

Chapter 2

Smallpox and Bioterrorism

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2.1 Introduction

This chapter will focus on information regarding smallpox and smallpox vaccination since 2001, notably, the persisting threat of smallpox as a bioterrorist agent, international preparedness for a smallpox outbreak, vaccine adverse event issues including myopericarditis, second- and third-generation smallpox vaccines, HIV/AIDS issues, similarities with other microbial threats such as monkeypox and SARS, and an example of hospital and city smallpox preparedness efforts beginning in late 2001. Recent reviews by us and others, including the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), have addressed the history, clinical features, pathogenesis, prevention, diagnosis, and management of smallpox [1–7]. In addition, reference is also made to classic comprehensive texts on smallpox from 1962, 1972, and 1988 [8–10].

After the terrorist airplane hijacking attacks of September 11, 2001, and the subsequent anthrax bioterrorism attacks, additional international efforts were undertaken to reassess the threat of smallpox being reintroduced into the human population a quarter century after its eradication. These efforts, including those of the WHO, were focused on recognition of the clinical aspects of smallpox, the public health response, smallpox vaccination, and the need for expanded smallpox vaccine stockpiles [11–20].

In the United States, the CDC and the Department of Defense (DoD) initiated extensive educational training regarding smallpox and smallpox vaccination [21–29]. According to CDC [30], between January 2003, the beginning of the civilian vaccination program, and October 31, 2004, at least 39,597 civilians were vaccinated against smallpox. The civilian program declined by the summer of 2003, temporally linked with three events that began in March 2003: These were the unexpected finding of myopericarditis in a small

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percentage of vaccinees, a growing appreciation of the risks of vaccination, and the apparent absence of biological weapons in Iraq, as confirmed subsequent to invasion. In the DoD, between December 13, 2002, and October 14, 2004, over 656,000 smallpox vaccinations were administered [24] (www.smallpox.army.mil/event/SPSafetySum.asp). Unlike the civilian program, the DoD smallpox vaccination program has continued without pause, and in fact, expanded in the latter half of 2004. On June 28, 2004, a memorandum from the Pentagon by the Deputy Secretary of Defense directed the expansion of the vaccination programs in the military for both smallpox and anthrax. Expansion of vaccination included “all uniformed DoD personnel serving in the Central Command Area of Responsibility,” which includes central Asia, parts of east Africa, and the Korean peninsula areas considered at special risk for military personnel [31] (www.smallpox.army.mil/resources/policies.asp).

2.2 Smallpox: A Persisting Bioterrorist Threat

The primary source threat of smallpox being reintroduced into the world was the former Soviet Union because of its alleged former massive program to weaponize smallpox. As reported by Alibek [32], the former deputy director of this Soviet effort, after the WHO announced in 1980 that smallpox had been eradicated [33], the Kremlin provided the resources and planning to produce and store up to 20 tons of smallpox per year. This alleged illegal and secret smallpox production effort, involving tens of thousands of persons over multiple years, was cited again in October 2003 at an international smallpox vaccine meeting in Geneva [34] by Henderson. According to Alibek, this viral production facility was located at Zagorsk, now known as Sergiyev Posad, located less than an hour northwest of Moscow [32]. This is still a top-secret facility under the Russian Ministry of Defense, according to Henderson, and it is unknown whether smallpox is still present in this facility [35].

There are only two facilities that are approved by the WHO for storage of variola virus and for limited research: the CDC and the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Novosibirsk, Siberia [35]. Increased laboratory research on smallpox at these two locations since 2001, including monkey and other animal model studies and efforts to develop antiviral drugs and attenuated vaccines against smallpox, inevitably carry an intrinsic risk of an accidental laboratory-associated infection. A laboratory-associated variola virus infection would trigger international public concern such as the one that occurred with SARS coronavirus infection in lab workers in Singapore, Taipei, and Beijing in late 2003 and 2004 [36].

In addition, there is concern that some workers in the former Soviet smallpox weapons program have left Russia, and may have taken variola virus with them and shared their expertise on smallpox with other nations or organizations [34, 35]. Such linkages could serve as a means whereby smallpox could be

reintroduced into a now largely unvaccinated and susceptible human population. In an attempt to decrease the risk of smallpox and other biological weapons, the United States and European nations are reported to be devoting \$90 million each year to assist Russia to employ approximately 6,000 former bioweapons scientists and to secure better its large bioweapons complex. One example of these funding initiatives is the planned construction in 2005 of new and more secure laboratories to study high-risk pathogens, although not smallpox, in Kazakhstan [37].

Prior to the 2003 war with Iraq, the *Washington Post* reported in a front-page article on November 5, 2002, that unnamed sources in the US government suggested that Iraq and North Korea, as well as the United States and Russia, possessed the variola virus [38]. No specific information was provided. On February 5, 2003, the US Secretary of State Colin Powell, in his detailed presentation at the United National Security Council regarding specific concerns about Iraqi weapons of mass destruction, mentioned smallpox only once, in referring to Saddam Hussein: "And he also has the wherewithal to develop smallpox" [39].

Even though the war with Iraq did not reveal any smallpox stockpiles or weapons of mass destruction, concern persists regarding the possible use of smallpox as a bioterrorist weapon. In the summer of 2003, Richard Danzig, former secretary of the US Navy and a biodefense expert, argued that aerosolized smallpox and aerosolized anthrax are two of the four major catastrophic bioterrorism threats for which the United States needs to prepare better [40]. He discussed specific measures and made recommendations for dealing with an emergency of 200,000 smallpox-infected persons. These difficult issues include rapid detection of the aerosol smallpox attack and rapid vaccination of large numbers of persons within a 4-day (96 h) window after infection, the period when vaccination can prevent or decrease the severity of clinical illness. Similarly, Alibek and Charles Bailey, bioweapons experts from the former Soviet Union and the United States, respectively, have recently emphasized the threat of an aerosolized attack with a bioterrorism weapon [41].

In the summer of 2004, the CDC and Federal partners began the planning and implementation with Departments of Health in multiple US cities, including the Washington, DC, National Capital Region of the new "Cities Readiness Initiative (CRI)." The specific funding and rationale for the CRI, listed on the CDC website in June 2004, is to enhance readiness in at least 20 US cities and their surrounding regions for a catastrophic event, such as an aerosol release of a bioterrorist agent over or within one or more cities [42].

Multiple organizations continue to create and critique computer models of smallpox outbreaks [43–47]. A recent review article on smallpox modeling by Ferguson and colleagues discussed the benefits and drawbacks of different types of smallpox vaccination policy options in controlling a smallpox attack [43]. These options included quarantine/isolation, movement restrictions, containment by "ring" vaccination, targeted vaccination, mass vaccination, and prophylactic vaccination.

In 2004, Dr. Alibek published a paper [48] on smallpox as a disease and as a weapon, in which he reviewed in detail specific aspects of the former Soviet Union's program to weaponize smallpox such as field testing at Vozrozhdenie island until the late 1970 s and production and testing using large reactors (up to 630 L) during the 1980 s. He also presented information on methods that might be used to release smallpox virus as a bioterrorist weapon, such as the use of mechanical devices to generate an aerosol, explosive devices, contamination of food or various articles, or release within a subway to generate an aerosol by evaporation of a liquid smallpox formulation or a dry powder.

2.2.1 Genetic and Immunologic Scenarios

Alibek concludes his paper with a discussion of genetically modified variola virus, designed to enhance its effectiveness as a weapon of mass destruction. This scenario builds on the work published from Australia in 2001 involving mousepox (ectromelia) with a gene inserted for interleukin (IL)-4 [49]. The IL-4 cytokine weakens the cell-mediated immune response against viruses such as orthopoxviruses, by inhibiting cytotoxic T-cells and interferon (IFN)- γ production. Clinically, the mice infected with this IL-4-modified mousepox had increased mortality and significantly decreased protection against mousepox by prior vaccination [49].

Similar laboratory work with variola virus, such as inserting the gene for IL-4 or related cytokines such as IL-13, has not been performed or approved by WHO for future experiments, given the risk that the findings with mousepox-IL-4 might be similar to that with variola-IL-4. However, IL-4-modified vaccinia virus has been studied recently in a mouse model. In these experiments, reported in 2004 by NIH researchers, an otherwise fatal challenge with vaccinia virus that had been modified to express murine IL-4 could be prevented by prior immunization with the non-replicating, attenuated vaccinia virus, Modified Vaccinia Ankara (MVA) [50]. This was an important finding because vaccinia virus, with or without the expression of IL-4, can infect humans (as well as mice), whereas mousepox virus does not infect humans.

Additional research has applied the immunologic model [51, 52] of type 1 cytokines (Th1) such as IFN- γ and type 2 cytokines (Th2) such as IL-4 to mousepox and vaccinia; such studies in mice and inferences from human conditions such as atopic dermatitis, immunocompromising diseases, and pregnancy could lead to a rationale for novel immunologic therapies for orthopoxviruses including vaccinia and variola.

In a paper published in 2004 from Australia [53], mousepox (ectromelia) infection of virus-resistant mice (C57BL/6) resulted in IFN- γ production and a strong cytotoxic T-cell cellular immune response. In contrast, mousepox infection of susceptible mice (BALB/c and A/J) resulted in little or no IFN- γ , but instead resulted in production of IL-4. Deletion of the IL-4 gene did not change

the disease in the susceptible mice, but loss of IFN- γ function in the resistant mice lead to 100% mortality. Similar earlier studies [54] from Australia and Japan found that mousepox-susceptible mice (BALB/c) were made less susceptible when “STAT-6” (signal transducer and activator of transcription), the intracellular signaling molecule for IL-4, was deleted.

A common theme in these studies is that a strong Th1 response that is exemplified by IFN- γ is needed to prevent or decrease mousepox disease. Since the Th1 IFN- γ and the type 2 cytokine IL-4 are cross-inhibitory [51, 52], the impairment of IFN- γ may be at least as important as the enhancement of IL-4. A strong cellular immune response (controlled by type 1 or Th1 cytokines) may be more critical than a predominant antibody immune response (controlled by type 2 or Th2 cytokines); this is particularly true when response is directly associated with a weak cell-mediated immune response as evidenced by impaired IFN- γ and cytotoxic T-cell production. In the early 1990s, IFN- γ itself had been reported to have antiviral activity against vaccinia [55–57].

In extending the type 1/type 2 cytokine model to humans, the situation is often less clear cut than in mice [51, 52]. There are data to support the view that atopic dermatitis, immunocompromising diseases, such as HIV/AIDS and some malignancies, and even normal human pregnancy are characterized by a relative decrease in the normal ratio of IFN- γ (type 1 cytokine) to IL-4 (type 2 cytokine) [52, 58–62]. These conditions have been associated with a relative decrease in cell-mediated immune responses. In addition, they are all associated with an increased risk of adverse events due to smallpox vaccination with vaccinia.

These observations on the importance of IFN- γ for the control of orthopoxviruses could generate the hypothesis that subcutaneous IFN- γ , already FDA licensed since 1990 for chronic granulomatous disease and specific medical conditions [63], could be beneficial to control some life-threatening adverse effects of smallpox vaccination including progressive vaccinia. Specifically, for the rare cases of progressive vaccinia that do not respond to Vaccinia Immune Globulin (VIG), and for which surgical therapy (resection) is being considered, along with VIG, subcutaneous IFN- γ could be administered under an investigational new drug (IND) protocol, if one were available. If successful in such a clinical setting, IFN- γ use would avoid surgical resection.

2.3 The Two Viruses: Vaccinia virus (Smallpox Vaccine) and Variola virus (Smallpox)

Vaccinia virus is the virus found in smallpox vaccine, while variola virus is the causative agent of smallpox; they are two distinct viruses. Table 2.1 compares these two related orthopoxviruses, their routes of transmission, virus–immune system interactions, and potential therapy. Vaccinia virus never causes smallpox. Vaccinia virus is not spread via respiratory droplets, and therefore no

Table 2.1 Comparison of vaccinia virus and variola virus

Vaccinia virus: smallpox vaccine	Variola virus: smallpox disease
VACCINIA : the vaccine virus	VARIOLA: the disease virus
Definitions	
Vaccinia: The virus in the smallpox vaccine	Variola: The virus that causes smallpox disease
Vaccinia does not cause smallpox	Variola is not used in smallpox vaccines
Distinct from cowpox (L. “vacca,” cow)	Two forms: variola major and variola minor
Transmission	
Vaccinia is spread only by direct contact	Variola is usually spread by direct contact, occasionally in bedding or clothes
Vaccinia is not spread through the air	Variola is spread through the air by droplet aerosols
Virus–immunity interactions	
Vaccinia induces immunity against variola	Variola major is often (30%) fatal if unvaccinated
Immunocompromised persons can develop more severe vaccine reactions, e.g., “progressive” or “necrotic” vaccinia	Immunocompromised and pregnant persons may have more severe smallpox and present with atypical skin lesions: “Hemorrhagic”, or “flat” smallpox (both with >90% mortality).
Therapy	
Vaccinia Immune Globulin (VIG) is effective for some, not all, serious vaccine reactions.	Vaccination within 3–4 days can protect against disease [166]. VIG is not used against variola.
No FDA-licensed antiviral drugs.	No FDA-licensed antiviral drugs
Cidofovir may be tested as an antiviral on an investigational new drug (IND) basis.	Cidofovir may be tested as an antiviral on an investigational new drug (IND) basis.

respiratory precautions are needed for persons vaccinated with vaccinia. Some serious vaccination reactions can be treated with VIG, whereas smallpox disease due to variola virus is not responsive to VIG. An intravenous formulation of VIG has replaced the older intramuscular formulation (IM) [64–66] after approval by the FDA on February 18, 2005 (www.fda.gov/cber/products/vigivdyn021805.htm) [64–66]. There are no FDA-licensed antiviral drugs to prevent or treat illness due to either vaccinia virus or variola virus. Table 2.2 lists 10 ways that smallpox vaccine differs from other FDA-licensed vaccines, including routine use of a bifurcated needle (Fig. 2.1), and that a successful vaccination (a “take”) is documentable on the skin by day 6–8 (Fig. 2.2).

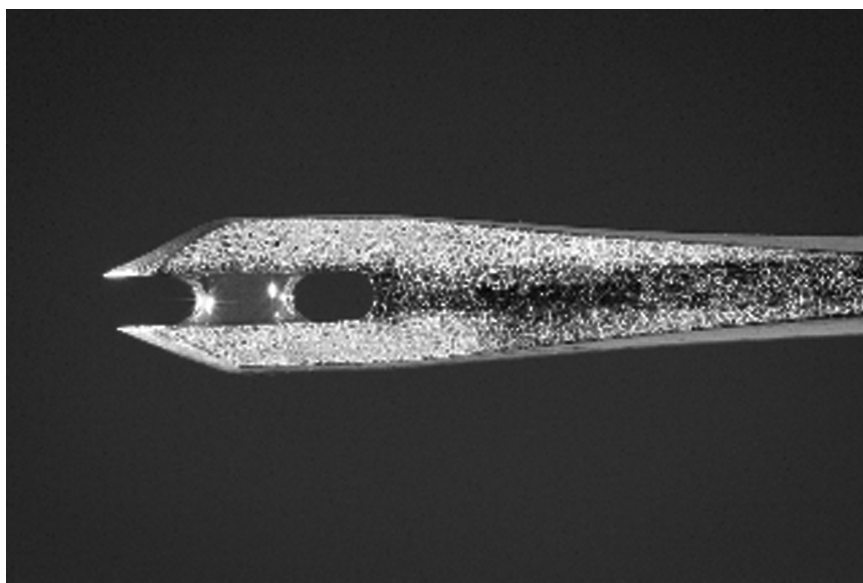
Variola virus can be transmitted in multiple ways: By far, the most common is via respiratory droplets, but transmission by fomites such as clothing or bed linens, has occurred [1]. Transmission as an aerosol, involving droplet nuclei, is rare but occasionally has been documented such as in a hospital in Meschede, Germany [67].

The incubation period of variola virus is 7–17 (mean = 12) days, after which a febrile prodrome begins with headache, backache, nausea, and prostration

Table 2.2 Ten (10) ways that smallpox vaccine differs from other vaccines

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1. Rationale for use: to protect against a disease eradicated over 25 years ago.
 2. One virus (vaccinia) protects against disease due to a second virus (variola).
 3. Contraindications (absent smallpox exposure) include any history of eczema.
 4. A “bifurcated” needle is routinely used for vaccination.
 5. Either 3 (naïve) or 15 (revaccinee) intradermal jabs of the needle are recommended.
 6. A trace of blood must be seen after last intradermal jab, or vaccination is repeated.
 7. A successful vaccination (a “take”) is documentable on the skin by day 6–8.
 8. The vaccine site is infectious to self and “contacts” until the scabs are fully formed: ~3 weeks.
 9. Least safe FDA-licensed vaccine: 15 life-threatening reactions, and one or two deaths, per million primary vaccinations.
 10. Some, but not all, serious vaccine reactions can be treated with Vaccinia Immune Globulin (VIG).
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(Table 2.3) [1, 9, 68]. Some have speculated that the incubation period may be shorter if a highly virulent and high-dose exposure to smallpox is accomplished by an aerosol release as a bioweapon [48]. After 1–4 days of the febrile prodrome, a rash begins in the oral mucosa and then in the skin; typically the rash is concentrated centrifugally, including the face, palms, and soles. Infectivity prior to the clear-cut onset of rash is rare, and the highest degree of infectivity occurs once the rash is present [1].

**Fig. 2.1** Bifurcated needle with smallpox vaccine liquid (CDC)

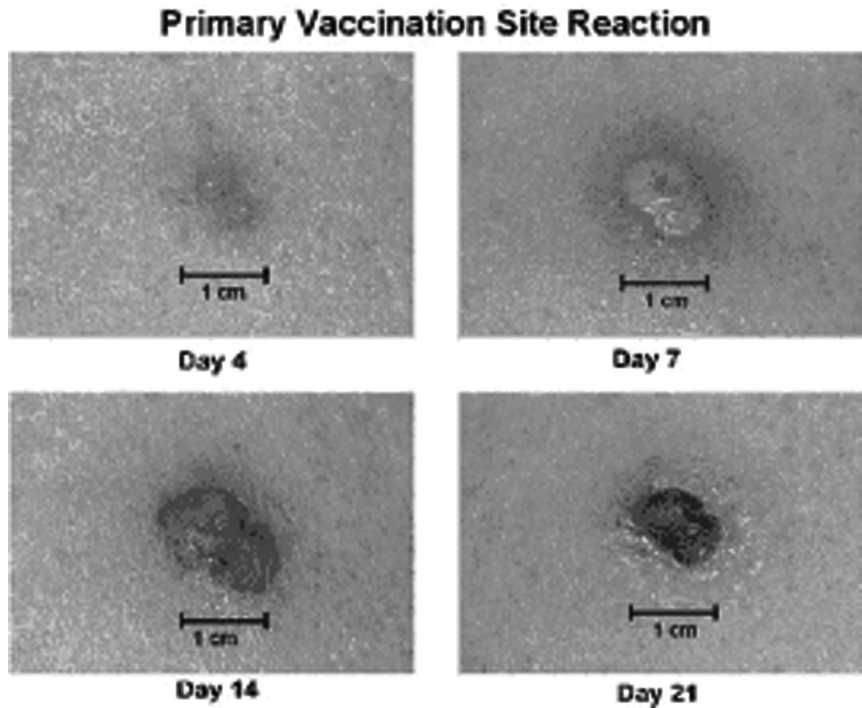


Fig. 2.2 Time course of typical skin reactions to smallpox vaccination in a vaccinia-naïve person (CDC)

Table 2.3 Major and minor criteria for the diagnosis of smallpox

Major criteria (3)
1. Febrile prodrome: occurs 1–4 days before rash. Fever >101F and at least one of the following: prostration, headache, backache, chills, vomiting, or severe abdominal pain.
2. Classic smallpox lesions: deep-seated, firm-hard, round well-circumscribed vesicles or pustules as they evolve lesions may become umbilicated or confluent.
3. Lesions in the same stage of development: on any one part of the body lesions are all in the same stage, e.g., all vesicles or all pustules at the same time.
Minor criteria (5)
1. Centrifugal distribution with greatest concentration of lesions on face and distal extremities.
2. First lesions on the oral mucosa/palate, face, or forearms.
3. Patient appears toxic or moribund.
4. Slow evolution of lesions evolving from macules to papules to pustules over several days.
5. Lesions on the palms and soles.
“High,” “Moderate,” and Low” risk of smallpox defined using these major and minor criteria
“High” Risk: all three major criteria
“Moderate” Risk: Febrile prodrome and either one other major criteria or 4–5 minor criteria.
“Low” Risk: either no febrile prodrome or febrile prodrome and <4 minor criteria.

Reference [21] <http://www.bt.cdc.gov/agent/smallpox/diagnosis/pdf/spox-poster-full.pdf>.

2.4 Myopericarditis and other adverse events after vaccination: 2002–2004

Prior to 2003, cases of myocarditis and/or pericarditis after smallpox vaccination were seldom reported in the United States, but were reported from Europe, especially Scandinavia, and from Australia [69–76]. One possible explanation was the use of a different vaccinia strain in the United States (New York City Board of Health strain) from those used in Europe and Australia. In a study of military conscripts from Finland [76], all of whom were routinely vaccinated against smallpox, an incidence of symptomatic myocarditis after the smallpox vaccination was approximately 1:10,000. This figure was based on 12 cases of myocarditis occurring 8–14 days after vaccination, without any other etiology for the myocarditis being found on investigation.

In the United States, the initial reports of myopericarditis after smallpox vaccination appeared in March 2003 and triggered immediate investigation by the CDC and the DoD [27, 77–80]. While investigations were ongoing to assess causality, new safeguards were implemented to avoid vaccination in persons with a history of either cardiac disease or stroke, or in those in whom three or more risk factors were present (Table 2.4). These traditional risk factors for heart disease included high blood pressure, elevated cholesterol, diabetes, smoking, and a positive family history of heart disease before the age of 50 years [27].

As of October 14, 2004, the DoD had diagnosed 82 cases of myopericarditis in over 656,000 vaccinees (about 1:8,000), most of whom were primary vaccinees. Out of 39,213 vaccinees, the CDC identified 5 probable and 16 suspected cases of myopericarditis after smallpox vaccination [81]. The DoD published

Table 2.4 CDC updated (November 15, 2003) smallpox guidelines for “Smallpox Pre-Vaccination Information Packet: Contents and Instructions.” “Smallpox Vaccination Patient Medical History and Consent Form.” Heart Problems

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1. Have you ever been diagnosed by a doctor as having a heart condition with or without symptoms such as a previous myocardial infarction (heart attack), angina (chest pain caused by a lack of blood flow to the heart), congestive heart failure, or cardiomyopathy?
 2. Have you ever had a stroke or transient ischemic attack (a “mini-stroke” that produces stroke-like symptoms but no lasting damage)?
 3. Do you have chest pain or shortness of breath when you exert yourself (such as when you walk up stairs)?
 4. Do you have any other heart condition for which you are under the care of a doctor?
 5. Do you have three or more of the following risk factors?
 - a. You have been told by a doctor that you have high blood pressure.
 - b. You have been told by a doctor that you have high blood cholesterol.
 - c. You have been told by a doctor that you have diabetes or high blood sugar.
 - d. You have a first-degree relative (for example, mother, father, brother, or sister) who had a heart condition before the age of 50).
 - e. You smoke cigarettes now.
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their initial findings in June 2003 [82] and in two updated articles [83, 84] in 2004 as well as on the DoD website dedicated to their smallpox vaccination program (www.smallpox.army.mil/event/SPSafetySum.asp). A causal relationship has been accepted for the myopericarditis, in part because it is more common in the DoD after primary vaccination than revaccination, because of its occurrence within 7–14 days after vaccination, and because of the absence of other etiologies. The DoD reported in 2004 a statistically significant association between developing myopericarditis and being male and white. Among primary vaccinees there was a significantly increased risk of myopericarditis within 30 days after smallpox vaccination, with an observed incidence of 16.11/100,000. In contrast, the DoD found no increased risk of myopericarditis in revaccinees [83]. No deaths have occurred, and the prognosis has been good for a full recovery after vaccine-associated myopericarditis. The DoD reported that 64 of the initial 67 patients (96%) had normalization of their functional status, echocardiography, EKG, and graded exercise testing at a mean of 32 weeks' follow-up. Atypical, but non-limiting, persistent chest discomfort was reported by 8 of the 67 patients (13%) [84].

No causal relationship has been found for myocardial infarction or other ischemic events after smallpox vaccination [24, 85]. Likewise, a retrospective analysis of cardiac deaths after the 1947 mass vaccination program of approximately 6.4 million persons in New York City revealed no evidence of an increase in cardiac deaths [86].

Among other adverse events following vaccination, the DoD reported as of October 14, 2004, that one death may have been attributable to smallpox vaccination, although the results are inconclusive, according to two independent civilian physician panels. This patient was a 22-year-old reservist who received five vaccines, including smallpox vaccine, at the same time and developed a lupus-like illness prior to her death 33 days after the five vaccinations [84] (www.smallpox.mil/event/panelreport.asp). Six other deaths after vaccination were judged to be clearly unrelated to vaccination. Sixteen other cases of "ischemic heart disease" such as angina or myocardial infarction occurred within 6 weeks after smallpox vaccination in the 656,000 vaccinees, but these cases were judged to be "similar to what normally occurs among unvaccinated military personnel of similar age" [86, October 14, 2003, summary at www.smallpox.mil/event/panelreport.asp]. Forty cases of generalized vaccinia were reported, and most were treated as outpatients; 50 cases of contact vaccinia were found, nearly all between spouses and adult intimate contacts outside the workplace [24]. More importantly, no cases of vaccinia transmission occurred between the 27,700 vaccinated health-care workers (HCWs) and patients or co-workers. Neither progressive vaccinia nor eczema vaccinatum cases were observed in military or civilian vaccinees (www.smallpox.army.mil/event/SPSafetySum.asp). Similarly, there were no episodes of vaccinia transmission from a civilian health-care worker to a patient or a co-worker.

2.5 HIV/AIDS and Smallpox Vaccination

In 2004, the DoD reported that 10 of the initial 438,000 patients who received smallpox vaccination since December 2002 also had undiagnosed infection with the human immunodeficiency virus (HIV) [88]. All 10 persons had a normal major reaction to the vaccination and normal healing. More importantly, however, none of these persons had AIDS-defining CD4-cell counts (<250 cells/ul), opportunistic infections, or malignancies. Their CD4 counts ranged from 286 to 751 cells/ul. In addition, 7 of 10 patients had previously been vaccinated against smallpox.

Only one patient with HIV infection has been reported to have had a serious, but nonfatal, adverse event after receiving the standard smallpox vaccination [89]. This occurred in 1984 in the US Army, prior to the availability of HIV antibody testing. At that time, smallpox vaccination was still routinely administered in the military due to concern about the potential use of smallpox virus as a bioweapon. Soon after smallpox vaccination, the first for this 19-year-old army recruit, the patient presented with AIDS-defining cryptococcal meningitis, and oral candidiasis. His CD4 T-cell count was <25 cells/ul. Four weeks after vaccination, while hospitalized for meningitis, a 3 cm \times 4 cm ulcer developed at the vaccination site, and then over the next 3 days, 80–100 pustular lesions appeared on the posterior legs and buttocks. These lesions also ulcerated, and vaccinia virus was cultured from the lesions. After 12 weekly intramuscular treatments with VIG, the skin lesions completely resolved. When this case report was published in 1987, the accompanying editorial by Halsey and Henderson [90] commented that several hundred HIV-infected military recruits must have received multiple immunizations, including vaccinia, without complications prior to the mandatory HIV-antibody testing and exclusion of HIV-positive recruits.

Although no other instances of complications of smallpox vaccination in patients with HIV infection have been reported, the use of recombinant vaccinia to express HIV proteins as an investigational form of cell immunotherapy did raise concerns about vaccinia virus potentially contributing to the deaths of three patients with AIDS and CD4 T-cell counts <50 cells/ul in a Phase I trial in Paris in 1989–1990 [91, 92]. According to Zagury, earlier clinical trials at the Cliniques Universitaires, Kinshasa, Democratic Republic of the Congo, and Paris had not shown similar toxicities [93]. This particular HIV cell immunotherapy, using paraformaldehyde inactivation and recombinant vaccinia to express HIV proteins in autologous EBV-transformed B-cells, was reviewed in 1991 and the decision was made to discontinue its use [92, 93].

Since 2001, concerns regarding complications of smallpox vaccination and smallpox infection in persons with immunocompromised conditions, such as HIV/AIDS or transplantations [94], have been discussed by Bartlett and others [95–97]. Issues regarding HIV/AIDS and smallpox and smallpox vaccination were presented at the 2002 International AIDS conference in Barcelona, Spain

[98]. In the event of a smallpox attack and possible exposure, the possibility of rapid testing for HIV infection has been considered as a possible screening tool. However, the critical point is that if a person has been exposed to smallpox, there are no contraindications to vaccination with vaccinia, including HIV infection.

One such rapid test for HIV antibody using fingerstick specimens, called OraQuick Rapid HIV-1 antibody test, was approved by the FDA on November 7, 2002 [99, 100]. The FDA later approved the OraQuick test for use with routine whole blood venipuncture samples in September 2003. The FDA revised the time during which the results of the test would be interpreted to between 20 and 40 min.

How would the ongoing global HIV/AIDS pandemic impact the global public health response if smallpox was to reenter the human population as a bioterrorist weapon? The potential public health, societal, and economic implications in terms of trying to contain and control smallpox in this setting, especially in parts of the world with the highest prevalence of HIV infection, such as sub-Saharan Africa and India have been debated. Some [101] have viewed with alarm the potential for dual infections with HIV and variola, or vaccinia, but there are others who doubt that this would pose an insuperable problem.

Safer smallpox vaccines are needed. Third-generation smallpox vaccines, using attenuated vaccinia viruses such as MVA or the LC16m8 strain used in a smallpox vaccine that was licensed in Japan in 1975, are being reevaluated as options for immunocompromised patients in the event of a smallpox attack. An immunocompromised animal model has been studied in which rhesus macaques are infected with simian immunodeficiency virus (SIV) or a SIV/HIV hybrid virus. They are vaccinated with an attenuated, replication-deficient vaccinia and/or with the standard non-attenuated first-generation Dryvax vaccine. Giving the attenuated vaccinia vaccine first, followed by Dryvax, decreases the adverse events seen with Dryvax alone [102].

Given the global susceptibility to smallpox since its eradication 25 years ago and subsequent cessation of routine vaccination, the threat of smallpox being reintroduced as an act of bioterrorism makes the insufficient amount of smallpox vaccine in most nations of the world today particularly concerning. For these and other reasons, it is evident why destruction of all stocks of smallpox virus has been called for and its use as a bioterror weapon has been characterized as a “crime against humanity” as one of several recommended international measures to prevent the return of smallpox [68, 103].

2.6 Hemorrhagic Smallpox

The rare and highly fatal (92–100%) form of smallpox known as “hemorrhagic smallpox” deserves specific consideration. Due to the striking and rapidly progressive clinical illness, some have speculated that terrorists would attempt

a smallpox attack that will cause a high incidence of hemorrhagic smallpox [104, 105], if such a strain of variola were able to be identified. At this time no strain of variola has been reported that reproducibly causes hemorrhagic smallpox.

The “early” form of hemorrhagic smallpox was found by Rao in his 1964 report of 100 such patients to have a much shortened time (mean of 5.95 days) from the onset of the smallpox febrile prodrome until death [106]. If an aerosol attack with smallpox occurs in which tens or thousands of persons are infected, some of the index cases may be patients with early hemorrhagic smallpox. Recognition of this rare (~1%) manifestation of smallpox would be critical to trigger an immediate public health response to such a smallpox outbreak.

A monkey model of hemorrhagic smallpox has been developed at the CDC by the US Army researchers [107–109]. High-dose intravenous challenge with variola virus caused a hemorrhagic form of smallpox with a monocyte-associated viremia. Analysis of the immune response in these animals suggested marked impairment of both the tumor necrosis factor alpha (TNF- α) response and the expression of the transcription factor NF- κ B, in these animals [109]. Cidofovir did not confer any prophylactic protection in this hemorrhagic smallpox model—a finding that the authors attributed to the overwhelming nature of the hemorrhagic smallpox with 100% lethality and a mean time to death of 4 days [108]. The high-level viremia with variola associated with hemorrhagic smallpox in the above-mentioned monkey models is reminiscent of the findings published in 1969 by a team of researchers from India, England, and the United States working in Madras, India, that patients with hemorrhagic smallpox also had high-level viremia with variola [110].

As an example of concern regarding hemorrhagic smallpox as a clue to weaponization of the virus, in late 2001, information was made public in a Russian newspaper and to the West regarding a previously unreported smallpox outbreak involving 10 patients in Aralsk, Kazakhstan, in the former Soviet Union in 1971 [111]. The fact that 3 of the 10 patients were diagnosed with hemorrhagic smallpox was raised as a possible clue by one investigator that a more virulent smallpox virus was being developed and tested at that time near Aralsk, on Vozrozhdeniye island in the Aral Sea, by the Soviet military [111]. Henderson doubted this interpretation, pointing out that the one person who was ostensibly infected by the aerosol actually had a mild case. All other cases resulted from secondary transmission, and the three who subsequently manifested hemorrhagic smallpox did not transmit infection to others. This would be consistent with Rao’s thesis that the cause of hemorrhagic smallpox relates to host response rather than to the intrinsic character of the virus strain [8, 112].

Rao has reported the largest series of hemorrhagic smallpox cases [8, 106]. Although earlier clinicians, such as Osler in his 1892 textbook of Medicine, had recognized two types of hemorrhagic smallpox, a rapidly fatal “black smallpox” and a later pustular form [113], the most detailed description of the clinical and epidemiological aspects of the disease has been given by Rao. In 1964, he published a paper describing in detail 240 hemorrhagic cases seen between

1959 and 1963, representing 2.3% of 10,857 total smallpox cases [106]. In his 1972 smallpox monograph [8], Rao reviewed 200 patients with hemorrhagic smallpox beginning in 1961 (and thus partially overlapping with his earlier series from 1959 to 1963), for a total of 385 cases of hemorrhagic smallpox out of approximately 30,000 total smallpox cases he had seen over 30 years of his work in India. Rao reported that, of these 385 cases of hemorrhagic smallpox, not even one transmitted hemorrhagic smallpox to another person, suggesting that it was the host response rather than the virus strain that was the critical variable.[8].

This observation by Rao is also used to counter the argument advanced by Sarkar and Mitra in 1967 that hemorrhagic smallpox patients have a more virulent virus than patients with confluent or discrete smallpox. Working in Calcutta, they isolated variola virus from 75 patients, comparing 25 with hemorrhagic smallpox, 25 with confluent smallpox, and 25 with discrete smallpox [114]. They used four methods to assess virulence: at least 50% mortality in the chick embryo, at least 50% mortality in infant mice, the histopathology of pocks on the chorioallantoic membrane (CAM), and at least 1,000 pock-forming units (PFU) per gram of liver in an infected chick embryo. These four methods were used, in part, because there were no standard methods to assay virulence of the variola virus. A positive result leading to being classified as more virulent was found in all four assays in 48% of hemorrhagic smallpox cases, compared with 36% of confluent cases, and 0% of discrete-type smallpox cases. A virological mechanism for these findings has never been reported, but one methodological difference, albeit of uncertain significance, is that the variola virus from all 25 hemorrhagic smallpox cases was isolated from venous blood, whereas 49/50 confluent and discrete cases were isolated from vesicular or pustular fluid. These studies from 1967 have not been replicated or restudied.

Salient clinical points from Rao's 240 hemorrhagic smallpox patients [106] distinguish the "early" and "late" form of hemorrhagic smallpox. The "early" form (100 of 240 patients) presented with fever and severe prodromal symptoms including excruciating backache and severe headache with hemorrhages into the mucous membranes and skin, which was described as having a "velvety touch and colour." Death occurred in 100% of these patients on average 5.95 days later. Classic smallpox skin lesions never developed, an important point because the current CDC algorithm for evaluating a rash would likely miss a patient with early hemorrhagic smallpox because of the lack of an acute, generalized vesicular or pustular rash, which is one of the major criteria in the CDC algorithm [21] (Table 2.3). Potentially diagnostic clues to even atypical forms of smallpox would still be recognized at autopsy [115, 116].

Rao's description of "late" hemorrhagic smallpox, based on the remaining 140 of the total 240 hemorrhagic smallpox patients, starts with the febrile prodrome that may or may not be severe, but with a rash actually developing to a papulovesicular stage [106]. The average time to death, which occurred in 92% of the 140 cases, was 10.2 days, considerably longer than the 5.95 days found in the early hemorrhagic smallpox form. In both early and late forms,

pregnant women were especially vulnerable to hemorrhagic smallpox. This was most striking with the early form in which 44 of the 100 patients (44%) were pregnant women, compared with 14 of the 140 patients (10%) with the late form.

In both early and late forms, death occurred despite past successful vaccination, at least a few of which were recent. However, the vaccines used in India during Rao's studies were of variable quality, and an apparent vaccination scar could be caused by trauma to the skin when a rotary lancet was used even when the smallpox vaccine was impotent. An illustrated historical review of devices and tools used to administer smallpox vaccines was provided by Baxby in 2002 [117]. This visually striking review emphasizes that the bifurcated needle (Fig. 2.1), successfully used during the smallpox global eradication program, became available only in the late 1960 s.

Increased hormone levels associated with pregnancy were considered by Rao and other researchers to contribute to the predisposition and high case fatality rate of pregnant women for hemorrhagic smallpox [118]. In 1963, Rao and colleagues in India reported on their extensive clinical experience with smallpox and pregnancy, totaling 244 pregnant women. They also compared in detail 94 consecutive pregnant women admitted over a 12-month period from 1961 to 1962 with a comparison group of non-pregnant women and men. Their multiple findings included that the highest risk of premature termination of pregnancy occurred if the woman was infected with variola in the very early or very late months of pregnancy. The incidence of hemorrhagic smallpox was much higher in pregnant women than in non-pregnant women or in men. The specific manifestation of hemorrhagic smallpox was reported to be lowest in the first trimester, then increasing to a peak in the sixth month, declining in the seventh and eighth month, but rising again at the end of pregnancy.

In his 1972 monograph, Rao summarized results of his experiments, published in 1968 in the Indian Journal of Medical Research [119], using a monkey model to define the pathogenesis of smallpox in pregnancy and in immunocompromised hosts, for example, by administering corticosteroids (cortisone) prior to infection with variola [8]. He concluded: "Thus cortisone has been shown to enhance the disease of variola in monkeys. Adequate doses of cortisone before and after variolation produced a fatal form of smallpox, associated with internal as well as external hemorrhages. Pregnant monkey and cortisonised monkey reacted to smallpox infection in the same way as a pregnant woman to smallpox. The mechanism by which cortisone enhances the disease is still vague."

The monkeys used in these experimental variola infection studies were Indian bonnet monkeys (*Macacus radiata*), 2–4 kg in weight, and caught in and around Madras. Only one of these monkeys was pregnant. A fourth egg passage variola virus suspension derived from vesicular fluid from a patient with smallpox was used to infect these monkeys by variolation on the abdomen, using a tuberculin syringe and injecting the variola suspension intradermally. A total of 30 monkeys were variolated, 16 of whom also received varying doses

of cortisone before and after infection, while 14 others received a placebo rather than cortisone. Twelve of sixteen (75%) of the cortisoned animals died of smallpox, whereas 0/14 of the control monkeys died. All 16 of the cortisonized monkeys developed varying degrees of generalized smallpox rash, as did 13/14 control animals albeit less extensive. Comparing the time course of viremia in both groups showed that higher percentage of cortisonized animals were viremic on days 4, 6, and 8 after infection. Autopsies of the 12 cortisonized monkeys that died revealed macroscopic petechial hemorrhages in the lungs and gastrointestinal mucosal membranes. Variola was found in the viscera of multiple cortisonized animals at autopsy, but no virus was found in the single control animal sacrificed. The pregnant monkey did not receive cortisone, aborted on the sixth day after variolation and died on the twelfth day with extensive hemorrhages in the lung and intestinal mucous membranes at autopsy.

For unknown reasons, few other viral infections, with the exception of Lassa Fever virus and hepatitis E [120], have such an increased case fatality rate in pregnant women as does smallpox. Whether the immunologic paradigm described during pregnancy of increased type 2 cytokines such as IL-4 and decreased type 1 cytokines such as IFN- γ plays an etiologic role in hemorrhagic smallpox is uncertain [59–62]. Interestingly, progesterone, a hormone elevated during pregnancy, has been reported to increase IL-4 production from T-cells, including those not normally producing this cytokine [62]. A speculative analogy to pregnancy and increased risk of severe smallpox exists in the recent experiments with mousepox engineered to express IL-4 and inhibit cytotoxic T-cells that produce IFN- γ , causing more virulent disease and overcoming the protective effect of prior mousepox vaccination.

2.7 International Preparedness for Smallpox

In October 2001, the director of the WHO, Dr. Gro Harlem Brundtland, stated [121]: “I want to emphasize that should an outbreak of smallpox be detected in any country, this should be considered an international emergency. WHO will help countries to pool available resources so as to contain the disease as rapidly and effectively as possible.”

The WHO has continued to provide international support to efforts related to smallpox detection and vaccination, including the provision of educational resources on its website and the sharing of results of annually sanctioned research on variola virus in Russia and the United States. Worldwide, the WHO provides support to surveillance networks for smallpox and other outbreaks via their Global Outbreak Alert and Response Network (GOARN). Preparations for a possible smallpox virus release and other potential bioterrorist events were initiated by a number of nations. These planning efforts involved the WHO, US scientists, public health officials, politicians, regulatory officials, and others.

Fig. 2.3 Smallpox skin lesions (WHO): day 5 of smallpox vs. chickenpox



The WHO has provided regular updates on smallpox and smallpox vaccination since 2001. These updates include addressing smallpox as a global public health emergency if even one case occurs [11–13], posting on their website photographs of smallpox and smallpox vaccination responses, including comparisons with chickenpox (Fig. 2.3), updating in 2004 the WHO 1970 review of the risks posed by biological weapons [5], and posting annually the laboratory research on variola virus in Russia and the United States. Such work has included the pathogenesis of variola infections, serological assays, PCR-based diagnostic assays, animal model development, studies of new vaccines, and antiviral drugs including the tyrosine kinase inhibitors such as the anti-leukemia drug “Gleevec” (5, 13 and www.who.int/csr/disease/smallpox/research/en/).

Israel was the first nation to begin smallpox vaccination following the anthrax attacks of September–October 2001 in the United States. An initial phase of vaccination was carried out between September 2002 and January 2003 when 17,000 first responders, including HCWs, were vaccinated using the Lister strain of vaccinia [122]. A study of a subset of these vaccinees, all of whom had

been previously vaccinated, showed that only 96/158 (61%) of these vaccinees had a successful clinical take, but this was understandable because vaccine titers were much lower than international standards..

On September 5–6, 2002, a multinational (G7+) Global Health Security Initiative (GHSI) workshop was held at the Paul Ehrlich Institute in Langen, Germany, on “Best practices in vaccine production for smallpox and other potential pathogens” [10]. National and regional information on current and projected smallpox vaccines and antiviral drugs was presented by scientists from Japan, the Pan American Health Organization, the European Union, Germany, France, Belgium, the United States, the WHO, and others. The US Food and Drug Administration (FDA) regulatory requirements for licensure of smallpox vaccines were presented [123].

On September 8–10, 2003, an international command post exercise named “Global Mercury,” involving a scenario with multiple “terrorists” who were inoculated with smallpox and traveled to different parts of the world was conducted. Canada, Mexico, Japan, Italy, Germany, France, the UK, the United States, the European Commission, and the WHO were involved with this real-time exercise, details of which were posted on the Health Canada website (www.hc-sc.gc.ca/english/media/issues/global_mercury_summary.html). Six recommendations resulted from this exercise. One of the most important was the recognition of the need to strengthen already existing national smallpox response plans by “greater elaboration of their international components.” It was also found that all forms of communications were to maintain in an adequate real-time manner during the exercise across multiple continents and nations. Better communications infrastructure, improved information management processes, and trained public health personnel were needed [14, 15].

In October 2003, an international conference was held in Geneva on past smallpox weapons development, current threats, and smallpox vaccination issues. Copies of slide presentations were posted online at www.smallpoxbiosecurity.org and partly in a special supplement of the International Journal of Infectious Diseases [124].

In November 2003, at a symposium on smallpox and smallpox vaccination held in Hong Kong, sponsored by the Health Department of Hong Kong, one of us (DL) presented both pre-event and post-event smallpox vaccination scenario discussions. Lessons learned from the US smallpox vaccination program were also presented. The smallpox preparedness program in Hong Kong had already been initiated prior to the onset of the SARS epidemic in February 2003. Issues regarding sharing of smallpox vaccine if needed to help control an international outbreak were also discussed.

As of December 2003, the UK had posted on its Department of Health website (www.dh.gov.uk) its updated smallpox plan [125]. This is a valuable reference document, with 17 appendices and a large amount of detail regarding the common and critical public health issues that would occur anywhere in the world once a smallpox outbreak had occurred. Particularly helpful are highly specific algorithms for how to manage initial suspected smallpox cases

depending on where they are first located. Separate algorithms are given if the suspected case is at home, in the emergency department (“Accident and Emergency”), on a general hospital ward, in a surgery clinic, in an intensive care unit, in an infectious disease unit, or in a port health control unit.

Additionally, detailed recommendations are provided for vaccination strategies at each level of alert, for example, if smallpox is reported outside the UK (alert level 2) or in the UK (alert level 3). Notably, the UK plan includes a specific smallpox outbreak alert level for when a large-scale outbreak occurs that is not contained by “ring” vaccination (alert level 4). Whereas “surveillance and containment” (sometimes referred to as “ring” vaccination) is recommended by the UK for alert levels 2 and 3, as it is done by the WHO, and the CDC mass vaccination is to be considered for alert level 4, depending on the circumstances and the risk/benefit analyses at that time.

In January 14, 2005, a smallpox tabletop exercise named “Atlantic Storm” was carried out, as described online at www.upmc-biosecurity.org. In this hypothetical scenario, terrorists released smallpox virus in six target areas via a commercially available dry powder dispenser hidden in a backpack. The targets included crowded public sites in Frankfurt, Istanbul, Rotterdam, Warsaw, Los Angeles, and New York City [17–21].

Two observations from this Atlantic Storm exercise deserve particular notice: (1) With respect to the availability of vaccine, only 40 of more than 200 nations now have any stocks of smallpox vaccine, and no country has more vaccine than what it believes it would need for its own citizens. The total global stockpiles of vaccine amount to about 750 million doses (about 10–12% relative to the global population); the WHO stockpile consists of only 2.5 million doses; there are only five vaccine production laboratories and, under emergency conditions, output would not be much more than 40 million doses per month. At present, there is, in place, no mechanism for deciding on priorities for global allocation of vaccine in case of an emergency. (2) There is, at present, no forum wherein different countries, in case of an emergency, could work to effect common policies with respect to restrictions on travel, harmonization of national policies, and mobilization of non-health resources.

In sum, the need to work collaboratively on an international basis, including discussion of sharing smallpox vaccine supplies, prior to a smallpox outbreak, is critical to prepare best for what could rapidly become a global public health emergency.

2.8 The Centers for Disease Control and Prevention: Smallpox algorithm for generalized vesicular or pustular rash

After the events of 2001, the CDC developed an algorithm to evaluate suspected patients with smallpox, focusing on patients with fever followed by a generalized vesicular or pustular rash [21]. During 30 months (from January 2002 until

June 2004), the CDC was consulted on 43 patients with suspected smallpox as part of this algorithm [126]. Major and minor criteria were developed (Table 2.3), and “high,” “medium,” and “low” risk patients identified. To decrease the number of false-positive laboratory tests for variola virus, only persons classified by this algorithm as “high-risk” for smallpox underwent testing for variola. Any “high-risk” case was not to undergo laboratory testing for another disease until laboratory testing was completed for variola virus. According to this algorithm, all patients with a generalized vesicular or pustular rash and fever were immediately placed on airborne and contact precautions, and the infection control team was notified.

The CDC investigators reported that, during the 30-month period, none of the 43 cases of suspected smallpox met the criteria for “high-risk”; eight were classified as “moderate-risk”; and 35 as “low-risk”. Despite not being classified as “high-risk,” one patient did have variola testing performed and the result was negative; the final diagnosis was HSV-2. The most common diagnosis was varicella. Of the eight “moderate-risk” cases, five were due to varicella, one due to a drug reaction, one due to erythema multiforme, and one due to eczema. On seven occasions, hospital or emergency department closures or diversions occurred. Use of the algorithm facilitated the prompt reversal of these closures and diversions.

The CDC authors added an important caveat to this algorithm, that it “is not designed to detect the most severe and atypical forms of smallpox – that is, flat-type or hemorrhagic type.” A color poster and online version of this CDC smallpox algorithm, with rapid interactive individual patient classification options, is available via the CDC website at: www.bt.cdc.gov/agent/smallpox/diagnosis/pdf/spox-poster-full.pdf.

The CDC website on smallpox, www.bt.cdc.gov/agent/smallpox/index.asp, contains extensive information on smallpox, smallpox vaccination, contact tracing, quarantine and isolation, criteria for a “contagious” facility where smallpox patients could be hospitalized or otherwise cohorted for care, VIG, cidofovir, and multiple other related issues. Several hundred photographs illustrating key points about smallpox and smallpox vaccination are available (e.g., Figs. 2.1 and 2.2) at www.bt.cdc.gov/agent/smallpox/smallpox-images/.

The CDC has sought outside guidance from the Institute of Medicine (IOM) on smallpox vaccine-related issues. Reports of at least six meetings have been submitted to the CDC director by The Institute of Medicine Committee on Smallpox Vaccination Program Implementation. Each of the six IOM reports can be found online at www.iom.edu/report.asp?id=21243.

The committee recommendations and assessments have traced a course from focusing on smallpox and smallpox vaccination, including active surveillance for adverse events, to recommending that smallpox preparedness be incorporated into a more general “all-hazards” emergency preparedness program. The committee has emphasized that smallpox vaccination is only one aspect of preparedness for smallpox and that detailed preparedness plans for smallpox and other public health emergencies should be written, critiqued, and assessed via training exercises [127].

2.9 Smallpox Vaccines 2004: First, Second, and Third Generations

Smallpox vaccine development can be divided into three generations of vaccines. The first generation vaccines are those that use vaccinia virus grown on the skin of a calf and subsequently purified after harvest. In the United States, through 2007, this vaccine was a lyophilized (freeze-dried) preparation produced by Wyeth–Ayerst that was reconstituted before use [128]. This has been replaced by a second-generation vaccine, ACAM 2000 (see below) [129].

Another first-generation smallpox vaccine, available in the United States, was stored frozen, rather than lyophilized, and approximately 80 million doses were provided to the US government by Aventis Pasteur. Dilution studies in 340 vaccinia-naïve adults using 1:5 and 1:10 dilutions of this vaccine showed equivalent vaccination take rates with this vaccine (undiluted: 100%; 1:5 dilution: 98.2%; and 1:10 dilution: 100%) [130]. Under field conditions, lower take rates would be expected; thus, the vaccine is recommended for use at a 1:5 dilution at most and only under emergency circumstances.

So-called second-generation smallpox vaccines are grown in tissue cell cultures. The vaccinia virus strains are plaque purified strains derived from those used in preparation of the animal lymph vaccines; the frequency of adverse events is expected to be comparable. At least two different tissue cell culture vaccines of this type entered clinical trials. These are ACAM 2000 grown in African green monkey kidney (“Vero” cells) made by Acambis in partnership with Baxter and a chick embryo cell-culture smallpox vaccine made by Bavarian Nordic, a Danish company. The ACAM 2000 vaccine uses a vaccinia virus derived from the New York City Board of Health seed strain [131, 132]; Bavarian Nordic uses a Lister-derived vaccinia strain. On September 1, 2007, the FDA announced licensure of this second-generation smallpox vaccine, ACAM 2000, including a medication guide (www.fda.gov/cber/products/acam2000qa.htm). This six-page medication guide includes information regarding possible side effects of the vaccine, what are the medical conditions that predispose to some of these side effects, how to care for the vaccination site, and what to avoid after getting vaccinated. As of 2007, Acambis has produced and supplied to the US stockpile some 200 million doses of their new vaccine.

Initial Phase I clinical trials of ACAM 2000 demonstrated comparable safety and immunogenicity with the Dryvax first-generation vaccine. In 2004, during the larger Phase III trials required for licensure, patients with myopericarditis were diagnosed, both in the Dryvax comparator-control vaccine recipients and in the tissue culture vaccine recipients. The symptoms were mild and transient, and no sequelae were detected.

Third-generation smallpox vaccines include those made from attenuated vaccinia virus strains, such as MVA and LC16m8, and more recently, DNA-based vaccines [133–135], using only selected DNA segments of the vaccinia virus, rather than the entire virus [133]. The central concept behind the

third-generation vaccines is to increase the safety profile by using attenuated vaccinia viruses or DNA-based vaccines rather than non-attenuated live, replicating vaccinia virus as are used in the first- and second-generation vaccines. The DNA subunit vaccine approach reported in 2004 by Hooper et al. demonstrated that rhesus macaques monkeys were protected from severe disease when a normally lethal challenge with monkeypox virus was administered [135].

MVA is a non-replicating attenuated vaccinia strain. It was developed in Germany in the 1960s and 1970s to be used prior to vaccinating with the traditional Lister lymph vaccine strain in expectation that MVA might protect against vaccine complications caused by the Lister strain. It has not been tested for efficacy during a smallpox outbreak. Immunocompetent monkeys given either two doses of MVA or one dose of MVA followed by one dose of Dryvax [136] have been successfully protected against intravenous infection with monkeypox, an orthopoxvirus closely related to smallpox. The precise vaccine-associated correlates of protection are unknown [137–140], but these authors found that antibody binding and neutralization titers, as well as vaccinia-virus-specific IFN- γ producing T-cells, were equivalent or higher in the immunized monkeys compared with those who received a single dose of the standard lymph vaccine (Dryvax) [136].

Extending the lethal monkeypox virus challenge to immunocompromised monkeys, specifically macaques infected with the AIDS-causing SIV, failed to show vaccine protection if the animals had become severely immunodeficient (CD4+ T-cell counts <300 cells/ul) [141].

On June 4, 2007, the US government announced purchase of approximately 20 million doses of MVA to stockpile in the event of a smallpox bioterrorist attack. Using a two-dose regimen, this stockpile would be sufficient to vaccinate the estimated 10 million immunocompromised people in the USA (www.hhs.gov/news/press/2007pres/06/pr20070604a.html).

Another attenuated vaccinia virus third-generation vaccine, LC16m8, is a smallpox vaccine developed in Japan by repeated low-temperature tissue cell culture passage of Lister strain vaccinia; it was licensed for use in Japan in 1975. The vaccine is a replicating strain, cultured in primary rabbit kidney cell cultures. It has been administered to more than 50,000 Japanese children and produces a smaller primary vaccination lesion and fewer secondary symptoms and signs than Dryvax (www.who.int/entity/csr/disease/smallpox/lance_gordon.pdf).

2.10 Vaccinia Immune Globulin and Antiviral Drug Development

VIG is being produced and tested as an intravenous formulation rather than the intramuscular form that has been traditionally used. In 2004, a study using IV-VIG showed that, compared with the IM product, IV-VIG is very safe and well

tolerated, yielding higher peak levels sooner than the 3–7 days seen after administration of lyophilized IM-VIG. A liquid form of IV-VIG was found to have a comparable adverse reaction rate to the lyophilized formulation [64]. The FDA licensed an intravenous formulation of VIG (“VIGIV” by DynPort Vaccine Company LLC) in February 2005.

In an historical review of VIG use and efficacy, Hopkins and Lane found that there have been no randomized controlled trials of VIG prior to FDA licensure [65]. Thus, recommendations for use of VIG are based on observational data. VIG is believed to prevent or decrease vaccinia complications in persons at increased risk, such as those with eczema or atopic dermatitis. In studies performed in Dutch military recruits, ~50,000 of whom received VIG before smallpox vaccination using a European strain of vaccinia, compared with the same number receiving a placebo before vaccination, only three recruits developed encephalitis in the group receiving VIG compared to 14 controls. The significance of this observation is puzzling as it has been believed that the pathogenesis of post-vaccinal encephalitis is an auto-immune response rather than the result of vaccinia infection of the brain.

No antiviral drug has yet been licensed by the FDA for the prevention or treatment of smallpox, monkeypox, or vaccinia virus disease. In June 2003, the IOM convened a committee to review and discuss the possibilities for the development of smallpox of an antiviral smallpox drug. Several new candidate antiviral drugs and strategies were reviewed, and seven recommendations with regard to future initiatives were made. These included the expansion of broad international collaborations, centralized resources, pharmaceutical company engagement, the training of a new cohort of investigators, and the formation of a high-level oversight panel, much like the AIDS Vaccine Research Working Group that would report to the directors of the NIH, CDC, and other federal agencies [142].

The antiviral drug, cidofovir, has been extensively studied for its possible uses in treatment or prevention of orthopoxvirus infection as well as other DNA viruses. The drug is licensed by the FDA only for the treatment of cytomegalovirus (CMV) in immunocompromised patients with HIV/AIDS. Recent literature on cidofovir for poxvirus infection, including investigational oral formulations, has been published by Bray and colleagues [143–145]. The possible role of cidofovir in prophylaxis is limited as it prevents infection in experimental animals (and presumably man) only if given at the time of actual infection or before. Since vaccination itself serves to protect even when given several days after infection, cidofovir offers no advantage. It has not been shown to have any effect in animal studies after infection is established. The current IV form of cidofovir is highly toxic, including renal toxicity. Renal shut down has occurred after the administration of only a single dose, and an oral formulation is under development.

2.11 Smallpox Preparedness in Hospitals and Public Health Partners

To illustrate one hospital's effort to implement a smallpox response plan, an example is given from Washington Hospital Center, the largest (909 bed) hospital in Washington, DC, where one of us (DRL) has worked, in partnership with the DC Department of Health. By noon of September 11, 2001, a 30-min education program about the major bioterrorism agents, including anthrax, plague, and smallpox, had begun in the Department of Medicine, including trainees and senior physicians. This effort was directed by Infectious Diseases and Infection Control Services and by late September 2001, included many other disciplines. Photographic and written information regarding the clinical recognition and diagnosis of smallpox, issues related to smallpox vaccine and bifurcated needle access limitations, and infection control issues to prevent transmission of variola were discussed. By September 21, thousands of N-95 respirators had been stockpiled with thousands of bottled doses of doxycycline for management of potential bacterial bioterrorism threats. On October 1, the interim biodefense plans were discussed in a meeting with the director of the DC Department of Health and President of the DC Hospital Association. A multi-dimensional educational program about bioterrorism, including smallpox, was initiated throughout the hospital in September and accelerated in October and November after the anthrax attacks.

By December 2001, an initial protocol for the management of patients with suspected or confirmed smallpox was completed by the Infection Control and Infectious Diseases services in coordination with the multidisciplinary bioterrorism preparedness task force working closely with the Department of Emergency Medicine. Information on the potential off-label use of the antiviral drug cidofovir for therapy of variola or vaccinia viruses was obtained and a protocol submitted to the hospital Institutional Review Board (IRB) for its use against smallpox or severe vaccinia reactions not resolved with VIG [146]. In addition, Medicine Grand Rounds was given on smallpox and vaccinia vaccination.

Beginning in January 2002, a series of 17 monthly bioterrorism 2-h continuing medical education (CME) public forums began for regional hospitals, clinics, and public health officials. These presentations were offered across the DC and surrounding areas of Virginia and Maryland within the National Capital Region. The subsequent monthly meetings included discussions on smallpox and smallpox vaccination and included smallpox experts from the NIH and Johns Hopkins University. These monthly meetings included hands-on opportunities to use the bifurcated needle on an artificial skin-covered deltoid teaching device by June 2002.

These hands-on training sessions were combined with a standardized power-point slide presentation and expanded to health-care settings across the region, including private clinics, hospitals, and the Medical Society of DC. Smallpox vaccination information and plans were shared between DC hospitals via the

DC Hospital Association Infectious Disease and Infection Control Committee. These hospitals included three DC-regional military medical facilities as well as the civilian hospitals in DC. The smallpox vaccination training slides were posted on a biodefense website, www.bepast.org, along with related information and frequently asked questions (FAQs).

As partners in this effort, the DC Department of Health began to co-sponsor the training exercises and to issue “smallpox immunization technician” certification cards to over 300 persons completing these hands-on educational sessions. The Department of Health initiated a written record of contact information for recipients of these training certificates, anticipating that in the event of a smallpox emergency these persons could volunteer to assist the Department of Health with vaccination efforts.

The nursing director and senior nurses within the hospital were trained in these same hands-on sessions, and copies of the training slides were provided for “train-the-trainer” exercises with other nurses. Similarly, senior members of the largest DC-regional Visiting Nurse Association (VNA) joined in the training on vaccination issues and how to use the bifurcated needle if they were needed for large-scale hospital or community-based smallpox vaccinations.

Bifurcated needle hands-on training exercises were also coordinated with colleagues in nearby areas of northern Virginia and Maryland starting in September 2002. These sessions included both the respective health departments and clinicians and emergency response volunteers. From this experience, we learned of one superb example of a large community-based “Bioterrorism Medical Action Team (B-MATS)” [147]. Initiated by a senior pediatrician, Dr. Daniel Keim, and his colleagues in Fairfax County, Virginia, and then integrated into, and expanded by, the Fairfax Department of Health, this B-MATS organization began with a focus on being able to administer smallpox vaccinations to everyone in the county on a round-the-clock basis. Thousands of volunteers were organized into teams of 75–80 people, only few of whom were physicians. Specific community facilities that were well known to each neighborhood in Fairfax County were identified as mass vaccination sites. Volunteers were recruited who were not full-time hospital employees to avoid any potential conflict of duties in the event of a major emergency. While the focus of these teams was initially on smallpox mass vaccination, the B-MATS concept was enlarged to include any type of bioterrorism. Details of the Fairfax County B-MATS are posted on their website at www.fairfaxcounty.gov/service/hd/actsurv_clinic.htm.

An illustrated teaching guide for the prevention, diagnosis, and management of the six CDC Category A bioterrorism agents, including smallpox, was created using the acronym “BE Past” for these six agents (botulism, ebola-viral hemorrhagic fevers, plague, anthrax, smallpox, and tularemia) in June 2002. This poster guide was disseminated throughout the hospital, and thousands were provided to regional and national hospitals, clinics, public health facilities, and fire/EMS stations, were posted on the website (www.bepast.org), and shared with colleagues in Italy, Hong Kong, China, Thailand, and the Czech Republic.

By December 2002, a separate nine-page illustrated community guide to smallpox and smallpox vaccine was co-authored with the DC Department of Health. This guide was subsequently distributed in the District of Columbia by the Department of Health. The guide was posted on their health department website [148] and translated into Spanish, Mandarin Chinese, and several additional languages.

In early March 2003, four persons volunteered to be the first to be vaccinated against smallpox by the Washington, DC, Department of Health. Two of the vaccinees, a DC health department pediatrician and a hospital infectious disease physician (DL), then worked with colleagues at Washington Hospital Center and the health department to immunize 40 HCWs on March 20. The following month, a 270-person multidisciplinary smallpox tabletop exercise was organized by the Washington Hospital Center for the DC National Capital Region. Issues ranging from clinical recognition of a smallpox outbreak to communication mechanisms across the region to vaccination plans and implementation were discussed. By 2004, however, only 105 non-military persons had been vaccinated by the DC Department of Health.

2.11.1 *Vaccination Coverage*

Reasons for the near-complete cessation of smallpox vaccination included the following: a lack of adequate liability and compensation protection in place at the time the vaccination program began [149] (Table 2.5); potentially mandated time away from clinical work (“furlough”) until the vaccination scab fell off; perceived health risk of transmitting vaccinia to colleagues or patients; health

Table 2.5 Compensation coverage for smallpox (Vaccinia) vaccine injuries*
Injury or condition in vaccine recipient or in a contact of the recipient.
1. Significant local skin reaction
2. Stevens–Johnson syndrome
3. Inadvertent inoculation
4. Generalized vaccinia
5. Eczema vaccinatum
6. Progressive vaccinia
7. Postvaccinial encephalopathy, encephalitis, or encephalomyelitis
8. Fetal vaccinia
9. Secondary infection
10. Anaphylaxis or anaphylactic shock
11. Vaccinial myocarditis, pericarditis, or myopericarditis
12. Death resulting from an injury referred to above in which the injury arose within the defined time frame.
*Above table and detailed definitions of each vaccine injury listed online at the Health Resources and Services Administration (HRSA) at www.hrsa.gov/smallpoxinjury/table.htm and in the Federal Register [149].

concerns of the candidate vaccinees; insufficient definition of the threat of smallpox being used as a bioterrorism weapon [167]; hospital legal concerns; competing obligations; priorities and resources by departments of health; unanticipated myopericarditis cases (nonfatal); the small number of cardiac deaths that were temporally, but not causally, linked to smallpox vaccination and the media coverage given to them; lack of weapons of mass destruction being found in Iraq after the March 2003 invasion; and the lack of additional terrorism attacks during 2002–2004.

To prepare for a potential bioterrorism agent such as smallpox is also to prepare for an emerging disease such as SARS (for similarities see Table 2.6) [36, 150, 151], or pandemic influenza [152], or another new respiratory infectious disease in terms of similarities in transmission, needed personal protective equipment (PPE), hospital preparedness, and public health responses. Accordingly, over the course of 5 months starting at the end of 2003, the Washington Hospital Center undertook a formal fit-testing program for N-95 respirators that successfully trained over 6,000 clinical and non-clinical workers in the appropriate use of these respirators. This better prepared the hospital to care for patients with a spectrum of droplet or aerosol-transmitted respiratory diseases, including smallpox, viral hemorrhagic fevers, pneumonic plague, and recently emerging diseases such as SARS, avian or pandemic influenza, Nipah virus, and traditional threats such as tuberculosis and measles. Similar to the smallpox hospital outbreak in Meschede, Germany [67], the SARS coronavirus was reported to be transmitted on at least some occasions via droplet

Table 2.6 Similarities between smallpox and SARS

1. Viral etiology: orthopoxvirus (smallpox) vs coronavirus (SARS)
2. No antiviral therapy or prophylactic drug proven effective.
3. Transmission by close contact face-to-face contact, including by respiratory droplets.
4. Transmission sometimes by airborne aerosol: (e.g., smallpox outbreak in a hospital in Meschede, Germany 1970 and SARS in Amoy Gardens apartments, Hong Kong, 2003).
5. Health care workers, family, and other close contacts at high risk or infection.
6. Infection control recommendations include: standard, contact, droplet, and airborne.
7. Personal protective equipment recommended by CDC includes: fit-tested N-95 (or higher) respirator, eye protection, gowns, and gloves.
8. Patients should wear a surgical mask to decrease transmission risk.
9. Patient isolation, contact tracing, and quarantine-monitoring of contacts.
10. Hospitals would actively screen persons entering hospitals for the disease and restrict entrance of visitors to non-essential personnel.
11. Require a “surge” in medical and public health response personnel and facilities.
12. Safeguards would be implemented by blood banks to prevent transfusion-related viral transmission.
13. Significant, and perhaps catastrophic, economic burden to affected nations.
14. On the list of mandated reportable diseases in the USA.
15. Would involve potential limitations on travel and gathering of large numbers of people.
16. Would require a coordinated global response, with the WHO, CDC, and National and Health Departments.

nuclei as an aerosol such as in the Amoy Gardens residential complex outbreak in 2003 [153].

At the city-wide level, while working at the DC Department of Health in the spring of 2004, one of us (DL) initiated the purchase and stockpiling of a large number of N-95 respirators (435,000) and surgical masks (2.5 million). Because of the experience of other nations hard hit by the SARS epidemic, acute shortages of N-95 respirators and surgical masks must be anticipated. These would need to be available immediately following the release of smallpox, certain hemorrhagic fevers, pandemic influenza, SARS, or other bioterrorist or emerging pathogens.

To prepare for smallpox is to prepare for many of the other public health threats that we face today and will face in the future. The integration of smallpox preparedness measures into broader public health preparedness locally, regionally, and nationally is one of the primary recommendations from the Committee on Smallpox Vaccination Program Implementation of the IOM of the National Academy of Science [127]. Explicit comparisons between smallpox, pandemic influenza, and SARS are made in the August 28, 2004, US draft pandemic influenza plan [152].

Preparing optimally for smallpox in the United States requires preparing on a global basis to prevent the return of smallpox. Whereas an intensified global program was required to eradicate smallpox from the human population in the 1960s and 1970s, today and in our future an intensified long-term global program of watchfulness and preparation for response is required to prevent smallpox from being reestablished as an endemic disease.

2.12 Monkeypox: An Emerging Disease in the United States in 2003 and in Sudan in 2005

In 2003, an outbreak of monkeypox occurred for the first time in the United States [154, 155]. The outbreak component involving humans began in May and was confirmed by laboratory testing at the CDC by early June 2003. Monkeypox, known in animals since 1958 and in humans since 1970, is an orthopoxvirus related to variola and vaccinia viruses. Monkeypox had never previously been reported outside of Africa. There, secondary attack rates were low and did not extend beyond four generations, making it a disease of low epidemic potential. Two clades had been found, one West African and one Congolese. Unlike smallpox, monkeypox is a zoonosis. As in Africa, the US outbreak in 2003 was linked to infected animals.

In general, clinical manifestations of monkeypox, including fever and the sequential appearance and resolution of the skin lesions, as well as the incubation period, are similar to smallpox. One specific finding that distinguishes monkeypox from smallpox is lymphadenopathy, especially in the cervical and inguinal regions, in patients with monkeypox [156]. Chickenpox (VZV) is

another disease that should routinely be considered in the differential diagnosis of monkeypox. Indeed, in 2007 Rimoin and colleagues from the United States, the Democratic Republic of the Congo (DRC), and the WHO reported in a laboratory study of 136 patients from the DRC with suspected monkeypox, 51 (37.5%) were confirmed to have monkeypox, whereas 61 (45%) had Varicella-Zoster Virus (VZV) and one had infection with both viruses [157].

The potential for monkeypox to be an emerging disease problem was discussed by Breman in 2000 and is related to the increasing contact of humans with animals in endemic areas and the waning of vaccinia-induced immunity [158]. In addition to the US outbreak in 2003, Inger Damon at the CDC and colleagues from the WHO and Medecins sans Frontiers (MSF) reported in 2006 laboratory confirmation of monkeypox virus for the first time in Sudan. They identified monkeypox virus isolated in November 2005 from the mother of a young child in southern Sudan, both of whom had clinically suspected monkeypox. Genetic sequencing of this isolate was most consistent with the clade from the Congo basin. Epidemiologic studies in Sudan by the MSF revealed “small clusters of self-limited disease compatible with monkeypox had occurred that were not widely spread within the community. No deaths were reported among patients with suspected cases” [159].

The endemic natural host for monkeypox is not known with certainty, but serologic evidence of orthopoxvirus infections, presumably due to monkeypox, has been found in some rodents in Africa, including the Gambian giant pouch rat and the rope squirrel. The first outbreak in the United States was traced to imported wild rodents from Accra, Ghana, West Africa, that arrived in Texas on April 9, 2003. Subsequent testing of these animals by the CDC revealed PCR, and virus isolation demonstrated that at least one Gambian giant rat, three dormice, and two rope squirrels were positive for monkeypox. Some of the Gambian giant rats were housed with prairie dogs at an Illinois pet distributor [160]. These prairie dogs were found to be susceptible to monkeypox, and close contact between humans and infected pets, including prairie dogs, resulted in monkeypox infection of at least 37 persons. Initial studies of the pathogenesis of monkeypox in the North American prairie dog were published in 2004 [161].

Most of these 37 laboratory-confirmed infections in the United States were mild, manifest as fever and a rash with a limited number of lesions. Patients were confirmed with monkeypox in Illinois, Indiana, Kansas, Missouri, and Wisconsin. Occupational exposure to prairie dog–associated monkeypox infection in veterinary facilities in Wisconsin was reported in detail [162]. On June 11, 2003, a combined order from the CDC and the FDA prohibited the importation of African rodents and the sale or transport in the United States of six genera of African rodents and of US prairie dogs [160]. All patients had had direct exposure to infected animals; no evidence of person-to-person transmission occurred [163]. One-third of the patients had a known history of at least one smallpox vaccination in the distant past.

None of the patients died. Two children became seriously ill, but eventually recovered. One child had respiratory difficulty with marked cervical

adenopathy and pharyngeal lesions, but did not require mechanical ventilation. Severe encephalitis occurred in one previously healthy 6-year-old whose family purchased one of the monkeypox-infected prairie dogs [164]. Two adults in this same family had mild monkeypox disease. One of these adults who had been vaccinated as a child had minimal symptoms and very few skin lesions. All three members of the family had one or more skin lesions on the palms. The child with encephalitis also had cervical lymphadenopathy, an uncommon finding with smallpox. She developed seizures and was placed on a ventilator. Her cerebrospinal fluid was IgM positive for orthopoxvirus, while PCR and culture of the CSF were negative. Eventually, she made a full recovery. As with smallpox and vaccinia-associated encephalitis, neither VIG nor cidofovir are recommended in the treatment of monkeypox encephalitis. Neither agent was given to this patient.

On June 12, 2003, the CDC issued interim guidance regarding use of smallpox vaccine, VIG, and the antiviral drug cidofovir for prevention and therapy of monkeypox during this outbreak [165]. Regarding smallpox vaccination (vaccinia virus), it was known from studies in Africa to confer protection ($\geq 85\%$) against monkeypox when given before exposure to monkeypox. Unlike smallpox disease [166], no data exist on the efficacy of giving smallpox vaccine after exposure to monkeypox, in terms of preventing or decreasing the severity of illness although it is believed that it would be effective. Accordingly, the CDC offered guidance for use of smallpox vaccination in different groups at risk for exposure to monkeypox.

HCWs caring for proven or suspected cases of monkeypox, and veterinarians exposed to animals (such as prairie dogs) with monkeypox, were advised to receive the smallpox vaccine within 4 days of initial exposure and to consider vaccination up to 2 weeks after the most recent exposure. CDC also advised that even previously vaccinated HCWs workers should continue to use PPE including a fit-tested N-95 respirator and should follow airborne, contact, and standard infection control precautions. HCWs who could be assigned to care for patients with monkeypox in the future were advised to receive smallpox vaccine and to have a confirmed take before caring for such patients. If this had not been done, then vaccination just before caring for these patients was indicated.

Similar to smallpox, vaccination of close contacts of monkeypox patients was addressed by CDC. The same working definition for close contact was applied to monkeypox as has been used for smallpox.

Some, but not all, medical contraindications to smallpox vaccination were maintained even for persons with close or intimate contact with a symptomatic, laboratory-confirmed case of monkeypox within the prior 2 weeks. Persons with T-cell immunodeficiencies were advised not to be vaccinated, including AIDS-defining CD4 T-cell counts, solid or bone marrow transplant recipients, or other persons receiving high-dose immunosuppressive medications, hematologic malignancies, or congenital T-cell defects. Otherwise, neither pregnancy, nor age or a history of active eczema were to be considered contraindications to smallpox vaccination.

VIG was not recommended for either prophylaxis or treatment of monkeypox patients because no data existed on its role in either setting. Consideration of cidofovir was only to be given as a last resort in the clinical setting of a life-threatening monkeypox infection, and not for prophylaxis.

The pathologic findings and clinical presentation of monkeypox were reported in two of the prairie dogs infected during this outbreak in the United States [161]. Evidence of viral replication was found in both the lungs, where a necrotizing bronchopneumonia was found, and in ulcerative lesions of the tongue. The potential for transmission of monkeypox from both mucocutaneous exposures and from the respiratory route is evident. Given the susceptibility of the prairie dog to severe monkeypox disease, this animal could serve as a model for further research into antiviral drug and new vaccine development against monkeypox [161]. There is no evidence to date that monkeypox has become endemic in US animals such as the prairie dog, as occurred with *Yersinia pestis* (plague) after emerging in California in the early 1900s following its spread from China (Guangdong Province to Hong Kong [167]) to San Francisco.

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