazolinone CID15997213. They found that this small molecule inhibited VEEV and WEEV by inhibiting viral RNA, protein, and progeny synthesis, specifically by targeting the nsP2 protein. In mice, administration of CID15997213 did not result in any signs of acute toxicity, and it provided complete protection from a lethal VEEV challenge at 50 mg/kg/day.[232]

Postevent Countermeasures: Current Options

No treatment has been shown to alter the course of VEE, WEE, or EEE in humans once disease has been contracted. At this time, treatment is limited to supportive care. No PEP exists for the New World encephalitic alphaviruses. In the context of a laboratory exposure, previously vaccinated individuals who are exposed to EEEV, WEEV, or VEEV may be offered a booster dose of the appropriate vaccine if their antibody levels are inadequate[66]; however, in a mass casualty event, limited vaccine supplies would likely preclude large-scale vaccination.

Smallpox

Smallpox is caused by variola virus, a DNA virus of the genus *Orthopoxvirus*. Once distributed globally, this disease was the greatest infectious cause of human mortality for centuries. In 1980, after an intensive vaccination program, the WHO declared that the disease was eradicated.[233] Subsequently, all known stocks of variola virus were destroyed, with the exception of stocks at two WHO collaborating centers: (1) the CDC and (2) the Russian State Research Center of Virology and Biotechnology.

Smallpox is readily transmitted from person to person via direct contact, droplets, aerosol, and contaminated fomites such as clothing and bedding.[234,235] Smallpox has been designated a category A biothreat agent because of its high mortality, high transmissibility, the potential for aerosol dissemination and transmission, and history of massive weaponization by the former Soviet Union.

Vaccination

History of Smallpox Vaccination. Vaccination against smallpox was recorded in 1,000 BCE in India and China, where individuals were inoculated with scabs or pus from smallpox victims (in either the skin or the nasal mucosa), producing disease that was milder than naturally occurring smallpox. In the 18th century in Europe, scratching and inoculation of the skin with material taken from smallpox lesions, known as variolation, was performed, resulting in a 90% reduction in mortality and long-lasting immunity. (Variolation was also performed using the pustules of a previously variolated individual.) In 1722, variolation of 242 individuals in Boston resulted in a smallpox death rate of 2.5% (6 persons) compared to a death rate of 14% in unvaccinated persons (849 deaths among 5,889 cases).[236]

In 1770, Edward Jenner noticed that milkmaids who had been exposed to cowpox virus (another orthopoxvirus) rarely had smallpox scars. Subsequently, Jenner discovered that inoculation of the skin with cowpox virus taken from a milkmaid's hand resulted in immunity. This early form of vaccination began in 1796. Beginning in the mid-1840s, the smallpox vaccine was manufactured in calfskin. The virus used as the vaccine, though originally cowpox virus, changed over time and eventually was found to be a distinct virus whose precise origins were unknown; this virus became known as vaccinia virus.[236] Production of the vaccine became regulated in 1925, with the New York City Board of Health strain of vaccinia as the primary US vaccine strain. Global vaccination efforts eventually led to eradication of the disease; the last known case of naturally occurring smallpox was reported in 1977.[233] 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.[234,236,237]

Because of renewed concerns over the risk of bioterrorism, vaccination against smallpox in at-risk military personnel was resumed in 2003 using Dryvax (Wyeth Laboratories, Marietta, PA), a live-virus preparation of vaccinia virus (the New York City Board of Health strain) made from concentrated, lyophilized calf lymph.

Dryvax and similar first-generation smallpox vaccines, which had been used in the global smallpox eradication campaign, were known to prevent smallpox. However, Dryvax was 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 was preferable to the animal-derived product.[238] Dryvax was replaced by ACAM2000 in 2007.[239]

Current Smallpox Vaccine. The smallpox vaccine used in the United States today, ACAM2000 (Sanofi Pasteur Biologics, Cambridge, MA), is a cell culture–based live vaccinia virus vaccine licensed by the FDA for prophylaxis against variola virus (see Table 27-1).[238] ACAM2000 is a lyophilized preparation that is free of adventitious agents and contains trace amounts of neomycin and polymyxin B. The diluent for the vaccine contains 50% glycerin and 0.25% phenol in US Pharmacopeia sterile water.[240]

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. Clinical trials have shown that administration of ACAM2000 results in cutaneous, antibody, and T cell responses that are comparable to those elicited by Dryvax. The safety profile of the two vaccines also appears to be similar.[241,242]

ACAM2000 is administered by scarification (percutaneously) to the upper arm over the deltoid muscle area with 15 jabs using a bifurcated needle.[14,240] The individual is followed after vaccination to document a take reaction, a vesiculo-papular response that indicates immunity against smallpox. Six to 8 days after the primary vaccination, a primary major reaction to the vaccine develops—a clear vesicle or pustule with a diameter of approximately 1 cm. The site then scabs over by the end of the second week, with the scab drying and separating generally by day 14 to 21 (Figure 27-4). First-time vaccinees who do not exhibit either a primary major reaction or an immune response require revaccination. If no primary reaction is noted after revaccination (and after ensuring that proper technique in vaccine administration was used), these revaccinees are considered immune.[240] At some point in the future (which may be years), the immunity of vaccinated individuals may wane, and revaccination at that time may again result in a take.

The CDC recommends vaccination with confirmation of a take at least every 10 years for laboratory researchers working with nonhighly attenuated vaccinia
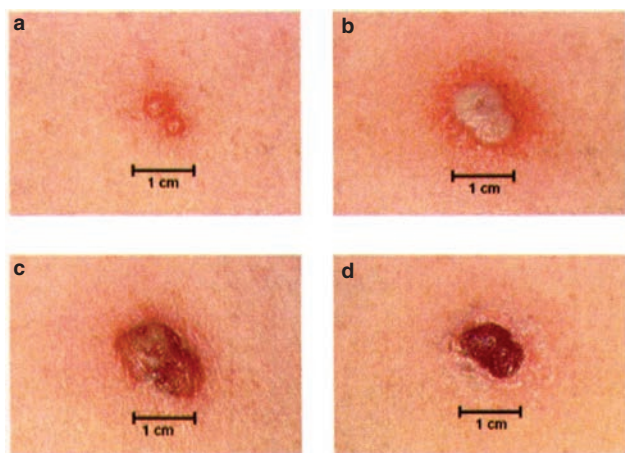
Figure 27-4. Take reaction in response to primary smallpox vaccination at (**a**) day 4, (**b**) day 7, (**c**) day 14, and (**d**) day 21. Reproduced from: Centers for Disease Control and Prevention website. http://www.bt.cdc.gov/agent/smallpox/smallpox-images/vaxsit5a.htm. Accessed September 16, 2014.

viruses, recombinant viruses developed from vaccinia viruses, and other nonvariola orthopoxviruses. For increased protection against more virulent nonvariola orthopoxviruses, such as monkeypox, revaccination every 3 years may be appropriate.[190,243] Individuals working with variola virus in the laboratory (at CDC) are required to receive a smallpox vaccination every 3 years (K S Meadows, Centers for Disease Control and Prevention, written communication, December 2015).

In the event of a smallpox release from a bioterrorism attack, individuals would be vaccinated according to the national policy. The current national policy[244] recommends vaccination initially of higher risk groups, including individuals directly exposed to the agent, household contacts or individuals with close contact to smallpox cases, and medical and emergency transport personnel. Ring vaccination—vaccination of contacts and contacts 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. In a postevent setting, 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 also 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.

Secondary attack rates (ie, estimates of the risk of transmission from a primary case to secondary contacts of that case) 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 a higher risk for acquiring infection. In an outbreak recorded in the Shekhupura District of Pakistan during the smallpox era, the secondary attack rate was only 4% in persons vaccinated with a first-generation vaccinia virus vaccine within the previous 10 years (5/115) and 12% in persons vaccinated more than 10 years before (8/65) compared with 96% in unvaccinated persons (26/27).[245,246] 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 (CFR) of only 1.4% in individuals who had received the smallpox vaccine within the previous 10 years, compared with 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 more than 20 years before. Postexposure vaccination resulted in 27% less mortality when compared (retrospectively) with smallpox patients who were never vaccinated.[245]

The effectiveness of postexposure vaccination appears to be greatest in the first 3 to 4 days after exposure to variola virus. In a recent review of historical data before the eradication of smallpox, Keckler and colleagues[247] found that vaccination of contacts decreased mortality and/or reduced morbidity in 100%, 75%, 67%, 58%, and 42% of reports when the (first-generation) smallpox vaccine was administered less than 1, 3, 5, 7, or 9 days postexposure, respectively. However, these historical data have a number of limitations, including the potential underestimation of prior immunity (from previous vaccination or exposure) in the patients described. Thus, Keckler et al also analyzed modern studies using animal models to assess the efficacy of postexposure vaccination (which, in these surrogate models, is also postinfection). In several of these studies using NHP and murine models, vaccination on postexposure day 0 or 1 resulted in 80% to 100% survival, and vaccination on postexposure day 2 or 3 resulted in 15% to 100% survival. However, in two studies, survival was consistently nearly 0% regardless of the day of vaccination. Conclusions from the animal models are difficult because of the diversity among models and the variability across species in the course of the disease.[247]

Vaccine Contraindications. According to the Advisory Committee on Immunization Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee,[248] smallpox vaccination is contraindicated in the pre-event setting for individuals who:

TABLE 27-3

CONTRAINDICATIONS TO AND PRECAUTIONS FOR PRE-EVENT SMALLPOX VACCINATION

Condition	Contraindication or Precaution
Allergy	Do not administer to those with allergies to vaccine components (eg, neomycin, polymyxin B). Where risk is great, vaccine should be administered with subsequent use of antihistamine or other appropriate medication.
Eczema (atopic dermatitis) or Darier disease (keratosis follicularis)	Do not administer to those with a history of eczema or Darier disease, even if no rash is present.
	Recent vaccinees should be counseled to avoid contact with individuals who have eczema or Darier disease.
Other skin conditions	Do not administer to those with disruptive or eruptive skin conditions, such as: • Severe acne • Burns • Impetigo • Contact dermatitis or psoriasis • Chicken pox The vaccine may be administered after the condition resolves or if the (noneczema/atopic) skin condition is sufficiently small and the patient is counseled to take great care to prevent transfer of vaccinia virus from vaccination site to affected skin. Vaccinees should be counseled to avoid contact with individuals who have a disruptive or eruptive skin condition.
Pregnancy or breastfeeding	Do not administer to patients who are pregnant or breastfeeding. Advise vaccinees not to become pregnant for ≥1 month after vaccination.
	Recent vaccinees should be counseled to avoid contact with individuals who are pregnant or breastfeeding.
Infancy	Do not administer to patients younger than 1 year old.
	Recent vaccinees should be counseled to avoid contact with infants.
Immunodeficiency	Do not administer to patients with diseases that have an immunodeficiency component, such as: • Human immunodeficiency virus infection • AIDS • Many cancers • Autoimmune diseases
Immunosuppressive therapy	Do not administer to patients who are currently taking immunosuppressive therapies, such as: • 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), including medication for treatment of inflammatory eye disease Immunosuppression from some medications may last for up to 3 months after discontinuation.
Cardiovascular disease or risk factors	Do not administer vaccine to patients with a history of, or significant risk factors for, ischemic heart disease, myocarditis, or pericarditis or those with significant cardiac risk factors (eg, hypertension, high cholesterol, or diabetes).
Simultaneous administration of varicella vaccine	Do not administer these vaccines simultaneously because the resulting skin lesions are difficult to distinguish.
Moderate or severe illness	Do not administer vaccine to patients who are moderately or severely ill at the time of vaccination.

(**Table 27-3** *continues*)

Table 27-3 continued

Active eye disease of the conjunctiva or cornea	Patients with inflammatory eye diseases may be at increased risk for autoinoculation of the eye by touching or rubbing the eye after touching the vaccination site. Such patients can be vaccinated but should be counseled to take great care to prevent transfer of vaccinia virus to the eye.

Notes: During an outbreak, after a biological terrorism event, or for individuals with a high risk of exposure to variola virus, no contraindications are absolute. Persons at greatest risk of complications from vaccination are those for whom smallpox infection poses the greatest risk. Data sources: (1) Dembek Z, ed. *USAMRIID's Medical Management of Biological Casualties Handbook*. 7th ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (2) 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.

- have a history or presence 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;
- have conditions associated with immunosuppression;
- have a serious allergy to any of the vaccine components;
- are younger than 1 year old; or
- have close physical contact with a person who (*a*) has a history or presence of atopic dermatitis or other acute, chronic, or exfoliative skin conditions; (*b*) has a condition associated with immunosuppression; or (*c*) is pregnant (Table 27-3).[248]

The CDC has recently added underlying cardiac disease (eg, a history of ischemic heart disease, myocarditis, or pericarditis) or significant cardiac risk factors (eg, hypertension, high cholesterol, or diabetes) as relative contraindications to the vaccine in a pre-event setting; however, these exclusions may be temporary, pending the results of further research into the possible link between smallpox vaccination and cardiac disease.[249]

In addition to the contraindications listed above, the ACIP does not recommend vaccination of persons younger than 18 years old in the pre-event setting.[248] Furthermore, although the presence of an infant in the household is not an absolute contraindication for vaccination of an adult, the ACIP recognizes that vaccination programs should defer vaccination of individuals whose households include infants younger than 1 year old because of data indicating a higher risk for adverse events among primary vaccinees in this age group. Because skin lesions resulting from the varicella vaccine may be confused with vaccinia lesions, simultaneous administration of the smallpox and varicella vaccines is not recommended.[248]

During an outbreak or after an intentional release of variola virus, there are no absolute contraindications to vaccination for any person who has been exposed to smallpox. However, if pregnant or eczematous persons are vaccinated under such circumstances, vaccinia immune globulin (VIG) could be administered concomitantly.[234]

Complications of Vaccination. Vaccinia virus can be transmitted (shed) from a vaccinee's unhealed vaccination site—or from lesions caused by autoinoculation, generalized vaccinia, eczema vaccinatum, or progressive vaccinia (see below)—to other persons by close contact. The virus can survive on fomites for at least several days.[250] Contact transmission can lead to adverse events that are identical to those that could be caused by intentional vaccination. In addition, viral shedding from the vaccination site can cause autoinoculation, in which the vaccinee spreads infection from the vaccination site to other areas, such as the eye, where vaccinia virus infection is associated with significant morbidity (Figures 27-5 and 27-6).

Although medical personnel are currently taught that vaccinia virus is shed from the vaccination site only until the scab (from the take reaction) separates,[240,251] Pittman et al[252] recently found that up to 23% of vaccinees continued to shed vaccinia virus after scab separation and as late as postvaccination day 42. From December 2002 to March 2011, a period when approximately 2.1 million military personnel and 40,000 civilian emergency responders were vaccinated against smallpox with Dryvax or ACAM2000, the incidence of vaccinia transmission through contact was 5.4 per 100,000 vaccinees. Generally, the virus was transmitted to household members, intimate contacts, or sports contacts. Most cases were mild; only 1 of 115 cases was life threatening.[253] Between March 2008 and August 2010 (when only ACAM2000 was used) another group reported an incidence of contact transmission of 4.4 per 100,000 vaccinations and an incidence of autoinoculation of up to 20.6 per 100,000 vaccinations (Table 27-4).[254] To avoid inadvertent transmission, vaccinees

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 27-4 and 27-5).[256,257]

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 27-6).[258]

Generalized vaccinia is characterized by a disseminated maculopapular or vesicular rash, frequently on an erythematous base and typically occurring 6 to 9 days after primary vaccination (Figure 27-7). Generalized vaccinia must be distinguished from other postvaccination exanthems, such as erythema multiforme and roseola vaccinatum (Figure 27-8). Lane et al reported 242.5 cases per million primary vaccinations and 9.0 cases per million revaccinations in a 1968 ten-state survey of smallpox vaccination complications.[259] The rash usually resolves without therapy. Contact precautions should be used to prevent further transmission and nosocomial infection.[258]

Eczema vaccinatum may occur in individuals with a history of atopic dermatitis, regardless of current disease activity, and it can be a papular, vesicular, or

Figure 27-5. 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 website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Allen W Mathies, MD, and John Leedom, MD, California Emergency Preparedness Office, Immunization Branch.

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 of in household trash. Two recent studies have demonstrated that the application of povidone iodine ointment to the vaccination site can reduce viral shedding.[252,255]

Smallpox vaccine adverse reactions are diagnosed by clinical exam. Most reactions can be managed with observation and supportive measures. Self-limited

Figure 27-6. 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 website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Allen W Mathies, MD, and John Leedom, MD, California Emergency Preparedness Office, Immunization Branch.

TABLE 27-4

RATES OF ADVERSE EVENTS AFTER SMALLPOX VACCINATION

Type of Event	Rate per 100,000 Vaccinations				
	Mar 2008–Jun 2013[*] (only serious AEs)	Mar 2008–Aug 2010[†]	Dec 2002–Mar 2011[‡]	Dec 2002–May 2003[§]	Historic Estimates[¥]
Autoinoculation	0.6	6.4–20.6	—	10.7	60.6
Contact transmission	0.5	3.5–4.4	5.4	4.7	0.8–2.7
Myo/pericarditis	1.9	—	—	8.2	10
Ischemic cardiac event	1.8	—	—	—	—
Eczema vaccinatum	0.1	—	—	0	0.2–3.5
Progressive vaccinia	0.1	—	—	0	0.1–0.7
Meningitis	0.5	—	—	—	—
Encephalitis	—	—	—	0.2	0.3–0.9
Death	0.1	—	—	0	0.1–0.2

Note: Dash indicates event not assessed.

[*]During this period, a total of approximately 834,465 doses of ACAM2000 were administered (approximately 832,035 to US military service members and 2,430 to US civilians). Events noted here are only those considered "serious" (those resulting in permanent disability, hospitalization or prolongation of hospitalization, life-threatening illness, or death). Reports of events were those submitted to the Vaccine Adverse Event Reporting System (VAERS) (see data source reference 4).

[†]During this period, 451,518 doses of ACAM2000 were administered (450,284 to US military service members and 1,234 to US civilians). The first number in each range includes only "suspect" and "confirmed" cases; the second number also includes "possible" cases. Reports included those submitted to VAERS as well as other sources (see data source reference 2).

[‡]During this period, approximately 2.1 million doses of smallpox vaccine (Dryvax until 2008, ACAM2000 thereafter) were administered to US military personnel and approximately 40,000 doses were administered to US civilians. Reports of contact transmission were assessed using the medical literature, VAERS, and the Defense Medical Surveillance System (see data source reference 1).

[§]During this period, approximately 450,293 doses of Dryvax were administered to military service members. Events noted here include those the authors considered "moderate or serious" (encephalitis, myopericarditis, eczema vaccinatum, progressive vaccinia, and death) as well as those they considered "mild or temporary" (inadvertent autoinoculation and contact transmission). Adverse events were collected in a variety of ways, including from VAERS (see data source reference 3).

[¥]From smallpox vaccinations in US civilians (adults and adolescents) and (for myopericarditis) Finnish military personnel. See Grabenstein and Winkenwerder (data source reference 3) and sources cited therein.

AE: adverse event

Data sources: (1) Wertheimer ER, Olive DS, Brundage JF, Clark LL. Contact transmission of vaccinia virus from smallpox vaccinees in the United States, 2003–2011. *Vaccine*. 2012;30:985–988. (2) Tack DM, Karem KL, Montgomery JR, et al. Unintentional transfer of vaccinia virus associated with smallpox vaccines ACAM2000 compared with Dryvax. *Human Vaccin Immunother*. 2013;9:1489–1496. (3) Grabenstein JD, Winkenwerder WJ Jr. US military smallpox vaccination program experience. *JAMA*. 2003;289:3278–3282. (4) McNeil MM, Cano M, Miller ER, Petersen BW, Engler RJ, Bryant-Genevier MG. Ischemic cardiac events and other adverse events following ACAM2000 smallpox vaccine in the Vaccine Adverse Event Reporting System. *Vaccine*. 2014;32:4758–4765.

pustular rash (Figure 27-9). Historically, eczema vaccinatum occurred at a rate of 14.1 and 3.0 per million primary and revaccinations, respectively[259]; however, in more recent military experience, no cases of eczema vaccinatum occurred in 450,293 smallpox vaccinations (of which 70.5% were primary vaccinations).[257] The rash may be generalized or localized with involvement anywhere on the body, especially areas of previous atopic dermatitis lesions.

Progressive vaccinia is a rare, severe, and often fatal 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 27-10 and 27-11). Those at highest risk include persons with congenital or acquired immunodeficiencies, HIV infection/AIDS, cancer, or autoimmune disease and

those who have undergone organ transplantation or immunosuppressive therapy. Estimated rates of progressive vaccinia ranged from 1 to 3 per million vaccinees historically,[259] no cases in 450,293 US military vaccinees,[257] and no cases (that met case definition) in 38,440 US civilian vaccinees in 2003.[260]

Although rare, central nervous system disease, which includes postvaccinial encephalopathy and postvaccinial encephalomyelitis, is the most frequent cause of death related to smallpox vaccination.[259] 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 after vaccination and include seizures, hemiplegia, aphasia, and transient amnesia. Histopathologic findings include cerebral edema, lymphocytic meningeal

TABLE 27-5

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

Indicated	Not Recommended
• Inadvertent autoinoculation, extensive lesions or severe ocular vaccinia (without evidence of vaccinial keratitis)	• Inadvertent autoinoculation, mild
	• Generalized vaccinia, mild (most cases)
	• Erythema multiforme
• Eczema vaccinatum	• Vaccinial keratitis*
• Generalized vaccinia, severe or recurrent	• Central nervous system complications
• Progressive vaccinia	

Note: Data are not available on the efficacy of prophylactic vaccinia immune globulin (VIG) for a pregnant woman to prevent fetal vaccinia or on the efficacy of VIG as a treatment for an infant born with fetal vaccinia.

*VIG is contraindicated for persons with vaccinial keratitis as it may produce severe corneal opacities. An exception may be made in persons with additional, potentially fatal complications that may respond to VIG; in such cases, the choice may be one of life vs vision.

Data sources: (1) Rusnak J, ed. *Occupational Health Manual for Laboratory Exposures to Select (BSL-3 & BSL-4) and Other Biological Agents*. 3rd ed. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases; 2011. (2) 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. (3) Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis*. 1970;122:303–309. (4) Lane JM, Ruben FL, Abrutyn E, Millar JD. Deaths attributable to smallpox vaccination, 1959 to 1966, and 1968. *JAMA*. 1970;212:441–444. (5) 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. (6) Ruben FL, Lane JM. Ocular vaccinia: an epidemiologic analysis of 348 cases. *Arch Ophthalmol*. 1970;84:45–48. (7) Fulginiti VA, Winograd LA, Jackson M, Ellis P. Therapy of experimental vaccinal keratitis: effect of idoxuridine and VIG. *Arch Ophthalmol*. 1965;74:539–544. (8) Kempe CH. Studies on smallpox and complications of smallpox vaccination. *Pediatrics*. 1960;26:176–189. (9) Military Vaccine Agency. *Pregnancy Discovered after Smallpox Vaccination–IV-VIG*. Silver Spring, MD: Vaccine Healthcare Centers Network; 2013. Information Paper.

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 after vaccination.[258,261] Neff's 1963 national survey[262] detected 12 cases of postvaccinial encephalitis among 14,014 vaccinations. Symptoms progress to amnesia, confusion, disorientation, restlessness, delirium, drowsiness, and seizures. The cerebrospinal fluid has normal chemistries and cell counts. 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.[258,263,264]

Fetal vaccinia, which results from vaccinial transmission from mother to fetus, is a rare but serious complication of smallpox vaccination during or immediately before pregnancy (Figure 27-12). Fewer than 40 cases have been documented in the world's literature.[256]

Myopericarditis, although previously reported as a rare complication of vaccination using vaccinia strains other than the New York City Board of Health strain,

Figure 27-7. Generalized vaccinia. This 8-month-old infant developed a generalized vaccinia reaction after vaccination. Generalized vaccinia is a widespread rash involving sores on parts of the body away from the vaccination site, which results from vaccinia virus traveling through the bloodstream. Image 4644.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Allen W Mathies, MD, California Emergency Preparedness Office, Immunization Branch.

Figure 27-8. After receiving a smallpox vaccination on the small of his back, this 14-month-old child manifested a nonspecific rash in the form of extensive, roseola-like erythematous macules and patches over his entire body. Eruptions such as this one are common after vaccination; although often dramatic in appearance, they are largely benign and usually self-limited. There is no evidence of systemic or cutaneous spread of the vaccinia virus, and live virions cannot be recovered from the involved sites. Image 3318.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Arthur E Kaye, Centers for Disease Control and Prevention.

was not well recognized until reported during active surveillance of the 2002–2003 US Department of Defense (DoD) vaccination program (Figure 27-13).[265,266] In 2003, reports of myocarditis among vaccinees raised concerns about carditis and cardiac deaths in military personnel receiving the smallpox vaccine (which, at the time, was Dryvax). Among 450,293 vaccinees, 37 individuals (all men receiving their first smallpox vaccination) experienced myopericarditis, for a rate of 82 per million vaccinees.[257] Ischemic cardiac events, including (rarely) fatalities, have also been reported following vaccination with both Dryvax and ACAM2000. Most recently, McNeil and colleagues[267] reviewed ACAM2000 reports in VAERS for March 2008

through June 2013. During this period, when more than 830,000 vaccinations were administered to DoD personnel and an additional 2,430 were administered to civilians (such as research personnel), 169 reports of serious adverse events were submitted to VAERS, including 138 for which a diagnosis was verified. Of these reports, cardiac diagnoses were the most frequent, representing 54.4% of the reports; among cardiac events, myopericarditis (40.2% of cardiac reports), myocarditis (35.9%), and pericarditis (14.1%)

Figure 27-9. Eczema vaccinatum. This 28-year-old woman with eczema vaccinatum contracted it from her vaccinated child. She had a history of atopic dermatitis, which was inactive when her child was vaccinated. As a therapy, she was given vaccinia immune globulin, idoxuridine eye drops, and methisazone, which resulted in healed lesions, no scarring, and no lasting ocular damage. Image 4621.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Allen W Mathies, MD, California Emergency Preparedness Office, Immunization Branch.

Figure 27-10. Progressive vaccinia. This patient presented with progressive vaccinia after receiving a smallpox vaccination. Progressive vaccinia, though rare, is one of the most severe complications of smallpox vaccination and is almost always life threatening. Image 4592.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Centers for Disease Control and Prevention, California Department of Health Services.

were the most common diagnoses.[267] Although no clear association with vaccination has been found, a history of ischemic heart disease and the presence of significant cardiac risk pose relative contraindications for smallpox vaccination.[265–268] Consequently, individuals with a history of myocarditis, pericarditis, or ischemic heart disease should not be vaccinated.

Vaccine research. Because of the contraindications and adverse events associated with first- and second-generation smallpox vaccines, including ACAM2000, development of third- and fourth-generation smallpox vaccines is ongoing.

The highly attenuated modified vaccinia virus Ankara (MVA), a third-generation smallpox vaccine, was produced by 572 serial passages in chicken embryo fibroblasts, which rendered the virus unable to replicate in most mammalian cells. MVA, which was used toward the end of the worldwide smallpox eradication campaign, is immunogenic and safe for use even in immunocompromised individuals. MVA has been safely given to approximately 150,000 persons since it was first developed in the 1970s.[242,269,270] The safety and immunogenicity of recently developed versions of MVA, such as Imvamune (Bavarian Nordic, Martinsried, Germany), which is stored in the Strategic National Stockpile (SNS), are currently being assessed in clinical trials.[271,272] In completed clinical trials, Imvamune has been safe,

well tolerated, and immunogenic, producing immune responses comparable to those elicited by Dryvax[273,274]; a trial comparing Imvamune to ACAM2000 is underway. Imvamune has received marketing authorization from the European Commission and Health Canada for immunization against smallpox in adults, including healthy individuals as well as those with immune deficiencies and skin disorders, such as atopic dermatitis and HIV infection.[275] The CDC has submitted a pre-EUA request to the FDA for potential use of Imvamune during a public health emergency; if granted, it would allow Imvamune to be administered to HIV-infected individuals and those with atopic dermatitis.[275]

Aventis Pasteur smallpox vaccine (APSV, or Wet-Vax) is a live, replication-competent liquid calf lymph-derived vaccinia virus vaccine that results in strong humoral and cellular immune responses[276]; APSV is also stored in the SNS. The CDC also has submitted a pre-EUA for the use of diluted APSV during a public emergency to increase the supply of smallpox vaccine.[275]

Fourth-generation vaccine candidates include subunit and DNA vaccines composed of vaccinia virus membrane and/or virion proteins or variola homologs.[239,270]

Figure 27-11. Progressive vaccinia after debridement. Image 4594.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Centers for Disease Control and Prevention, California Department of Health Services.

Passive Immunotherapy

VIG, which is administered intravenously, is used primarily for complications from the smallpox vaccine (Table 27-5); it does not currently play a role in smallpox prevention.[277] In particular, VIG may be recommended in severe cases of ocular vaccinia; however, it is contraindicated in individuals with vaccinial keratitis because of the risk of corneal clouding. Corneal clouding was observed in 4 of 22 persons with vaccinial keratitis who received VIG.[278] A subsequent study in rabbits showed that treatment of vaccinial keratitis with VIG was associated with both corneal scarring and persistent and larger satellite lesions compared with control animals.[279] VIG should not be withheld from a patient with keratitis if a comorbid condition exists that requires VIG administration and if the risk
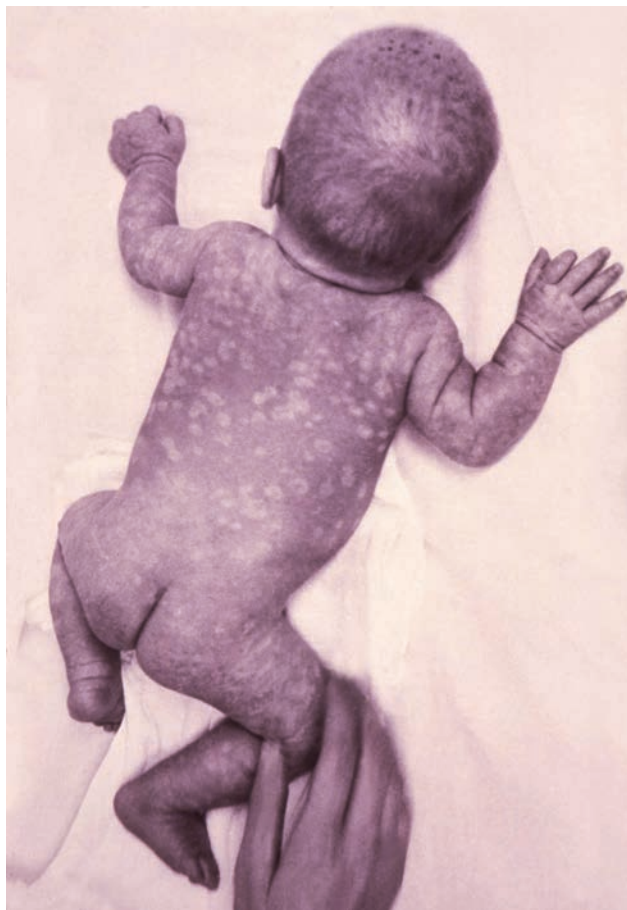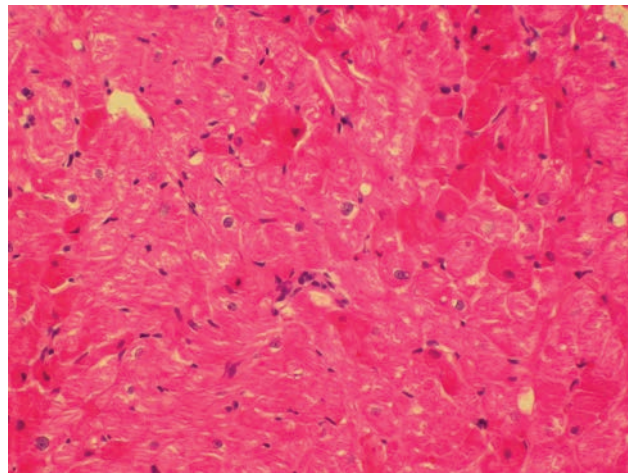
Figure 27-13. Histopathology of vaccine-related myocarditis showing a nonspecific lymphocytic infiltrate. Reproduced with permission of Department of Pathology, Brooke Army Medical Center, Texas.

of the comorbid condition is greater than that of VIG-associated complications of keratitis.[258]

Treatment of generalized vaccinia with VIG is restricted to those who are systemically ill or have an immunocompromising condition.[258]

Individuals with eczema vaccinatum are generally systemically ill and require immediate therapy with VIG, which is the only currently approved treatment for this condition.[258] The mortality rate of individuals with eczema vaccinatum was 7% (9/132), even with VIG therapy. A measurable antibody response developed in 55 of 56 survivors who had antibody titers obtained after VIG administration. No antibody response was detected in five persons with fatal eczema vaccinatum cases who had post-VIG antibody titers measured.[280]

Progressive vaccinia carries a high mortality rate and should be aggressively treated with VIG, debridement, intensive monitoring, and tertiary medical center–level support.[258] However, anecdotal experience has shown that, despite treatment with VIG, individuals with cell-mediated immunity defects have a poorer prognosis than those with humoral defects.

Prophylactic VIG to prevent fetal vaccinia could be considered for a woman who discovers she is pregnant shortly after receiving the smallpox vaccine; however, data on the efficacy of this approach are not available.[281] VIG could be considered for an infant born with lesions, but again, data regarding efficacy or the appropriate dosage are not available.[258]

Limited historical data are available on the effect of VIG in conjunction with the smallpox vaccine for postexposure prevention of smallpox in contact

Figure 27-12. Fetal vaccinia. This child contracted vaccinia virus while in utero. Image 3338.
Reproduced from: Centers for Disease Control and Prevention Public Health Image Library website, http://phil.CDC.gov. Accessed September 16, 2014. Photograph: Courtesy of Arthur E Kaye, Centers for Disease Control and Prevention.

cases.[282–284] A 1961 study by Kempe et al[282] 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 with 1.5% of contacts (5/326) who received both the smallpox vaccine and VIG therapy. Research published a year later by Marennikova[283] studied the effect of antivaccinia gamma globulin given to 13 of 42 persons who had been in close contact with smallpox patients. 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. Of the 29 persons not given antivaccinia gamma globulin, 13 developed smallpox.[283] However, no clinical trials have provided evidence that giving VIG in conjunction with the smallpox vaccine as prophylaxis has a greater survival benefit than vaccination alone.[285–287]

Monoclonal antibodies represent another approach to passive immunotherapy. Postexposure administration of human monoclonal antibodies has, for example, protected rabbits from a lethal dose of an orthopoxvirus.[288]

Antiviral Agents

Antiviral agents have been used successfully, sometimes in combination with VIG, in the treatment of complications of smallpox vaccination. Animal studies suggest that some of these antivirals would also be helpful in treating smallpox infection.

Cidofovir has broad-spectrum activity against DNA viruses, including the herpes viruses, papillomavirus, adenovirus, and poxviruses.[289–291] Cidofovir provides a pronounced, long-lasting inhibition of viral DNA synthesis, allowing for infrequent (weekly or bimonthly) dosing.[292] Cidofovir has been approved by the FDA for treating cytomegalovirus retinitis in patients with AIDS. Treatment of vaccinia complications or smallpox with cidofovir would be an off-label use of the drug. However, both the DoD and the CDC currently have IND protocols for the use of cidofovir in these two conditions.

Studies of cidofovir have demonstrated improved or prolonged survival in BALB/c mice and in mice with severe combined immunodeficiency infected with vaccinia virus, as well as cowpox-infected mouse models, when treatment was initiated as long as 5 days before or up to 96 hours after infection.[293] The greatest benefit of cidofovir prophylaxis was observed when it was administered within 24 hours before or after exposure.[294–296] NHP studies have demonstrated improved survival in monkeypox and smallpox models.[297]

In humans, cidofovir has been effective in the treatment of the poxvirus infection molluscum contagiosum in patients with AIDS. Dose-related nephrotoxicity has been associated with cidofovir therapy in humans; however, this may be minimized by concomitant intravenous hydration with saline and oral probenecid (generally administered as a 2-g dose 3 hours before the cidofovir infusion, and again at 2 and 8 hours after infusion).[298–300] An investigational drug, brincidofovir (or hexadecyloxypropyl-cidofovir, previously referred to as CMX-001; Chimerix Inc, Durham, NC), is an oral formulation of cidofovir that has shown no evidence of a link to nephrotoxicity.[299] The US Government recently announced plans to add brincidofovir to the SNS for the treatment of patients with smallpox.

Tecovirimat, previously known as ST-246, is a potent and specific inhibitor of orthopoxvirus replication under development by SIGA Technologies (Corvallis, OR). The drug is active against multiple species of orthopoxviruses, including variola virus and cidofovir-resistant cowpox variants. In animal models, this oral drug has been effective in preventing death from infection with variola virus and other orthopoxviruses; it also reduced shedding of vaccinia virus after smallpox vaccination.[301,302] A recent study found that tecovirimat resulted in survival of 100% of cynomolgus macaques challenged with intravenous variola virus, whether the antiviral was administered beginning on the 2nd or 4th day after infection (only 50% of placebo-treated macaques survived). Disease in tecovirimat-treated macaques was milder, and oropharyngeal viral shedding was reduced, compared with placebo-treated survivors.[303] Clinical trials have demonstrated that tecovirimat is safe and well tolerated.[304] For treatment of orthopoxvirus infections, tecovirimat and brincidofovir may be more effective if used in combination. Furthermore, based on experience with the use of tecovirimat to treat progressive vaccinia,[305] topical tecovirimat may need to be administered instead of—or in addition to—oral tecovirimat.[304] The US Government recently added tecovirimat to the SNS for the treatment of patients with smallpox.

For the treatment of eczema vaccinatum, cidofovir can be used off-label or under an IND protocol, and the investigational antiviral drugs, tecovirimat and brincidofovir, are available under an emergency IND application.[305,306]

One animal study showed that both topical and intravenous cidofovir were effective in treating vaccinia necrosis in mice deficient in cell-mediated immunity. Topical cidofovir was more effective than intravenous cidofovir, and the administration of both

cidofovir preparations was superior to either preparation alone.[307] Again, tecovirimat and brincidofovir are available under an emergency IND application.[305,306] Because of the potential for renal toxicity with cidofovir, brincidofovir may be a better choice to treat progressive vaccinia, especially in immunocompromised individuals. It may be necessary to administer tecovirimat (particularly the topical preparation to improve absorption in individuals unable to eat) in addition to brincidofovir and repeated doses of VIG, as described by Lederman et al.[305]

The animal and human data suggest that cidofovir may be effective in therapy and in short-term prophylaxis of smallpox if initiated within 4 days after exposure. One dose of intravenous cidofovir may provide protection for 7 days.[292]

Topical treatment with trifluridine (viroptic; Catalytica Pharmaceuticals, Greenville, NC) is often recommended for ocular vaccinia resulting from inadvertent inoculation, although the FDA has not specifically approved this use of trifluridine.[258,308]

Postevent Countermeasures: Current Options

A suspected or confirmed case of human smallpox would be considered an international emergency and should be immediately reported to local and state public health authorities and the CDC. Individuals who have been exposed to smallpox patients or to animals infected with variola virus and laboratory workers with an aerosol or percutaneous exposure to variola virus must be quarantined and monitored (for fever, rash, or flu-like symptoms) for at least 17 days after the last contact with the index case or exposure, regardless of whether they have been vaccinated.[14,66]

Treatment. The FDA has not approved any antiviral agent to treat smallpox. However, tecovirimat and brincidofovir could be made available under an IND protocol to treat patients with smallpox. Tecovirimat is also available under a DoD IND and expanded access protocol for the treatment of smallpox, complications resulting from smallpox vaccination, or other orthopoxvirus infections. Intravenous cidofovir also is available to treat smallpox under an IND protocol. Clinical guidelines from the CDC for the use of antiviral medications in the event of a smallpox release or outbreak are under development.

Postexposure Prophylaxis. The CDC recently provided detailed clinical guidance on postevent use of smallpox vaccines for individuals who have been exposed to smallpox virus and those who are at high risk for smallpox infection but have not had a known exposure.[275] A brief summary of these recommendations follows.

For children and adults (including pregnant women) without severe immunodeficiency or relative contraindications who have been exposed to smallpox virus or are at high risk for infection, the CDC recommends the administration of ACAM2000. The CDC guidelines describe in detail the recommended procedure for vaccinating, in a postevent context, persons with a severe allergy to ACAM2000 or a component of that vaccine who have been exposed to the virus or are at high risk for infection. Briefly, such individuals should be vaccinated with ACAM2000, ASPV, or another vaccine, depending on the situation and the vaccines available; vaccination of such individuals may need to occur in a facility capable of treating an anaphylactic reaction. Individuals with atopic dermatitis who have been exposed to smallpox virus should be vaccinated with ACAM2000; those with atopic dermatitis who are at high risk of infection but without a known exposure should receive Imvamune instead unless they have previously received ACAM2000 without complications.

Some immunocompromised individuals—such as recipients of a solid organ transplant within the previous 3 months—are expected to benefit from vaccination with ACAM2000 (if exposed to smallpox virus) or Imvamune (if at high risk for smallpox infection without a known exposure). However, severely immunodeficient persons—such as those with HIV infection whose CD4 cell count is less than 50 cells/mm^3 or those who recently received a bone marrow transplant—are, in general, not expected to benefit from vaccination with any of the smallpox vaccines currently available. Vaccination of severely immunocompromised individuals with Imvamune may be considered if antiviral agents are not available.[275]

Viral Hemorrhagic Fevers

Viral hemorrhagic fevers (VHFs)—severe illnesses characterized by fever, vascular dysregulation, and vascular damage—are caused by a subset of the lipid-enveloped RNA viruses belonging to four families: (1) *Arenaviridae*, (2) *Bunyaviridae*, (3) *Filoviridae*, and (4) *Flaviviridae*. Transmission to humans occurs in a variety of ways, such as via infected aerosols of rodent excreta, contact with infected blood or body fluids, or through the bites of infected arthropods. Not all patients infected with these viruses develop VHF.[14,165,309,310] Because of their ability to cause widespread, severe illness and death and the potential for either aerosol dissemination and infectivity or person-to-person transmission, the viral agents of VHFs are considered potential agents of biological warfare.

Vaccination

The only vaccine for any VHF that is licensed in the United States is the live attenuated yellow fever vaccine, YF-Vax (Sanofi Pasteur Biologics, Cambridge, MA), derived from the 17D yellow fever virus strain (see Table 27-1). This vaccine has substantially diminished the burden of yellow fever infection worldwide and is well tolerated, although it is contraindicated in infants and immunosuppressed patients and is used with caution in elderly people.[311] Because of yellow fever's short (3- to 6-day) incubation period, postexposure use of this vaccine is unlikely to be effective.[309]

A number of human vaccines developed and licensed in other countries may have efficacy against VHFs. In particular, a live attenuated Argentine hemorrhagic fever (AHF) vaccine, known as Candid #1, demonstrated efficacy against Junin virus in a field study among 6,500 agricultural workers in Argentina who were randomly assigned to receive either placebo or Candid #1. Of the 23 patients who developed AHF, 22 had received placebo compared to only 1 patient who had received the vaccine.[312] Candid #1, which is the first vaccine used to control an arenaviral hemorrhagic fever, is the first live viral vaccine to be manufactured and registered in Argentina.[313]

Hantavax, a suckling mouse brain-derived hantavirus vaccine (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[314]; however, the humoral immune response, when measured by PRNT80 antibodies, was protective in only 33.3% of vaccine recipients.[315] Clinical trials and animal studies of other hantavirus vaccine candidates, such as DNA vaccines[316,317] and vaccinia-vectored constructs,[318] have suggested other potential vaccine options.

In 1974, an inactivated Crimean–Congo hemorrhagic fever virus (CCHFV) vaccine developed by Soviet scientists was licensed in Bulgaria and is used in CCHFV-endemic areas of the country for military personnel and medical and agricultural workers. Although data are lacking on the total number of vaccinated civilians who have contracted CCHF, no vaccinated military personnel have contracted the disease since 1997, and none of the vaccinated laboratory personnel working with CCHFV have become infected, even after accidental exposure via needle prick.[319] However, a recent study found that, although CCHFV-vaccinated individuals developed high CCHFV-specific antibodies after a single dose, neutralizing activity against CCHFV was low even after repeated doses.[320]

A formalin-inactivated RVFV vaccine (TSI-GSD-200), currently under IND status, is used in the SIP at USAMRIID for laboratory workers who may be exposed to the virus (see Table 27-1).[321,322] However, no human RVFV vaccines are commercially available. The primary focus of RVFV vaccine research is the vaccination of livestock to prevent abortions and deaths in these species and spillover into humans during epizootic outbreaks.[323,324] A live attenuated vaccine for RVFV, the Smithburn strain, is the only vaccine approved for use in livestock.

Phase 1 and 2 studies have been conducted at USAMRIID on a live attenuated RVF MP12 vaccine, which was found to be safe and immunogenic in human volunteers.[325,326] Because inactivated vaccines are expensive (requiring multiple boosts) and live vaccines have side effects in animals (abortions and teratogenicity), other vaccine types are being explored for use in endemic areas. RVFV vaccines based on virus-like particles[327] or recombinant viral vectors[328] and DNA vaccines[329] have recently demonstrated potential.

A formalin-inactivated Kyasanur Forest disease virus vaccine, licensed in India since 1990, was 62.4% effective (with a 95% confidence interval of 26.1%–80.8%) and 82.9% effective (with a 95% confidence interval of 71.3%–89.8%) among those who received two doses and those who received an additional booster dose, respectively, compared with unvaccinated individuals.[330]

A neutralizing antibody study in humans and a viral challenge study in African green monkeys and crab-eating macaques demonstrated at least partial protection against two flaviviruses—Omsk hemorrhagic fever virus and tickborne encephalitis virus (TBEV)—using FSME-IMMUN, an inactivated TBEV vaccine licensed for use in Canada and Europe.[331,332]

Substantial research has focused on the development of an effective vaccine for protection against the five known antigenically distinct ebolaviruses:

1. Zaire ebolavirus (ZEBOV);
2. Sudan ebolavirus (SEBOV);
3. Taï Forest ebolavirus (also called Côte d'Ivoire ebolavirus);
4. Reston ebolavirus; and
5. Bundibugyo ebolavirus (BEBOV).[333,334]

Among the more promising approaches, several investigators have used vaccines based on viral vectors, such as recombinant vesicular stomatitis virus (VSV), that express the transmembrane glycoproteins of one or more ebolaviruses. For example, a single immunization with a replication-competent VSV vector expressing the ZEBOV glycoprotein, a vaccine candidate referred to as rVSV-ZEBOV, protected cynomolgus macaques from

lethal challenge with ZEBOV.[335,336] A single injection of a recombinant VSV vaccine expressing glycoprotein from BEBOV provided 100% protection against BEBOV challenge in macaques, as did a short prime–boost regimen using recombinant VSV–based ZEBOV and SEBOV vaccines.[337] A single immunization with a bivalent recombinant complex adenovirus vaccine (CAdVax), which expresses glycoproteins from both SEBOV and ZEBOV, protected macaques against ZEBOV challenge; with the addition of a boosting vaccination, CAdVax also provided protection against SEBOV challenge.[338] Two injections of a recombinant human parainfluenza virus type 3 vaccine vector encoding ZEBOV glycoprotein protected rhesus macaques challenged with ZEBOV.[339] Two intramuscular injections with a replicon vaccine based on SEBOV glycoprotein–expressing VEEV completely protected cynomolgus macaques challenged with aerosolized SEBOV.[340] In another approach, macaques challenged with ZEBOV were protected by three immunizations with virus-like particles containing ebolavirus glycoprotein, VP40, and nucleoprotein.[341]

Since the 2014 Ebola virus disease (EVD) outbreak in West Africa, several candidate Ebola vaccines have moved rapidly forward in development. One candidate, a recombinant chimpanzee adenovirus type 3–vectored ebolavirus Zaire vaccine (cAd3-EBOZ), recently underwent a phase 1 clinical trial in healthy adults. In this trial, a single intramuscular injection of cAd3-EBOZ was safe (2 of 10 subjects developed transient fever 1 day after vaccination) and resulted in antibody responses in the range reported to be protective in an NHP challenge model.[342] A phase 1 trial has also found rVSV-ZEBOV to be safe and immunogenic in healthy adults.[343] A phase 2/3 trial of cAd3-EBOZ and rVSV-ZEBOV is ongoing in Liberia.

Vaccines may also prove useful as PEP against some VHF-causing pathogens. In one case report,[344] a physician who experienced an accidental needlestick while working in an Ebola treatment facility in Sierra Leone during the 2014 Ebola outbreak was vaccinated 43 hours postexposure with VSV-ZEBOV through an emergency IND. Strong innate and Ebola-specific adaptive immune responses were detected after vaccination, and the patient survived.

Antiviral Agents

Antiviral medications prescribed to treat VHFs are important primarily after patients have developed symptoms because data are—in general—insufficient to support their use as PEP.

Ribavirin. The antiviral medication with the most evidence of efficacy is ribavirin, a nonimmunosuppressive nucleoside analogue with activity against a number of viruses, including at least some arenaviruses and bunyaviruses, but not filoviruses or flaviviruses.[309,345] Ribavirin inhibits the conversion of inosine 5'-phosphate (IMP) to xanthosine 5'-phosphate, disrupting the synthesis of guanosine monophosphate, a vital nucleotide needed to form viral nucleic acid.[346] However, because ribavirin does not efficiently cross the blood–brain barrier, it may not protect against neurologic effects of VHFs.[309,347] Another caveat to the use of ribavirin is its association with serious side effects, including hemolytic anemia, hypocalcemia, hypomagnesemia,[348] and genotoxicity.[349] Ribavirin has demonstrated teratogenicity and embryotoxicity in animal studies; for this reason, it is generally contraindicated during pregnancy.[309,350]

Ribavirin appears to be effective in the treatment of Lassa fever if it is begun early in the course of the illness. Among patients with Lassa fever who were treated within the first 6 days after the onset of fever, intravenous ribavirin was more effective than passive immunotherapy in reducing mortality: the CFR was reduced from 55% among patients treated with passive immunotherapy to 5% among those treated with ribavirin.[351] Results from NHP studies support this finding.[352,353] Ribavirin is less beneficial when administered starting after day 7 of illness.[345] Data are extremely limited regarding the efficacy of ribavirin PEP for Lassa fever in humans.[309,354]

Ribavirin's efficacy in treating AHF and other arenaviruses is less clear. In macaques inoculated with Junin virus on day 0, ribavirin treatment begun on day 6 (after viremia and clinical signs of illness were detected) provided minimal protection. Of the four animals, one died early in the course of illness; although initial improvement was observed in the three remaining animals, all three subsequently developed a CNS infection that was fatal in two animals.[347] However, Enria and colleagues found a survival benefit among humans with AHF who were treated with ribavirin.[355,356] An anecdotal report described recovery from Bolivian hemorrhagic fever, which is caused by Machupo virus, in two patients treated with ribavirin.[357]

In macaques, ribavirin appears to provide a benefit when used as PEP against AHF. Among macaques inoculated with Junin virus on day 0, four animals treated with ribavirin beginning on day 0 survived, whereas four that received placebo died during the 4th week after infection.[347]

In a double-blind, placebo-controlled trial, ribavirin effectively reduced mortality and viremia in patients with hemorrhagic fever with renal syndrome (HFRS).[358,359] However, a meta-analysis of the use of ribavirin in the treatment of HFRS and hantavirus pulmonary syndrome found mixed results.[360]

Ribavirin also has demonstrated in vitro activity against CCHFV.[348,361] The results of human studies assessing the efficacy of ribavirin in the treatment of CCHF are highly variable.[362–365] However, as Ergonul and colleagues argue, this variability may be due—at least in part—to variability in the delay between symptom onset and the start of treatment.[366,367] In one study, for example, patients admitted to a hospital and started on ribavirin within 2 days of symptom onset were less likely than others to become more severe cases.[367]

As PEP, evidence of ribavirin's efficacy in humans is limited. In one case study,[368] six healthcare workers who were exposed to CCHFV (via needlestick or contact with skin and mucosal surfaces) received ribavirin beginning within 1 hour of exposure; none of these individuals developed symptoms. One healthcare worker who was exposed to CCHFV (probably via aerosolization of contaminated blood or secretions) and did not receive ribavirin later developed CCHF (she recovered fully after treatment with ribavirin).[368]

Favipiravir. A viral RNA polymerase inhibitor, favipiravir (also known as T-705 and marketed as Avigan; Toyama Chemical Co, Shinjuku-ku, Tokyo) was initially developed as an antiinfluenza drug. It has been approved in Japan for specifically defined cases of influenza. However, it may also be effective against several other virus families, including arenaviruses, bunyaviruses, and flaviviruses.

During and after the 2014 EVD outbreak in West Africa, interest in favipiravir increased dramatically. A phase 2 clinical trial is underway in Guinea, aiming to assess the efficacy of favipiravir in reducing mortality in individuals with EVD. Preliminary results posted by the group Médecins sans Frontièrs suggest that 10 days of favipiravir may be beneficial among patients (children older than the age of 1 year and nonpregnant adults) with high or moderate levels of viral replication who have not yet developed severe visceral lesions. However, the drug appears not to be efficacious among those with a very high level of viral replication along with serious visceral involvement.[369] Based on the apparently greater benefit of favipiravir for patients with moderate to high viremia versus very high viremia, Van Herp et al[370] argue for the use of favipiravir as PEP for contacts of patients with EVD.

In animal models, favipiravir has also shown promise to treat AHF and CCHF. Favipiravir resulted in 78% survival of guinea pigs infected with Junin virus when administered intraperitoneally for 2 weeks beginning 2 days after challenge; by comparison, only 11% of placebo-treated animals survived. Among ribavirin-treated guinea pigs, survival ranged from 33% to 40%. Oral administration of favipiravir was less protective than was the intraperitoneal route, with 20% of orally

treated guinea pigs surviving; however, animals that succumbed survived longer than placebo-treated animals.[371] In a small animal model of CCHF, mice treated with favipiravir, initiated up to 2 days after infection with CCHFV, survived with no signs of disease and no virus detectable in blood or organs.[372,373]

BCX4430. The synthetic adenosine analogue BCX4430 has broad-spectrum antiviral activity against many viruses, including bunyaviruses, arenaviruses, flaviviruses, and filoviruses. In particular, Warren et al[373] recently found that BCX4430, administered as late as 48 hours after infection, completely protected macaques from disease caused by Marburg virus. This product also conferred significant protection in guinea pigs challenged with Marburg virus and in mice challenged with Ebola virus. A phase 1 clinical trial to assess the safety, tolerability, and pharmacokinetics of BCX4430 in healthy adults was recently completed.

Interferons. Stimulating the immune system is another potential therapeutic modality, but no human studies using this technique have been conducted for any of the VHF viruses. IFN combinations may be useful in such an approach, particularly with VHF infections in which the immune response is impaired. However, IFN compounds may be deleterious in some VHF infections, such as AHF, in which high IFN levels are associated with worse outcomes.[310,374] IFNs have demonstrated a benefit in bunyavirus murine models.[375] In NHPs inoculated with Ebola virus, early postexposure treatment with either IFN α-2b[376] or IFN-β[377] prolonged survival but did not prevent death. Similar findings were obtained in Marburg virus–inoculated macaques that received early postexposure treatment with IFN-β.[377]

Other Drugs. Although in vitro data suggest that the Mx family of proteins has antiviral activity against a wide variety of RNA viruses, further study is needed.[378,379] Recently, FDA-approved IND applications and phase 1 clinical trials have been initiated for two small-molecule therapeutics: (1) anti-sense phosphorodiamidate morpholino oligomers (AVI-6002, AVI-6003) and (2) lipid nanoparticle/small interfering RNA (TKM-Ebola). However, the need for multiple doses to achieve therapeutic efficacy makes these compounds less than ideal with regard to patient compliance and outbreak scenarios.[380]

Pathogenesis studies with Ebola virus have implicated tissue factor-induced disseminated intravascular coagulation as a critical component of fatal outcomes.[381] In a rhesus macaque model of Ebola virus infection, treatment with a factor VIIa/tissue factor inhibitor (recombinant nematode anticoagulation protein c2 or rNAPc2) led to a survival advantage[382]; however, rNAPc2 was not effective against Marburg virus.[383]

This compound has not been tested in humans for treating EVD, and tissue factor inhibitors have not been effective in the treatment of septic shock.[384]

IMP dehydrogenase inhibitors (similar to ribavirin) have been tested in both in vitro and animal models against arenaviruses; however, because of their toxicity, such compounds have been used only experimentally for cancer patients in crisis.[385,386] Other compounds that have demonstrated in vitro activity against arenaviruses include 3′-fluoro-3′-deoxyadenosine,[387] phenothiazines,[388] and myristic acid compounds.[389,390] When challenged with Lassa virus, guinea pigs treated with ST-193, a small-molecule inhibitor of arenavirus entry into cells, had an overall survival rate of 62.5% compared with 0% in the ribavirin-treated and vehicle groups.[391]

Although using steroids to treat VHFs has not been recommended,[309] evidence suggests that corticosteroids may be effective among severely ill patients with CCHF. In a recent study, among 16 severely ill patients with CCHF who received corticosteroid therapy in addition to ribavirin, 8 died (a CFR of 50%), whereas among 8 severely ill patients who did not receive additional corticosteroid therapy, all 8 died (a CFR of 100%; *P* = 0.014). Among moderately ill patients, corticosteroid was not associated with a reduced CFR.[392] Several antivirals have been tested in a bunyavirus (Punta Toro virus) murine model,[375] suggesting possible compounds for further testing.

Passive Immunotherapy

Studies on the benefits of passive immunotherapy for treating VHFs have yielded mixed results.[309] Serum collected from donors after infection with Junin virus has been used successfully to treat AHF.[355,393] In a cynomolgus macaque model of Lassa virus infection, treatment with serum from immune monkeys led to a survival advantage; the benefit was greater when this passive immunotherapy was combined with ribavirin.[353] In humans, however, serum from convalescent patients used to treat Lassa fever did not reduce mortality in patients with a high risk of a fatal outcome.[351] Human-derived antibodies against Bolivian hemorrhagic fever dosed in rhesus macaques to achieve neutralizing antibody titers of 1:4 to 1:8 protected monkeys against severe clinical manifestation of illness after Machupo virus challenge.[394]

Anecdotal evidence suggests that immunoglobulins and/or transfusions from convalescent patients may improve outcomes in human EVD.[395,396] Postexposure treatment with concentrated polyclonal IgG antibodies collected from vaccinated macaques that survived an Ebola or Marburg challenge was completely protective in macaques challenged with Ebola or Marburg virus[397]; in contrast, an earlier study using equine IgG did not produce a mortality benefit in NHPs.[376] ZMapp, a product composed of three humanized monoclonal antibodies produced in the plant *Nicotiana benthamiana* completely protected macaques when treatment was initiated up to 5 days after Ebola virus challenge.[398,399] ZMapp is now undergoing a clinical trial to assess its safety and efficacy in the treatment of EVD.

Substantial supportive data are lacking for the use of immunoglobulin from survivors for treating CCHF,[319] but 15 high-risk patients (viral load of at least 108 copies/mL) treated with convalescent hyperimmunoglobulin had a survival rate of 86.6%,[400] and an earlier small case series found 100% survival among treated patients.[401] Monoclonal antibodies against HFRS viruses have been effective in murine models,[402] and such treatment appears to be well tolerated in healthy human volunteers.[403]

Yellow fever virus immunotherapy data from human studies are lacking; however, specific monoclonal antibody therapy with MAb 2C9-cIgG resulted in substantial improvement in survival among hamsters infected with yellow fever virus.[404]

As with passive immunotherapy for treating other diseases, concerns about the transmission of bloodborne pathogens, such as hepatitis C,[405] may limit treatment with donated serum or may—at a minimum—necessitate a rigorous screening process. In addition, the impracticality of obtaining large quantities of donated serum from previously infected individuals with no such population available (particularly in the United States) limits the utility of this treatment. Revolutionary advances in plant virus-based transient expression to manufacture large quantities of monoclonal antibodies may facilitate passive treatment with antibodies to counteract the effects of VHFs.[405]

Other Countermeasures

Good infection control practices, particularly the isolation of patients and barrier precautions, are a crucial countermeasure in efforts to limit the impact of VHF viruses used as biological weapons. The specific infection control needed for each virus is discussed elsewhere in this volume. Management measures also must overcome the fear and panic associated with use of a VHF virus whose potential lethality tends to be exaggerated in popular culture, such as Ebola.[406] Modern intensive care unit support with careful fluid management will probably improve the outcome for patients infected with VHF viruses,[407] but access to this care may be limited in a mass casualty scenario.

Postevent Countermeasures: Current Options

Treatment. Supportive care is the primary form of treatment of individuals with VHFs. For adults (including pregnant women) and children with a VHF of unknown etiology, the Working Group on Civilian Biodefense[309] recommends treatment with ribavirin and supportive care, beginning as soon as possible after symptom onset. In the case of a VHF, the potential teratogenic and embryotoxic effects of ribavirin are thought to be outweighed by the benefits of treatment. If the VHF is found to be caused by an arenavirus or bunyavirus, then the ribavirin should be continued such that the patient is treated for 10 days. If the infection is caused by a filovirus or flavivirus, ribavirin should be discontinued. In a contained casualty situation, ribavirin should be administered intravenously (under an IND protocol); in a mass casualty situation, it should be administered orally (an off-label use).[309] The DoD maintains expanded access protocols for the IND use of intravenous ribavirin to treat Lassa fever, CCHF, and HFRS caused by Hantaan, Seoul, Puumala, and Dobrava viruses. Patients with AHF or Bolivian hemorrhagic fever may benefit from convalescent plasma, which is used as an investigational therapy.[14]

Postexposure Prophylaxis. In the context of a bioterrorism event, the Working Group on Civilian Biodefense[309] recommends careful observation of exposed patients for 21 days, with antiviral treatment begun only if fever or other signs and symptoms of infection appear. Persons with a high-risk exposure to a VHF-causing virus and close contacts of patients with a VHF (other than RVF or a flavivirus-caused VHF, which are not transmitted person to person) should be instructed to record their temperature twice daily and report any symptom of a VHF, including a temperature of 101°F or higher. The appearance of symptoms should prompt the initiation of treatment as described previously.[309]

For asymptomatic laboratory workers or healthcare workers, a high-risk exposure (eg, via needlestick) to Lassa virus, CCHFV, or a hantavirus could warrant PEP with ribavirin[408–410]; although this recommendation is not a product of a consensus process.

TOXINS

Botulinum Neurotoxin

Clostridium botulinum is an anaerobic, gram-positive, spore-forming bacillus that produces a potent toxin, botulinum neurotoxin (BoNT). The most poisonous substance known, BoNT is found in soil and water worldwide and is commercially available for cosmetic and medical uses.[165,411] By blocking the release of acetylcholine, a neurotransmitter that causes muscle contraction, BoNT may result in muscle weakness, flaccid paralysis, and subsequent respiratory impairment. Eight immunologically distinct toxin serotypes (A through H) are produced by discrete strains of the organism. Although botulism is generally acquired from ingestion of food contaminated with BoNT, it may also occur from toxin production by *C botulinum* if present in the intestine or wounds. Botulism is not acquired naturally by aerosolization; this route of acquisition would suggest a possible bioterrorism event but may also occur from exposure to aerosolized toxin in a research laboratory.[1]

Vaccination

Pentavalent Botulinum Toxoid. No FDA-licensed vaccines are available for preexposure vaccination against botulism. An investigational product, pentavalent botulinum toxoid (PBT), was used from 1959 through 2011 for persons at risk for exposure to BoNT serotypes A through E. The PBT was available as an IND through the CDC (IND-161, for at-risk laboratory workers) until it was discontinued based on data indicating a decline in immunogenicity of some of the toxin serotypes.[412] The PBT has also been available through the US Army Office of the Surgeon General (IND-3723, for at-risk military personnel). Although IND-3723 remains active, the PBT is now effective only against toxin serotype A. Thus, the PBT could still be used to vaccinate military personnel against toxin serotype A.[413] Derived from formalin-inactivated, partially purified toxin serotypes A, B, C, D, and E, the PBT was developed by the DoD and manufactured first by Parke Davis and later (beginning in the early 1970s) by the Michigan Department of Public Health. 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.[413–415]

Vaccine Research. Vaccine candidates include formalin-inactivated toxoids (A through F), which are made in nearly the same way as formalin-inactivated PBT, and recombinant BoNT vaccines.[416,417] The production of formalin-inactivated toxoids is expensive and relatively time consuming because it (*a*) requires partially purified culture supernatants to be treated exhaustively with formaldehyde and (*b*) must be

performed by a highly trained staff within a dedicated high-containment laboratory space. Furthermore, the resulting toxoid is relatively impure, containing only 10% neurotoxoid (the remainder is irrelevant material).

However, the use of pure and concentrated antigen in recombinant vaccines offers advantages—increased immunogenicity and decreased reactogenicity—over formalin-inactivated toxoids.[414] Recombinant techniques use a fragment of the toxin that is immunogenic but is not capable of blocking cholinergic neurotransmission. 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 (Hc) of the toxin.[414,418] Phase 1 trials on the bivalent recombinant vaccine (for protection against toxin serotypes A and B) have been completed, with promising preliminary serologic results at 12 months after two doses of vaccine (administered at 0 and 6 weeks).[419] DynPort Vaccine Company LLC (Frederick, MD) sponsored a phase 2 randomized, double-blind, placebo-controlled, multicenter study to evaluate the safety, dosing schedule, and antibody kinetics of recombinant botulinum vaccine A/B (rBV A/B-40) in healthy adults. It was completed in December 2010, but results have not been published. A phase 3 randomized study to assess the safety, lot consistency, and clinical benefit of rBV A/B is planned. Recombinant vaccines given by aerosol[420,421] and by the mucosal route[422] are also being investigated.

Because the BoNT Hc has been produced as a stable recombinant protein and is an excellent immunogen, it has been assessed in diverse viral delivery platforms.[413] In particular, BoNT Hc has been virally vectored using attenuated human adenovirus,[423,424] inactivated rabies virus virions,[425] and Semliki Forest virus (SFV) DNA replicon[426,427] or recombinant SFV viral replicon particles,[428] conferring substantial protection against lethal challenge in murine models.

Passive Immunotherapy

In March 2013, the FDA approved BAT (Botulism Antitoxin Heptavalent [A, B, C, D, E, F, G] – Equine) to treat individuals with symptoms of botulism following a known or suspected exposure. BAT was developed at USAMRIID, as one of two equine-derived heptavalent BoNT antitoxins, and manufactured by Cangene Corporation (Winnipeg, MB, Canada), which is now Emergent BioSolutions (Rockville, MD). The first approval of a plasma derivative under the Animal Rule, BAT is a sterile solution of fragments of antibodies to seven of the eight BoNT serotypes known to cause botulism (A, B, C, D, E, F, and G, but not H). The antibody fragments are derived

from the processing of whole antibodies obtained from horses previously immunized with a specific serotype. When administered to humans, the most commonly observed side effects include headache, fever, chills, rash, itching, and nausea. However, BAT has the potential to cause hypersensitivity reactions, including anaphylactic and anaphylactoid reactions, in individuals sensitive to equine proteins; delayed allergic reactions may occur 10 to 21 days after administration. Therefore, a skin test before administration of BAT and careful monitoring is advised. BAT is approved for use in adults and children, including infants with botulism caused by serotypes other than A or B. The safety of BAT in pregnant and lactating women is unknown; evidence regarding safety and efficacy in pediatric and geriatric populations is limited. BAT is maintained in the SNS and is available through the CDC's Drug Service.[429,430]

In October 2003, the FDA approved the Botulism Immune Globulin Intravenous (Human) (BabyBIG), a human botulism immune globulin derived from pooled plasma of adults immunized with PBT, 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 Infant Botulism Treatment and Prevention Program through the California Department of Health Services.[431]

Although passive antibody prophylaxis has been effective in protecting laboratory animals from toxin exposure,[432] 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.

Postevent Countermeasures: Current Options

Treatment. Immediately after clinical diagnosis of botulism, adults (including pregnant women) and children should receive a single intravenous infusion of antitoxin (BAT or, for infants with botulism from serotypes A or B, BabyBIG) to prevent further disease progression. The administration of antitoxin should not be delayed for laboratory testing to confirm the diagnosis.[14,66,430,433–435] Skin testing should be conducted

before the administration of BAT to detect sensitivity to serum or antitoxin.[430,435] Intensive supportive care (eg, artificial ventilation or feeding by enteral tube) should also be provided.[434] Although antibiotics may be necessary for the treatment of wound botulism or secondary infections, aminoglycosides and clindamycin should be avoided because they may further impair neuromuscular transmission.[434]

Postexposure Prophylaxis. Asymptomatic individuals with suspected exposure to BoNT should be carefully monitored, preferably near critical care services, for evidence of botulism; the patient's vital capacity and maximal expiratory force should be assessed frequently. Such individuals should be treated promptly with antitoxin at the first sign of illness.[434] In rare instances, it may be appropriate to administer antitoxin as PEP to asymptomatic persons after a high-risk laboratory exposure. PEP may also be appropriate for asymptomatic persons who are thought to have been exposed concurrently with persons already diagnosed with botulism.[14,66]

Staphylococcal Enterotoxin B

Staphylococcal enterotoxin B (SEB) is one of more than 20 antigenically distinct enterotoxin proteins produced by the bacterium *Staphylococcus aureus*. Ingestion of SEB is a common cause of food poisoning, with symptoms (including nausea, vomiting, and diarrhea) typically beginning within 1 to 6 hours of exposure. Ocular exposure can result in conjunctivitis and localized periocular swelling and sometimes gastrointestinal symptoms. Inhalation of SEB may cause fever, fatigue, respiratory symptoms, and sometimes gastrointestinal symptoms, generally within 2 to 12 hours of exposure, which may progress to overt pulmonary edema, acute respiratory disease syndrome, septic shock, and death.[66,436] Because it can be disseminated in a variety of ways and can cause lethal shock in humans, even at low doses (especially by the inhalational route), SEB is considered a potential bioterrorism agent.

Vaccination

No vaccine against SEB is 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. 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.[437] Intramuscular vaccination with recombinantly attenuated SEB using an Alhydrogel (Accurate Chemical & Scientific Corporation, Westbury, NY) adjuvant was protective in rhesus monkeys challenged by aerosols of lethal doses of SEB. All monkeys developed antibody titers, and the release of inflammatory cytokines was not triggered.[438] A phase 1 clinical trial assessing the safety and immunogenicity of a recombinant SEB vaccine has recently been completed.

A candidate SEB vaccine using a VEEV replicon as a vector has also been studied. The gene encoding mutagenized SEB was cloned into the VEEV replicon plasmid, and the product was then assembled into VEEV replicon particles. The vaccine elicited a strong antibody response in animal models and was protective against lethal doses of SEB.[439]

SEB toxoids (formalin-inactivated) incorporated into meningococcal proteosomes or microspheres have been found to be immunogenic and protective against aerosol SEB challenge in NHPs. The proteosome-toxoid, given intratracheally, elicited serum IgG and IgA antibody titers as well as a strong IgA response in bronchial secretions.[440] 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 the toxoid, which results in both a primary and an anamnestic secondary antitoxin response and thereby may require fewer doses.)[441] However, enteric symptoms such as vomiting still occurred in many vaccinees with both vaccine candidates.[440–442]

Passive Immunotherapy, Postexposure Prophylaxis, and Treatment

No PEP is available for SEB. The only current treatment modality is intravenous human immunoglobulin. This form of passive immunotherapy can reduce mortality in animal models if given within 4 to 8 hours after inhalation.[66]

Ongoing work is assessing whether currently FDA-approved medications provide effective PEP or treatment for SEB. One of the most promising lines of research focuses on antiinflammatory and immunosuppressant agents as well as antioxidants. In particular, the immunosuppressant rapamycin (also known as sirolimus) has protected mice from intranasal and systemic exposure to SEB.[443] A recent study in a murine model of SEB-induced lethal shock found that 75% of

mice receiving a combination of the antiinflammatory drug dexamethasone (at 2 and 5 hours after SEB challenge) and the antioxidative drug N-acetyl cysteine (at 24, 30, 48, 54, 72, 78, and 96 hours after challenge) survived; by comparison, only 10% of untreated mice survived.[444]

Postevent Countermeasures: Current Options

Treatment is limited to supportive care, which should focus on oxygenation and hydration; severe cases with pulmonary edema may require ventilation, vasopressors, and diuretics. At this time, no PEP is available; individuals potentially exposed to SEB should be closely monitored for symptoms of intoxication and treated accordingly.[14,66]

Ricin

Ricin is a protein toxin derived from castor beans (the seeds of the castor oil plant, *Ricinus communis*). Ricin, a cytotoxic lectin, consists of an A-chain, the toxic portion of the protein, bound to a B-chain, which serves to bind the toxin to surface receptors found on mammalian cells, enabling the A-chain to enter the cell. Once inside the cell, the A-chain inhibits protein synthesis, which ultimately results in cell death.[445–449] Ricin can be delivered by aerosol, ingestion, or injection.[450] Inhalation of ricin as a small-particle aerosol may produce pathological changes beginning 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, beginning within 3 to 20 hours, in severe gastrointestinal symptoms (nausea, vomiting, cramps, and diarrhea) followed by vascular collapse and death. Injection can result, beginning within 6 hours, in general weakness and myalgias, followed by vomiting, fever, multiorgan failure, and death.[14,66,448,449]

Vaccination

No vaccine is available, but several vaccine candidates are being studied.[451] Because passive prophylaxis with monoclonal antibodies in animals is protective against ricin challenge, the vaccine candidates are based on induction of a humoral response.[452,453]

The most promising development for a vaccine has been to genetically engineer the ricin toxin A chain (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 to protect mice against intraperitoneal challenge with large doses of ricin.[451] A pilot clinical trial in humans demonstrated that a recombinant RTA vaccine (RiVax), given as three monthly intramuscular injections at doses of 10, 33, or 100 μg (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.[454] However, the antibody response was of short duration. More recently, a phase 1B trial of Alhydrogel-absorbed RiVax found positive titers of anti-RiVax antibodies in four of five volunteers receiving three 10-μg doses and four of four individuals receiving three 100-μg doses. All of the eight individuals who seroconverted still had positive titers on day 252; five of these individuals continued to exhibit titers on day 364. The vaccine appeared to be safe and well tolerated.[455] A recently developed heat-stable version of RiVax could extend the vaccine's shelf life at high temperatures, potentially simplifying storage and distribution.[456]

A ricin vaccine candidate (RTA 1–33/44–198 or RVEc) developed at USAMRIID demonstrated high relative stability to thermal denaturation, no detectable cytotoxicity, and immunogenicity in animal studies. The vaccine demonstrated protective immunity against aerosol challenge with ricin in rodents, rabbits, and NHPs. Additionally, no toxicity was observed in two animal models.[457–460] In a phase 1 escalating, multiple-dose study, this vaccine was found to be safe, well tolerated, and immunogenic in healthy adults who received three doses of either 20 μg (10 volunteers) or 50 μg (10 volunteers) of RVEc. Among 10 volunteers who received a single 100-μg dose, 2 individuals developed elevated creatine phosphokinase levels, which resolved without sequelae; no further vaccinations were administered at this dosage. Four individuals in the 50-μg group received a single booster dose, which was safe and well tolerated; all booster recipients developed a robust anamnestic response.[461] Further studies are planned to optimize dose, scheduling, and route of administration.

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.[462] Oral vaccination of mice with the ricin toxoid vaccine encapsulated in poly(lactide-co-glycolide) microspheres was also protective against lethal aerosol ricin challenge.[463]

Treatment and Postexposure Prophylaxis

No therapeutic or PEP agent for ricin intoxication has been developed. 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.[452,453] The development of prophylactic and therapeutic medical countermeasures for ricin intoxication is challenging in part because ricin is taken up into cells rapidly[464] and has high enzymatic efficiency,[465] leaving a narrow treatment window.

Postevent Countermeasures: Current Options

Individuals who may have been exposed to ricin should be monitored closely. Diagnostic testing could include nasal swabs, sputum, and induced respiratory secretions for assay via polymerase chain reaction or antigen enzyme-linked immunosorbent assay (Ag-ELISA) and serum for baseline toxin assays via Ag-ELISA or polymerase chain reaction.[66] Treatment consists primarily of supportive care, such as oxygenation, maintenance of electrolyte balance, and hydration for inhalational exposure and gastric lavage, administration of cathartics, and volume replacement of fluid loss for gastrointestinal intoxication.[14,66,466]

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 protect both military and civilian populations. Generally, military personnel undergo prophylactic vaccination against a broad array of endemic diseases as deployments into areas not travelled by the masses could be required without significant advance notice. In addition, the military has recognized the benefit of vaccinating troops for protection against exposure to 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.

Vaccine recommendations for the civilian and military populations must weigh the risks and benefits as well as the logistics of maintaining immunity with vaccine booster doses. More studies to assess the long-term medical effects of repeated vaccination with multiple vaccines are needed to assure civilian and military populations about the safety of the long-term use of vaccines. 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 the distribution of medical supplies and the management of bioterrorism events within the continental United States; therefore, military physicians must be properly trained and prepared for managing bioterrorism events.

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