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Smallpox: a potential agent of bioterrorism

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Abstract

The events of 11 September 2001, in New York City, and subsequent identification of anthrax in the United States Postal System, have generated a new sense of awareness for the potential of biological terrorism, if not warfare. Among those agents identified by the Centers for Disease Control and Prevention as 'Class A Bioterrorist Threats', smallpox is among the most dangerous. The ease of transmission of this agent, the lack of immunity in the population at large to this agent, and rapidity of its spread, if released, all generate significant concern for its deployment. A vaccine directed against smallpox is available but it is also associated with significant adverse events—some of which are life-threatening. Further, no antiviral drug has proven efficacious for therapy of human disease, although one licensed drug, cidofovir, does have in vitro activity. Regardless, heightened awareness should lead to the development of a vaccine without significant adverse events and safe and efficacious antiviral drugs. The availability of a vaccine and antiviral drugs that are safe would significantly remove any major threat of smallpox deployment by a terrorist.

Keywords: Smallpox; Bioterrorism; Vaccination

1. Introduction

Smallpox is one of the oldest recorded infections of mankind. Likely, this agent, also known as *variola*, evolved by adaptation to humans from a rodent cowpox-like virus through an intermediate host, such as cattle. The earliest descriptions of smallpox date to 10,0000 B.C. in Asia and India. Subsequently, the spread of infection can be traced to Pacific Rim countries in the East and Europe and North Africa in the West. By the 17th century, smallpox was introduced into North America from Europe. At its peak, namely when the World Health Organization (WHO) decided to initiate an eradication program, 10–15 million cases occurred annually, as reviewed (Fenner, 2002).

Smallpox resulted in one of the first effective preventive measures for an infectious disease, namely immunization. Interesting, as early as 1000 A.D., dried smallpox scab material was utilized in China for intranasal inhalation in order to develop protective immunity. In India, the same material was used to generate pustules to cause variolation, resulting in disease protection. Importantly, and surprisingly, the mortality following vaccination by such procedures, even if the material contained live virus, was approximately 2% rather than the customary 30%. Prevention by vaccination, however, was not introduced as a standard procedure until the late 18th century when it was recognized by Edward Jenner that milkmaids who acquired cowpox were resistant to smallpox. By the middle to late 19th century, utilization of vaccinia for the prevention of smallpox was routine. By the 1950s most industrialized countries had eliminated endemic smallpox by the use of vaccine prepared on the skin of either cattle or sheep and suspended in bactericidal concentrations of glycerol.

As early as 1958, the possibility of global eradication was suggested. Eradication was accomplished in only a few developing countries by 1965; however, in 1967, WHO launched an 'Intensified Eradication Program'. Utilizing the unique epidemiologic intervention principle of Foege and colleagues, known as 'ring vaccination', cases gradually decreased in West Africa. Henderson and colleagues facilitated the WHO program leading to global eradication with the last case occurring in Somalia in October of 1977 (World Health Organization, 1980; Fenner et al., 1988). Routine smallpox vaccination of civilians was discontinued in the United States in 1982.

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With the worldwide eradication of smallpox, the WHO launched an effort to destroy the remaining stocks of virus known to exist at the Centers for Disease Control and Prevention in the United States and in Koltsovo at the State Center of Virology and Biotechnology (Vector). As the issues of destruction of smallpox were publicly debated, societies of the western world developed an increasing concern that stocks of smallpox existed in the hands of individuals for its clandestine use in an offensive biowarfare program, particularly in individuals and countries as Iraq, Korea, Iran, and Libya. Polar positions existed in the United States regarding the destruction of the smallpox samples. Ultimately, however, following an Institute of Medicine Advisory Committee, (Institute of Medicine, 1999) a recommendation to maintain the stocks for purposes of antiviral and vaccine development was put forward by then President Clinton and supported by the Department of Defense and the United States Congress. This recommendation receives continued support from the Department of Health and Human Service and the Department of Defense.

Initially, scientists believed that the possibility of the reappearance of smallpox was miniscule; however, the safety of the population at large changed dramatically after the events of 11 September 2001, with the destruction of the World Trade Centers in New York City, United States. Further, the subsequent deaths related to anthrax in postal workers and community members heightened the possibility of the deployment of microbes for bioterrorism, particularly in developed societies. Because of waning immunity to smallpox, this agent becomes one of the most likely for consideration as a microbe of bioterrorism. Its pathogenicity is so virulent that only 50–100 cases could lead to massive outbreaks of disease (Meltzer et al., 2001). The epidemiology, natural history, and its use as an agent of bioterrorism will be described.

2. Epidemiology

At the beginning of the 20th century, smallpox existed worldwide; however, its distribution was not uniform as areas of endemicity existed. Two principle forms of the disease exist: variola major and the much milder form variola minor (or alastrim), as reviewed (Dixon, 1948; Marsden, 1948; Mack, 1972; Fenner et al., 1988; Henderson et al., 1999; Fenner, 2002). In 1970, 1300 new cases occurred in 1000 villages in Southwest India and in 1973, 10,000 new cases occurred in India.

Smallpox is a viral disease that is unique to humans; no known animal reservoirs exist. In order to sustain itself, virus must be transmitted from person-to-person. The mechanism of spread is by droplet, aerosol or direct person-to-person contact. Typically, an infected individual will cough or sneeze and virus is transmitted to the oral mucosa of a susceptible host. Direct contact is also a route of transmission, including contact with contaminated clothing or bed linens. The disease is characterized by a seasonal distribution with spread occurring during the late winter and early spring; a time when chickenpox is prevalent in most communities. However, it can be transmitted in any climate and in any part of the world. As would be predicted, transmission within families is increased by overcrowding during rainy periods. On the other hand, transmission between communities was increased by the greater mobility of individuals during dry periods. The transmission of smallpox among populations is slower than that of chickenpox or measles. As noted, spread was primarily to family members and friends but not among classroom contacts. The reason for this latter observation is based on the fact that transmission did not occur until the onset of rash. Since disease onset was abrupt with fever and malaise, confinement occurred early in the course of illness.

After the early descriptions of smallpox, the distinction between variola major and variola minor was defined on epidemiologic grounds. In Asia, for example, variola major was associated with a mortality of 30% or higher. In contrast, in South America and sub-Saharan Africa, a similar clinical entity resulted in a mortality of 1% or less, and was designated variola minor. Importantly, through the end of the 19th century, variola major predominated throughout the world. However, at the turn of the century, variola minor was detected at the very southern extremes of Africa and, subsequently, Florida. The distinction between these two strains relates to genetic and growth characteristics of the causative viruses in vitro.

Smallpox was typically a disease of children as nearly one third of case occurred under the age of 5 years and nearly three quarters in individuals less than 14 years. However, in rural communities where vaccination and natural infection were less common, disease incidence paralleled the age distribution. Both sexes are equally affected. The incidence of smallpox was higher in lower socioeconomic groups, presumably secondary to overcrowding.

Patients suffering from smallpox are most infectious during the early stages of illness, namely the first 7–10 days after the onset of lesions but, not before. Transmission occurs most frequently 4–6 days after the onset of cutaneous lesions. At that time, the skin lesions are in a papulovesicular stage. However, as scabs formed, infectivity waned rapidly. Patients were considered infectious until all the crusts separated.

3. Clinical manifestations

Issues relevant to clinical variola are summarized in Table 1. Infection is initiated by viral replication on the respiratory mucosa. Primary viremia leads to seeding of the reticuloendothelial system (Dixon, 1948; Marsden, 1948; Mack, 1972; Mack et al., 1972; Henderson et al., 1999). Secondary viremia results in clinical disease associated with fever, malaise and myalgia. Virus localizes in small blood vessels of the dermis. The incubation period for smallpox is characteristically 12 days. The first clinical sign of infection

Table 1
Smallpox

Clinical features	Flu-like symptoms with 2–4 day prodrome of fever and myalgia Rash prominent on face and extremities including palms and soles Rash scabs over in 1–2 weeks Rash onset is synchronous
Mode of transmission	Person-to-person
Incubation period	1 day–8 weeks (average 5 days)
Communicability	Contagious at onset of rash and remains infectious until scabs separate (about 3 weeks)
Infection control practices	Contact and airborne precautions N95 respirator Private room or cohort Discharge when noninfectious
Prevention	Live-virus intradermal vaccine that does not confer lifelong immunity Contact CDC Previously vaccinated person should be considered susceptible
Supply assessment	Number of airborne precautions rooms available Number of N95 respirators available Vaccine availability
Postexposure prophylaxis	Smallpox vaccine only within 3 days of exposure If greater than 3 days, vaccine and vaccinia immune-globulin (VIG) Instruct exposed individuals to monitor self for flu-like symptoms or rash for 7–17 days
Treatment	There is no licensed antiviral for smallpox (cidofovir is experimental) Supportive care

is a prodromal illness which last 2–4 days, characterized by malaise, headache, high fever, vomiting, and delirium. Likely, prodrome coincides with the phase of secondary viremia. As prodrome progresses to the third or fourth day, buccal and pharyngeal lesions begin to appear. Rash begins on the face and spreads to the forearms and hands and, then, to the lower limbs and trunk. Lesions were always more numerous on the face than other areas of the body. Lesions begin as macules and quickly evolve to papules and, subsequently, to vesicles by about the fifth day of illness. Pustules appear about the eighth day of illness. The pustules are usually round and tense and deeply embedded in the dermis. Pustules are followed by scabs and, ultimately, scars.

Hemorrhagic smallpox does occur, being the most serious form of disease and usually fatal. As would be anticipated, hemorrhages into the skin or mucous membranes characterize this clinical presentation. Secondary bacterial infection was not common. Death usually occurred during the second week and was attributed to immune complex mediated shock.

The illness associated with variola minor is less severe with few constitutional symptoms and a less pronounced rash.

The disease most commonly confused with smallpox is chickenpox (described later). During the first 2–3 days of rash, it may be difficult to distinguish these two entities. Chickenpox is characterized by the development of a rash that involves lesions in all stages of development—maculopapules, vesicles, pustules and scabs. Nevertheless, smallpox lesions do not demonstrate all stages of evolution simultaneously. The lesions of chickenpox tend to involve the extremities to a greater extent than the trunk.

4. Diagnosis

The identification of a single suspected case of smallpox should be treated as an international health emergency and brought immediately to the attention of national officials through local and state health departments. As discussed above, the clinical findings resemble those encountered with chickenpox. Laboratory confirmation of the diagnosis in a smallpox outbreak is important. Specimens should only be collected by someone who has been recently vaccinated. Vesicular/pustular fluid should be harvested and transmitted immediately to state or local health department laboratories for confirmation. Laboratory examination requires high containment (BL-4) facilities and should only be undertaken by experienced personnel. Typical approaches to the identification to the agent include electronmicroscopy, polymerase chain reaction, and isolation in cell culture. Differentiation from chickenpox can be accomplished by staining of scraped skin lesions with monoclonal antibodies directed against varicella zoster virus (Meltzer et al., 2001). A potentially confusing diagnosis, and one which occurs in a period of global travel, is that of monkeypox which would be identified in typical diagnostic assays (Centers for Disease Control and Prevention, 1998). From 1970 to 1986, there were 400 cases worldwide with recent outbreaks in sub-Saharan Africa. This disease more closely resembles chickenpox but has a 5–10% mortality. Monkeypox is indistinguishable from smallpox with the exception of the enlargement of cervical and inguinal lymph nodes. Also, monkeypox resolves more promptly.

5. Vaccination

In the United States, vaccination against smallpox was discontinued in 1982 (Centers for Disease Control and Prevention, 1990). Thus, a significant portion of the American population is susceptible to smallpox. Persistence of detectable antibodies as detected by ELISA, particularly for older individuals, is approximately 5–10 years (El-Ad et al., 1990). However, persistence of neutralizing antibody has been documented in a few individuals >10 years after

Table 2
Complications of Smallpox Vaccination in the United States for 1968 (Henderson et al., 1999)

Vaccination status age (years)	Estimated number of vaccinations	Number of cases						
		Postvaccinial encephalitis[a]	Progressive vaccinia[a]	Eczema vaccinatum[a]	Generalized vaccinia	Accidental infection	Other	Total
Primary vaccination[b]	5,594,000	16 (4)	5 (2)	58	131	142	66	418
Revaccination	8,574,000	0	6 (2)	8	10	7	9	40
Contacts	. . .[c]	0	0	60 (1)	2	44	8	114
Total	14,168,000	16 (4)	11 (4)	126 (1)	143	193	83	572

Adapted from JAMA 1999, 281, 2127–2137.
[a] Data in parentheses indicate number of deaths attributable to vaccination.
[b] Data include 31 patients with unknown vaccination status.
[c] Ellipses indicate contacts were not vaccinated.

vaccination. Regardless, the following can be concluded. First, immunity is not life long (Kempe, 1960). Second, some persistence of immunity has been documented in a limited number of individuals. In studies performed in Scandinavia, individuals exposed to smallpox but immunized as children had a lower mortality upon exposure to variola major than those not vaccinated. The implications for these findings are unclear.

Vaccination consists of the administration of vaccinia virus grown on scarified scabs of calves. Vaccine production is, likely, the crudest of all vaccines available. After purification, virus is freeze dried in rubber-stopped vials that contain sufficient vaccine for at least 50 doses. Vaccine is administered with a bifurcated needle (Henderson et al., 1972). Vaccine should be stored at −20 °C. Currently, there are approximately 90–100 million doses of vaccine available for administration in the United States.

Vaccination is not without complications. First, and most importantly, no immunocompromised host should be vaccinated, as illustrated by an immunocompromised military recruit inadvertently vaccinated (Redfield et al., 1987). Second, the rate of postvaccine encephalitis is approximately 2.3–2.9 cases per one million vaccinations with an associated 25% mortality (Lane et al., 1969; Goldstein et al., 1975). In addition, vaccinia gangrenosum occurs in approximately 2.6 per one million vaccinations and is associated with a high mortality. Generalized vaccinia is usually not fatal but occurs in as many as 290 individuals per one million vaccinations. Vaccine complications are summarized in Table 2 (Henderson et al., 1999).

6. Postexposure treatment

At the present time, no antiviral drug has been shown to be effective in the prevention or treatment of smallpox. However, cidofovir, a licensed phosphonate analog has demonstrated in vitro activity against monkeypox, vaccinia and variola (Neyts and De Clercq, 1993; Lalezari et al., 1997; Bray et al., 2000; Smee et al., 2001a,b). Furthermore, it has activity against other pox viruses as well (Meadows et al., 1997; Davies et al., 1999; Zabawski and Cockerell, 1999; Ibarra et al., 2000; Toro et al., 2000; Geerinck et al., 2001). While this drug is active in vitro, cidofovir does have significant nephrotoxicity. In addition, lipid prodrugs of cidofovir that are orally bioavailable are under investigations (Neyts and De Clercq, 2001; Huggins et al., 2002; Kern et al., 2002; Smee et al., 2002). Cidofovir administration should be by physicians experienced with its use. In the opinion of this author, cidofovir is a logical current choice as a treatment. With the availability of vaccinia immunoglobulin, smallpox, at least, would at least be ameliorated if an outbreak occurred (Sharp and Fletcher, 1973).

7. Development of new vaccines

The original smallpox eradication campaigns used vaccines that were derived from many vaccinia virus strains, including the New York calf lymph virus, a New York City chorioallantoic membrane strain, EM-63 (USSR) and Temple of Heaven (China). By the late 1960s, over 70 produces used 15 principal strains of vaccinia virus for the development of vaccines. However, one strain, the ‘Lister’ or ‘Elestree’ derived from sheep in the United Kingdom, became the most prevalent used throughout the world. Historically, most of these vaccines were produced in live animals. More recently, the use of primary cell substrates, particularly embryonated chicken egg-produced smallpox vaccine, would help avoid some of the potential problems associated with vaccine production in animals. These problems include: harvesting, contamination, adventitious agents, allogenicity, and accompanying animal proteins. In addition, the Food and Drug Administration (FDA) has licensed live-virus vaccines that have been produced in diploid cell substrates (e.g. MRC-5, WI-38). The MRC-5 cell line was used for the preparation of a vaccine evaluated in a Phase I clinical trial (McClain et al., 1997). Likely, the FDA will consider acceptable the production of live smallpox vaccines produced in these diploid cell substrates.

Alternatively, the continuous cell line Vero has been used to prepare inactivated virus vaccines, particularly the inacti-

vated polio vaccine. While the FDA has not yet licensed this substrate for live-virus production, international experience suggests that it may be a suitable substrate for a smallpox vaccine. The selection of cell substrates of vaccine production in Vero cells has recently been addressed in an FDA letter (http://www.fda.gov/cber/letters.htm).

Strains selected for vaccine production warrants note. The LC16m8, an attenuated vaccinia virus strain was developed in Japan for primary vaccination in 1975. It was derived by passing the Lister strain 36 times through primary rabbit kidney cells at low temperature. Initial studies indicated lower reactive genicity with acceptable immunogenicity. Of note, there was lower neurovirulence in a monkey assay (Hirayama, 1975). The most highly attenuated vaccinia strain is the Ankara. It has been passaged over 570 times in chicken embryo fibroblasts. This virus is host restricted, being unable to replicate in human and other mammalian cells. Thus, for all intense purposes it behaves like an inactivated virus being acceptable in high-risk individuals. It has been safely used in over 100,000 persons in Turkey and Germany; however, its effectiveness in its prevention of smallpox is unknown.

Each of these constructs, as well as genetically engineered viruses is under consideration for use in humans. Of note, the recent availability of vaccinia pools at the Adventis laboratories in Swiftwater, PA, USA of classic vaccine stocks, remove some of the intense pressure for the immediate development of new constructs.

8. Bioterrorist use

Likely, smallpox was first initially deployed as a biological weapon during the French and Indian Wars (1754–1767) by British forces in North America. Apparently, blankets contaminated with smallpox virus (obtained from infected patients) were distributed to American Indians. The resulting epidemics led to a mortality of greater than 50% in the effected tribes, as reviewed (Henderson et al., 1999).

More recently, the potential spread of smallpox, if used as a biological weapon, was illustrated by two European smallpox outbreaks in the 1970s. These outbreaks were not thought to be intentional. The first occurred in Mashede, Germany in 1970 when aerosol deployment led to a widespread outbreak, even when low doses of smallpox were released.

The second outbreak occurred in Yugoslavia in 1972. In spite of routine immunization, a single case led to a logarithmic increase in the number of transmissions from person-to-person. From these two European studies, it would be anticipated that exposure of a limited number of individuals would result in an expansion factor of 10- to 20-fold. Inactivation of aerosol virus takes place over a period of approximately 48 h, as reviewed (Fenner, 2002).

9. Management of a smallpox outbreak

As soon as a diagnosis of smallpox is entertained, suspected infected individuals should be isolated and all household contacts vaccinated, if vaccine is available. Because of the potential of aerosol transmission, if feasible, patients should be managed in the home environment as it may prevent person-to-person spread. Vaccination administered within the first few days after exposure (up to 4 days) may prevent or significantly ameliorate subsequent illness. Currently, vaccine deployment, namely ‘ring vaccination’ versus universal, is under discussion to prevent the appearance of new cases.

Because of aerosol transmission, smallpox transmission within the hospital environment has been recognized as a problem for some time. As a consequence, many health care providers established two facilities for the delivery of health-related services during epidemics of smallpox. Standby hospitals dealt only with patients having smallpox.

10. Summary

The potential use of smallpox has ominous implications, particularly given its rapidity of spread amongst susceptible individuals. As we have learned from the deployment of anthrax in North America during the fall of 2001 and early 2002, aerosol release of a potentially life-threatening agent is both feasible and devastating. The terror engendered by such a series of events is such that it provides an agent with apt capability of serving the purposes of generating fear. With the recognition that improved vaccines will take time to develop and the lack of immediate antiviral therapy, research efforts to develop treatments and improve vaccines should be of high priority to the United Stated Research Establishment. As reported in *Emerging Infectious Diseases* in July of 2002, all of the above suggestions are being investigated by a team of investigators from the CDC (LeDuc et al., 2002).

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