Chapter 11

SMALLPOX AND RELATED ORTHOPOXVIRUSES

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INTRODUCTION

Variola, the virus that causes smallpox, is one of the most significant bioterrorist threat agents. During the 20th century, smallpox is estimated to have caused over 500 million human deaths.[1] Yet the disease and the naturally circulating virus itself were eradicated by the World Health Organization's (WHO) global eradication campaign, which was declared a success in 1980.[2] This program, which involved vaccinating all humans in a ring surrounding every suspected case of variola infection, was successful in part because smallpox is solely a human disease; there are no animal reservoirs to reintroduce the virus into the human population. The impact of a smallpox virus attack in the human population would be even more catastrophic now than during the 20th century, because most vaccination programs were abandoned worldwide in the 1970s, the prevalence of immunosuppressed individuals has grown, and mobility, including intercontinental air travel, has accelerated the pace of viral spread. Smallpox virus is stable, highly infectious via the aerosol route, and highly transmissible from infected to susceptible persons, and it has a relatively long asymptomatic incubation period, making contact tracing difficult.[3] Mathematical models of a variola reintroduction into contemporary human populations indicate dire consequences.[4] Public health experts have argued that a significant portion of the population should be prevaccinated to blunt the impact of such an attack.[5] However, the vaccine is associated with significant adverse events,[6] which are more serious in persons who are immunocompromised, and prerelease vaccination is contraindicated for a significant portion of the population.

Recent revelations that the former Soviet Union produced ton quantities of smallpox virus as a strategic weapon[3] and conducted open-air testing of aerosolized variola on Vozrozhdeniye Island in the Aral Sea have increased the plausibility of variola being used as a bioterrorism agent.[7] Considerable investment is being made in biopreparedness measures against smallpox and related orthopoxviruses, including emergency response plans for mass immunization and quarantine,[8] as well as development of improved countermeasures such as new vaccines and antiviral drugs.[9] These countermeasures are also needed to respond to the public health threat of the closely related monkeypox virus, which occurs naturally in western and central Africa and produces a disease in humans that closely resembles smallpox. Alibek claimed that monkeypox virus was weaponized by the former Soviet Union.[10] Monkeypox virus was imported inadvertently into the United States in 2003 via a shipment of rodents originating in Ghana, where, in contrast to the significant morbidity and mortality seen in the Democratic Republic of Congo, little morbidity was associated with infection. Over 50 human infections were documented in the United States as a result, demonstrating the public health importance of this agent and the potential bioterrorist threat.[11,12]

AGENT CHARACTERISTICS

Classification

Poxviruses infect most vertebrates and invertebrates, causing a variety of diseases of veterinary and medical importance. The poxvirus family is divided into two main subfamilies: (1) the *Chordopoxvirinae*, which infects vertebrates; and (2) the *Entomopoxvirinae*, which infects insects. Subfamily *Chordopoxvirinae* is divided into eight genera, one of which is *Orthopoxvirus*, which includes the human pathogens variola (Figure 11-1), monkeypox virus, and other species that infect humans such as cowpox and vaccinia viruses. Members of the *Orthopoxvirus* genus are mostly zoonotic pathogens, and a few of these viruses produce disease in humans (Table 11-1).

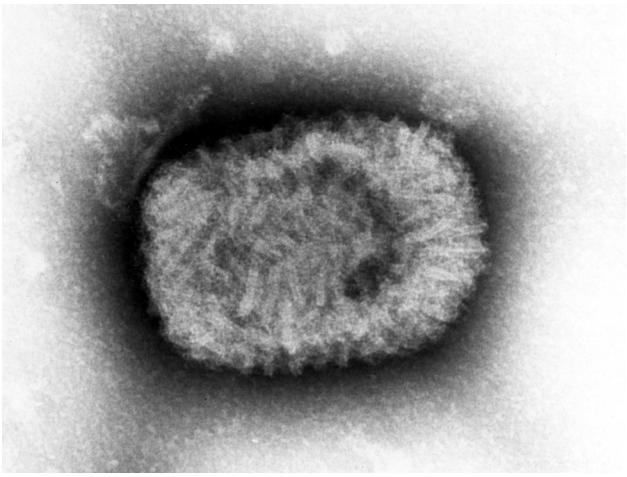

Fig. 11-1. A transmission electron micrograph of a tissue section containing variola viruses.
Photograph: Courtesy of FA Murphy, University of Texas Medical Branch, Galveston, Texas.

Morphology

Orthopoxviruses are oval, brick-shaped particles with a geometrically corrugated outer surface. Their size ranges from 220 nm to 450 nm long and 140 nm

TABLE 11-1

POXVIRUSES THAT CAUSE HUMAN DISEASE

Genus	Species	Animal Reservoir
Orthopoxvirus	Variola virus	None
	Vaccinia virus	Unknown (none?)
	Cowpox virus	Rodents
	Monkeypox virus	Rodents
Parapoxvirus	Bovine popular stomatitis virus	Cattle
	Orf virus	Sheep
	Pseudocowpox virus	Cattle
	Seal parapoxvirus	Seals
Parapoxvirus	Tanapox	Rodents (?)
	Yabapox virus	Monkeys (?)
Molluscipoxvirus	Molluscum contagiosum virus	None

to 260 nm wide. The outer envelope consists of a lipoprotein layer embedding surface tubules and enclosing a core described as biconcave because of an electron microscopy fixation artifact. The core contains the viral DNA and core fibrils, and it is surrounded by the core envelope and a tightly arranged layer of rod-shaped structures known as the palisade layer. Between the palisade layer and the outer envelope are two oval masses known as the lateral bodies (Figure 11-2). Two infectious forms of orthopoxviruses (described next) result from the replication cycle.

Phylogenetic Relationships

The evolutionary relationships among the poxviruses have been facilitated by the recent availability of complete DNA sequences for over 30 species. Phylogenetic analysis reveals that variola and camelpox viruses are more closely related to each other than any other members of the genus, and vaccinia is most closely related to cowpox virus strain GRI-90.[13,14] Cowpox virus strain GRI-90 appears to be less closely related to cowpox virus strain Brighton, indicating that at least two separate species are included under the name cowpox virus. Monkeypox virus does not group closely with any other orthopoxvirus, which indicates that it diverged from the rest of the genus members long ago. Yet vaccination prevents monkeypox. Minor modifications to the camelpox virus genome might result in a virus with variola attributes. Virulence or attenuation may hinge on a few genetic determinants. For example, variola major (associated with a 30% fatality rate) and variola minor (< 1% fatality rate) are greater than 98% identical over the length of the 185,000-kilobase (kb) genome.

As anticipated from the genomic homologies, members of the *Orthopoxvirus* genus are antigenically related. Serum absorption and monoclonal antibody studies have identified cross-reacting and species-specific neutralizing antigens.[15] Nine neutralizing epitopes have been identified among the intracellular

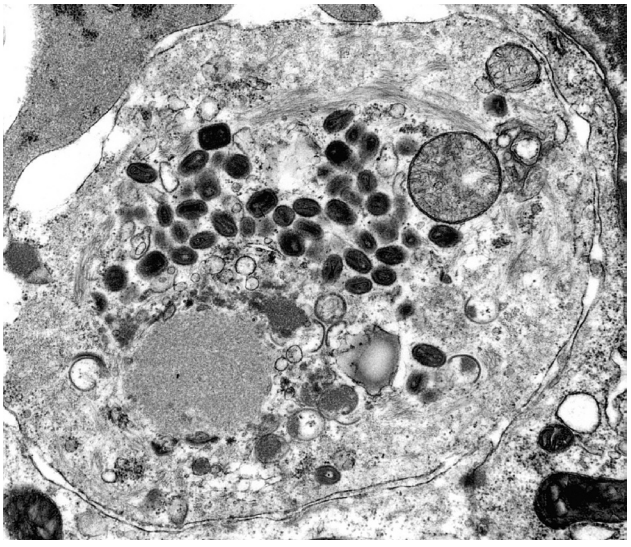

Fig. 11-2. Thin section of smallpox virus growing in the cytoplasm of an infected chick embryo cell of infected person. Intracellular mature virions (brick-shaped) and immature virions (spherical) are visible. Magnification is approximately x 25,000.
Photograph: Courtesy of FA Murphy, University of Texas Medical Branch, Galveston, Texas.

mature virion (IMV) particles of different species of orthopoxviruses[16]; additional epitopes, believed to be critical in protection against infection in vivo, exist on extracellular enveloped viral particles.[17,18] Viral envelope proteins are important in protective antibody responses: envelope antigens were absent from virion suspensions used for inactivated smallpox vaccines that proved to be ineffective.[19,20]

Replication

Orthopoxvirus genomes are linear, double-stranded DNA approximately 200 kb long. The genomes encode about 176 to 266 proteins, including enzymes and factors that are necessary for self-replication and maturation. The central region of the genome contains highly conserved genes that are essential for viral replication, and the terminal regions contain less conserved genes that are important for virus-host interactions. The virus contains a number of virus-encoded enzymes, in particular a DNA-dependent RNA polymerase that transcribes the viral genome.[21] Replication occurs in cytoplasmic factories referred to as B-type inclusions, in which virions at various stages of assembly are seen. Whether host cell nuclear factors are involved in viral replication or maturation is unclear. Cells infected with some poxviruses (eg, cowpox, avian poxviruses) also contain electron-dense A-type inclusions, usually containing mature virions; A-type inclusions are easily seen by light microscopy (Figure 11-3).

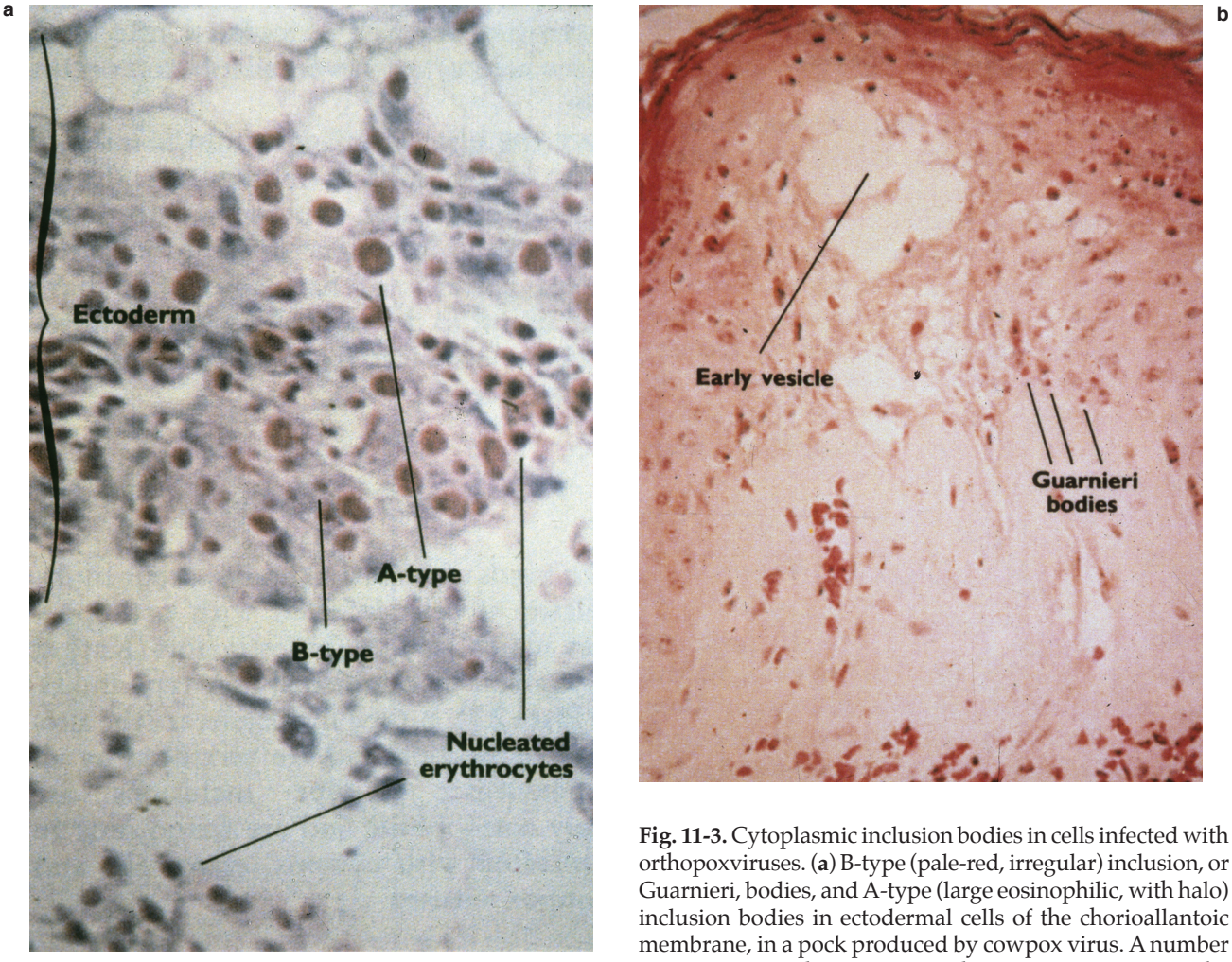

Fig. 11-3. Cytoplasmic inclusion bodies in cells infected with orthopoxviruses. (**a**) B-type (pale-red, irregular) inclusion, or Guarnieri, bodies, and A-type (large eosinophilic, with halo) inclusion bodies in ectodermal cells of the chorioallantoic membrane, in a pock produced by cowpox virus. A number of nucleated erythrocytes are in the ectoderm and free in the mesoderm, and the surface of the pock is ulcerated. Hematoxylin-eosin stain. (**b**) This section of the skin of a patient with hemorrhagic-type smallpox shows Guarnieri bodies and free erythrocytes below an early vesicle. Hematoxylin-eosin stain. Reproduced with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 85.

Viral replication begins with attachment of viral particles to the host cell surface, most likely through cell receptors, and involves expression of early, intermediate, and late genes.[21] Initial uncoating occurs during entry, followed by synthesis of early mRNAs, which are translated to facilitate further uncoating and transcription of intermediate mRNAs. Intermediate mRNAs, in turn, are translated to allow transcription of the late mRNAs. The late mRNAs are translated into structural and nonstructural proteins of the virions. These proteins, along with DNA concatemers that are formed during the early phase of replication, are assembled into genomic DNA and packaged into immature virions, which then evolve into brick-shaped infectious IMVs. IMVs are infectious only when they are released by cell lysis. IMV particles, which can acquire a second membrane from an early endosomal component to form the intracellular enveloped virion (IEV), migrate to the cell surface via microtubules and fuse with the cell membrane to form cell-associated virions (CEVs). CEVs induce polymerization of actin to form filaments that affect the direct transfer of CEVs to adjacent cells. If CEVs become dissociated from the cell membranes, they are called extracellular enveloped virions (EEVs). Although IMVs are produced in greatest abundance in cell culture and are the most stable to environmental degradation, CEVs and EEVs probably play a more critical role in cell-to-cell spread in the intact animal.[22]

Many of the *Orthopoxvirus* gene products, known as virokines and viroceptors, interact with and modulate essential functions of the host cells and immune processes.[21,23] The limited host range of variola may relate to the unique association of viral gene products with various host signaling pathways. Therefore, strategies that block such key pathways in the replication and maturation of poxviruses provide potential targets for therapeutic intervention.[24]

Pathogenesis

Most knowledge about smallpox pathogenesis is inferred from animal studies of mousepox,[25,26] rabbitpox,[26] and monkeypox[27,28] in their respective hosts, and from vaccinia in humans. Studies using primates infected with variola[29] corroborate these findings and lend further insight into human smallpox and monkeypox infections. In both natural and experimental infections, the virus is introduced via the respiratory tract, where it first seeds the mucous membranes, including membranes of the eye, and then passes into local lymph nodes. The first round of replication occurs in the lymph nodes, followed by a transient viremia, which seeds tissues, especially those of the reticuloendothelial system, including regional lymphatics, spleen, and tonsils. A second, brief viremia transports the virus to the skin and to visceral tissues immediately before the prodromal phase. In humans, the prodrome is characterized by an abrupt onset of headache, backache, and fever, and usually sore throat resulting from viral replication in the oral mucosa. Characteristic skin lesions develop following viral invasion of the capillary epithelium of the dermal layer. The virus may also be present in urine and conjunctival secretions.[30] At death, most visceral tissues contain massive virus concentrations.

In a review of all pathology reports published in English over the past 200 years,[31] Martin suggested that generally healthy patients who died of smallpox usually died of renal failure, shock secondary to volume depletion, and difficulty with oxygenation and ventilation as a result of viral pneumonia and airway compromise, respectively. Degeneration of hepatocytes might have caused a degree of compromise, but liver failure was not usually the proximate cause of death.

Much of the pathogenesis of smallpox remains a mystery because of the limited tools that were available when it was an endemic disease. Detailed analysis of the pathophysiology of the disease course using the monkeypox and variola primate models and in comparison with limited clinical and pathology data from human smallpox victims suggests a role for dysregulation of the immune response involving the production of proinflammatory cytokines, lymphocyte apoptosis, and the development of coagulation abnormalities. High viral burdens, which were identified in numerous target tissues in the animal models, were probably associated with organ dysfunction and multisystem failure. Immunohistochemistry studies showing the distribution of viral antigens as well as electron microscopy evidence of the replicating virus correlated with pathology in the lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive system, and liver. Apoptosis was a prominent observation in lymphoid tissues, with a striking loss of T cells observed. The cause of this widespread apoptosis remains unknown. However, strong production of proinflammatory cytokines at least in part likely contributed to the upregulation of various proapoptotic genes. The strong upregulation of cytokines may also have contributed to the development of a hemorrhagic diathesis. The detection of D-dimers and other changes in hematologic parameters in monkeys that developed classical or hemorrhagic smallpox suggests that activation of the coagulation cascade is a component of both disease syndromes. In human populations, however, the occurrence of hemorrhagic smallpox was approximately 1% to 3% of the total cases observed.

From these recent studies of variola and monkeypox virus infection in primates, the "toxemia" described by clinicians for human smallpox[2] may be fundamentally related to the processes underlying septic shock.[32] Common denominators include lymphocyte apoptosis; proinflammatory cytokines (exuberant production of type I interferon [IFN], interleukin-6, tumor necrosis factor-α, and IFN-γ measurable in plasma); and disseminated intravascular coagulation. Aberrant activation of these pathways, which contributes to toxic shock, is a hallmark of pathological activation of the innate immune system.

To facilitate viral replication, orthopoxviruses generally modulate their host's immune response to the pathogen's advantage. Poxviruses encode proteins that target or interrupt the natural inflammatory response and interfere with apoptosis, synthesis of steroids, and initiation of the complement system. In general, these proteins block either extracellular immune signals (by mimicking or interfering with cytokine/chemokine proteins and/or receptors), or they work intracellularly by interfering with apoptosis, targeting by the immune system, or intracellular immune cell signaling. A combination of these mechanisms may allow the virus to overcome immunological surveillance and establish clinical disease in the host.[33]

ORTHOPOXVIRUSES AS BIOLOGICAL WARFARE AND BIOTERRORISM THREATS

Using variola virus in warfare is an old concept. British colonial commanders used blankets from smallpox victims as a biological weapon, distributing them among Native Americans.[34-36] During the American Civil War, allegations were made about the use of smallpox as a biological weapon, although no definite evidence existed.[37,38] In the years leading up to and during World War II, the Japanese military explored weaponization of smallpox during the operations of Unit 731 in Mongolia and China. More recently, the former Soviet Union developed smallpox as a strategic weapon and produced ton quantities of liquid smallpox on a continuing basis well into the 1980s.[10,39] The former Soviet Union also conducted open air testing of weaponized smallpox virus and demonstrated that infectious virus could drift 15 km downwind and infect humans.[7]

Although declared stocks of smallpox virus exist only at the two WHO repositories (the Centers for Disease Control and Prevention [CDC] in Atlanta, Georgia, USA, and at the State Research Center of Virology and Biotechnology/Vector in Koltsovo, Russia), it is of concern that undeclared stocks may exist in military sites within the former Soviet Union, or that they were transferred from the Soviet program to programs in Iraq, Iran, North Korea, or elsewhere.[39] The probability that such stocks exist is impossible to assess, but the catastrophic consequences of smallpox release in a biological attack cannot be discounted.[4]

Variola is a significant threat for use as a biological weapon because of its stability, infectivity in aerosol form, small infectious dose, severe disease manifestations, and interhuman transmissibility. Furthermore, the anticipated morbidity and mortality for the general population may be higher than historical averages because of waning immunity following vaccinations in the distant past and immunosuppression resulting from HIV, cancer, organ transplants, and old age.[3] Other members of the *Orthopoxvirus* genus share many of variola's properties and are potential agents of a deliberate bioterrorist attack. Of the poxviruses other than variola, monkeypox virus presents the greatest threat for biological warfare or terrorism use. Monkeypox can naturally produce severe disease in humans that closely resembles smallpox, with mortality exceeding 15% in some outbreaks.[40] The disease is transmitted from person to person, is highly transmissible by aerosol and, in at least some nonhuman primate models, has an infectious dose as low as one tissue culture infecting dose ($TCID_{50}$).[27,41-43] Monkeypox virus, like variola, is relatively stable and can resist desiccation in both heat and cold.[44] The monkeypox virus also can grow to high titers in cell culture systems, including the chick chorioallantoic membrane of embryonated eggs, a simple methodology described in older microbiology texts using equipment and supplies available at agricultural supply stores. A large dose of monkeypox delivered by aerosol can produce a rapidly progressive and overwhelming pneumonia in nonhuman primate models.[28] Monkeypox virus may have already been weaponized by the Soviet military.[10]

Cowpox and buffalopox produce limited cutaneous disease in humans in natural infection.[45] Buffalopox, like cattlepox, may be essentially identical to vaccinia.[46] The effect of altering route of delivery, dose of virus, or the actual viral agent itself on human disease manifestation is unclear. Several studies demonstrate that orthopoxviruses produce different clinical syndromes and immunological responses in animal models depending on the route of infection.[28,47-51] Aerosol infection has the potential to produce more pronounced pulmonary disease.[28,42,52] In addition, all orthopoxviruses share a significant amount of homology with variola and monkeypox.[14] If the critical virulence factors for systemic human disease were found, then cowpox,

buffalopox, or other orthopoxviruses potentially could be genetically modified to express these critical factors. When designed as a weapon and delivered by aerosol, these viruses could have significant impact in humans, even without genetic modification.

Camelpox rarely, if ever, causes disease in humans. However, because of Iraqi admissions of research with camelpox as part of the country's biological warfare program, some concern exists over its potential use as a biological weapon.[53] Camelpox virus is the closest relative of variola virus; the major difference between camelpox virus and variola strain Bangladesh-1975 genomes is four additional insertions, elongated inverted terminal repeats, and a small area of gene rearrangement present in camelpox virus.[13] As with other orthopoxviruses, slight modifications in the camelpox virus genome might dramatically change its pathogenicity in humans. Although prohibited by US law, genetic modification of camelpox would be a likely starting point by any group that wanted to construct variola based on published sequences. In addition, it may soon be technically feasible to create infectious variola using an oligonucleotide synthesizer, analogous to the recent demonstration for creation of the much simpler polio virus.[54]

The possibility of genetically engineered orthopoxviruses remains unknown in biodefense research. Studies have shown increased mousepox and vaccinia virus virulence in mouse models by the incorporation of cloned host cytokine genes into the virus genome.[55,56] Whether these results represent findings unique to the virus-host model used or reflect a more general premise of enhanced virulence is unclear.[57,58] The possibility of similar genetic engineering only increases the threat of orthopoxviruses that are not significant natural threats for human disease. Further research is warranted to ensure that present and future countermeasures are effective with modified viruses.

CLINICAL ASPECTS OF ORTHOPOXVIRUS INFECTIONS

Smallpox

Variola virus is stable and retains its infectivity for long periods outside the host.[59] Variola virus is infectious by aerosol,[3] but natural airborne spread other than among close contacts is unusual.[60,61] Approximately 30% of susceptible contacts became infected during the era of endemic smallpox,[62] and the WHO eradication campaign was predicated upon the requirement of close person-to-person proximity for reliable transmission to occur. Nevertheless, two hospital outbreaks demonstrated that the variola virus can be spread through airborne dissemination in conditions of low relative humidity.[63] The patients in these outbreaks were infectious from the onset of their eruptive exanthem, most commonly from days 3 through 6 after fever onset. If the patient had a cough, then chances of infection were greatly increased. Indirect transmission via contaminated bedding or other fomites was infrequent.[64] Some people in close contact with patients harbored virus in their throats without developing disease and may have been a means of secondary transmission.[65,66]

After exposure to aerosolized virus, variola travels from the upper or the lower respiratory tract to regional lymph nodes, where it replicates and gives rise to viremia, which is followed by a rash.[67] The incubation period of smallpox averages 12 days (range 9–14 days). Those in contact with infected patients are quarantined for a minimum of 16 to 17 days following exposure.[67] Following infection via the respiratory route and replication in local lymph nodes, variola virus disseminates systemically to other lymphoid tissues, spleen, liver, bone marrow, and lung. During this asymptomatic, prodromal period, variola virus can be recovered from the blood, but the yield is lower than later in the illness. Clinical manifestations begin acutely with malaise, fever, rigors, vomiting, headache, and backache; 15% of patients develop delirium. Approximately 10% of light-skinned patients exhibit an erythematous rash during this phase. After 2 to 3 more days, an enanthem appears concomitantly with a discrete rash about the face, hands, and forearms. Because of the lack of a keratin layer on mucous membranes, lesions shed infected epithelial cells and give rise to infectious oropharyngeal secretions in the first few days of the eruptive illness, and occasionally 24 hours before eruption.[68] These respiratory secretions are the most significant but not the sole means of virus transmission. Following subsequent eruptions on the lower extremities, the rash spreads centrally during the next week to the trunk. Lesions quickly progress from macules to papules and eventually to pustular vesicles (Figure 11-4). Lesions are more abundant on the extremities and face, and this centrifugal distribution is an important diagnostic feature. In contrast to the lesions seen in varicella, smallpox lesions on various segments of the body remain generally synchronous in their stage of development. From 8 to 14 days after onset, the pustules form scabs, which leave depressed depigmented scars on healing. Although variola titers in the throat, conjunctiva, and urine diminish with time,[67] virus can readily be recovered from

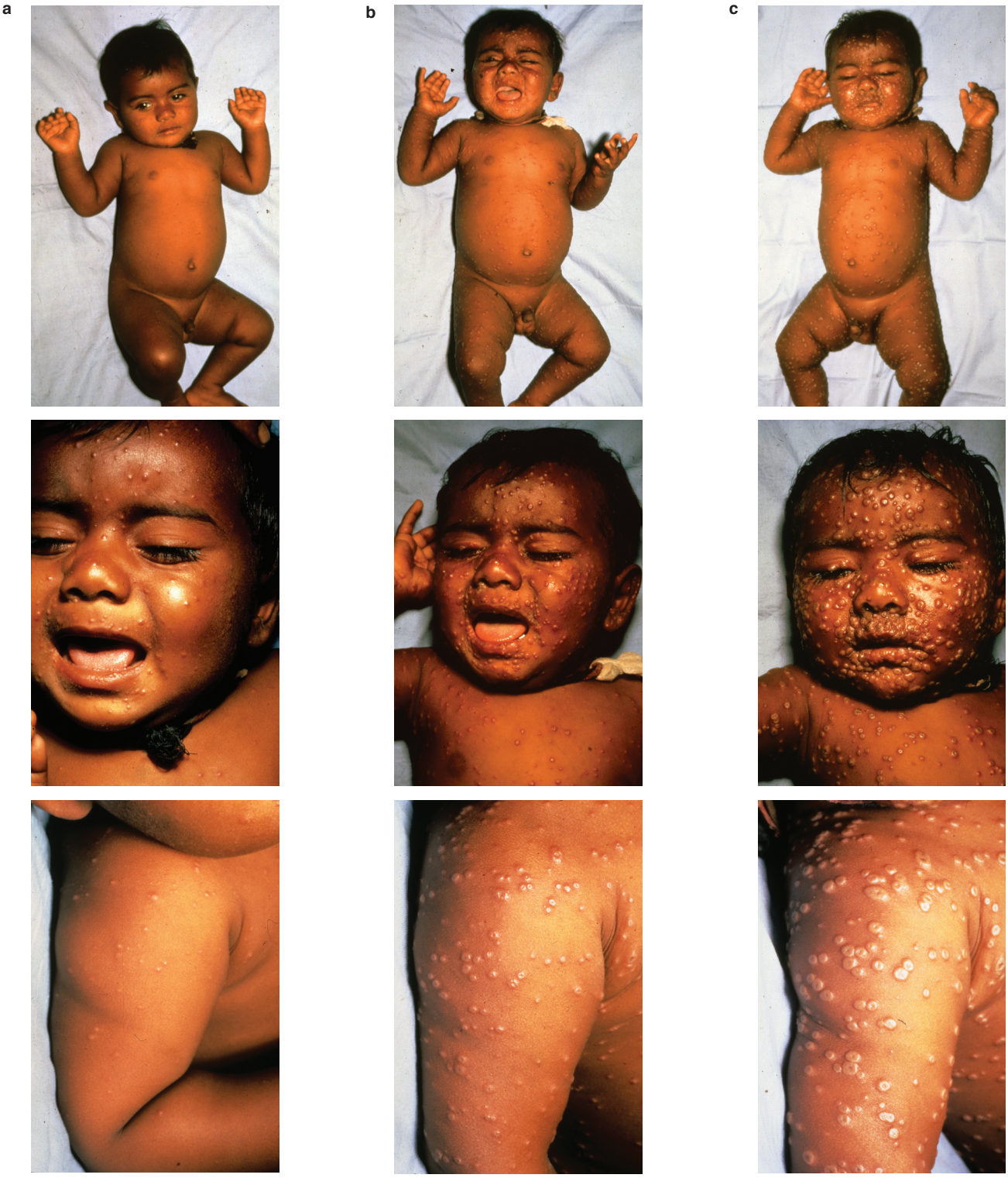

Fig. 11-4. This series of photographs illustrates the evolution of skin lesions in an unvaccinated infant with the classic form of variola major. (**a**) The third day of rash shows synchronous eruption of skin lesions; some are becoming vesiculated. (**b**) On the fifth day of rash, almost all papules are vesicular or pustular. (**c**) On the seventh day of rash, many lesions are umbilicated, and all lesions are in the same general stage of development. Reproduced with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 10–14. Photographs by I Arita.

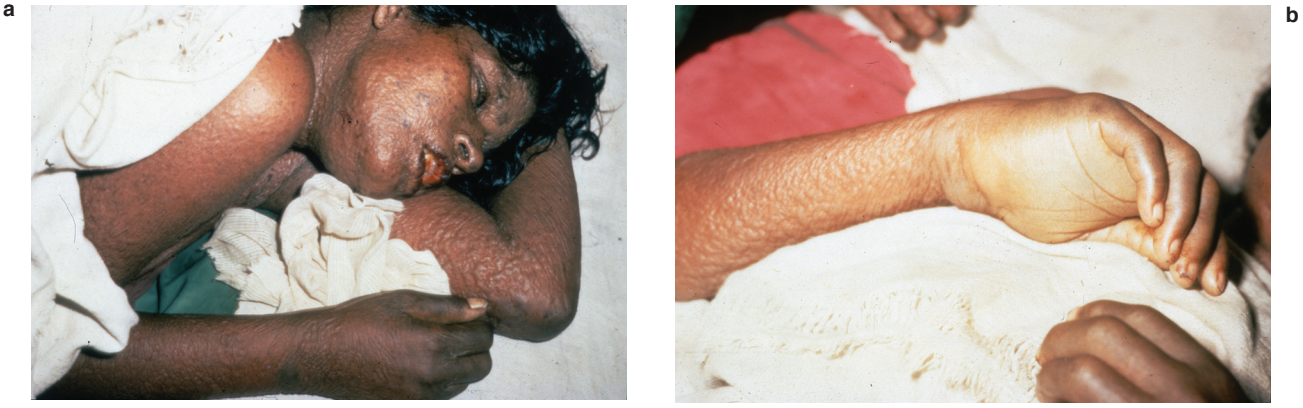

Fig. 11-5. Flat-type smallpox in an unvaccinated woman on the sixth day of rash. Extensive flat lesions (**a** and **b**) and systemic toxicity with fatal outcome were typical. Reproduced with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 33. Photographs by F Dekking.

scabs throughout convalescence.[69] Therefore, patients should be isolated and considered infectious until all scabs separate.

Two distinct forms of smallpox were recognized in the last century of smallpox occurrence. Variola major, the highly virulent, prototypical, and historically significant form of the disease, remained prevalent in Asia and parts of Africa during the 20th century. Variola minor was distinguished by milder systemic toxicity and more diminutive pox lesions.[2] However, Dixon reported many cases that were indistinguishable from variola major in his extensive comparison of lesion types.[70] Korte first described variola minor, found in Africa, in 1904.[2] Chapin found a similar mild form known as alastrim that occurred in North America as early as 1896 and subsequently was exported to South America, Europe, and Australia. Two distinct viral strains of reduced virulence caused variola minor and alastrim, and both typically caused 1% mortality in unvaccinated victims.[2]

The Rao classification specified five clinical presentations of variola.[71] Three quarters of variola major cases were designated classic or ordinary type (see Figure 11-4). After prodromal fever and constitutional symptoms appeared, patients developed the typical variola rash, centrifugal in distribution, with synchronous progression from macules to papules, to vesicles to pustules, and then to scabs. The fatality rate was 3% in vaccinated and 30% in unvaccinated patients. Other clinical presentations of smallpox occurred less frequently, probably because of the difference in host immune response. Flat-type smallpox, noted in 2% to 5% of smallpox patients, was characterized by both severe systemic toxicity and the slow evolution of flat, soft, focal skin lesions that did not resemble the classical variola exanthem (Figure 11-5). This syndrome caused 66% mortality in vaccinated patients and 95% mortality in unvaccinated patients. Fewer than 3% of smallpox patients developed hemorrhagic-type smallpox, which was accompanied by extensive petechiae (Figure 11-6), mucosal hemorrhage, and intense toxemia; death usually occurred before typical pox lesions developed.[72] However, on occasions hemorrhagic smallpox also occurred in the classic type later in the disease. Both hemorrhagic-type and flat-type smallpox may have indicated underlying im-

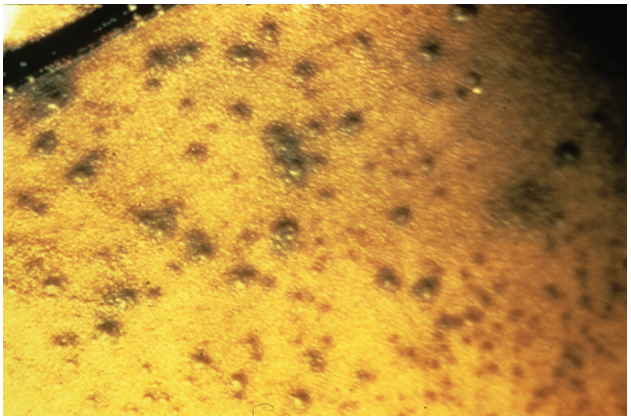

Fig. 11-6. Early hemorrhagic-type smallpox with cutaneous signs of hemorrhagic diathesis. Death usually intervened before the complete evolution of pox lesions. Reproduced with permission from Herrlich A, Munz E, Rodenwaldt E. *Die pocken; Erreger, Epidemiologie und klinisches Bild*. 2nd ed. Stuttgart, Germany: Thieme; 1967. In: Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 35.

munodeficiency; hemorrhagic forms occurred more commonly in pregnant women and young children.[73] The modified type, which occurred typically but not exclusively in previously vaccinated individuals, was characterized by moderation of constitutional symptoms, typically reduced numbers of lesions, and rapid evolution of lesions, with scabs formed by the 9th day of the illness. The *variola sine eruptione* was characterized by prodromal fever and constitutional symptoms. These patients, most of whom had been vaccinated, never developed a rash.[71] In actuality, the manifestations of variola infection fall along a spectrum, and classification is primarily for the purpose of prognosis.

Bacterial superinfection of pox lesions was relatively common in the preantibiotic era, especially in the absence of proper hygiene and medical care and in tropical environments.[2] Arthritis and osteomyelitis developed late in the disease in about 1% to 2% of patients, occurred more frequently in children, and often manifested as bilateral joint involvement, particularly of the elbows.[74] Viral inclusion bodies could be demonstrated in the joint effusion and bone marrow of the involved extremity. Cough and bronchitis were occasionally reported as prominent manifestations of smallpox, with implications for spread of contagion; however, pneumonia was unusual.[2] Pulmonary edema occurred frequently in hemorrhagic-type and flat-type smallpox. Orchitis was noted in approximately 0.1% of patients. Encephalitis developed in 1 in 500 cases of variola major, compared with 1 in 2,000 cases of variola minor. Keratitis and corneal ulcers were important complications of smallpox, progressing to blindness in slightly fewer than 1% of cases. Disease during pregnancy precipitated high perinatal mortality, and congenital infection was also recognized.

Partial immunity caused by vaccination resulted in modified-type smallpox, in which sparse skin lesions evolved variably, often without pustules, and quickly, with crusting occurring as early as the 7th day of illness. When exposed to smallpox, some fully immune individuals developed fever, sore throat, and conjunctivitis (called contact fever), which lasted several days but did not give rise to the toxicity or minor skin lesions that signify *variola sine eruptione*. Persons who recovered from smallpox possessed long-lasting immunity, although a second attack may have occurred in 1 in 1,000 persons after an intervening period of 15 to 20 years.[75] Both humoral and cellular responses are important components of recovery from infection. Neutralizing antibodies peak 2 to 3 weeks following onset and last longer than 5 years,[76] up to several decades in some individuals.[18]

Monkeypox

The clinical features of human monkeypox are classically described as being similar to those of smallpox.[77] Disease begins with a 2- to 4-day disruptive phase with high fever and prostration. The rash develops and progresses synchronously over 2 to 4 weeks, evolving from macules to papules, to vesicles and pustules, to scabs. Lesions are usually umbilicated, have a centrifugal distribution, and involve the palms and soles. Sore throat and frank tonsillitis frequently occur during the eruptive phase of human monkeypox.[77,78] Lymphadenopathy is a common finding that differentiates monkeypox from smallpox. Lymphadenopathy, which has been documented in up to 83% of unvaccinated persons with monkeypox, arises most frequently early in the course of infection, involving the submandibular and cervical nodes and less frequently the axillary and inguinal nodes.

Clinical manifestations of human monkeypox are likely more diverse and not as stereotypical as those of smallpox. Mild infections were frequent in the first recognized African cases, with 14% of patients having fewer than 25 lesions and no incapacity.[77] In a series of 282 patients, the exanthema first appeared somewhere other than the face in 18% of the vaccinated patients; 31% of vaccinated patients had pleomorphic or "cropping" appearance of rash lesions, and 9.4% had centripetal distribution.[79] All of these features are inconsistent with a mimic of smallpox. Patients in the recent US outbreak tended to have fewer mild lesions than most African patients. Patients were hospitalized in only 19 of 78 suspected cases in the United States, and only 2 had significant illness requiring some form of medical intervention.[80,81] None of the initial cases was suspected as a smallpox-like disease. A *sine eruptione* form of monkeypox has not been described, but the number of serologically diagnosed infections without consistent rash illness suggests that it is a possibility.[82] A hemorrhagic form of human monkeypox has not been documented.[83,84]

Complications of monkeypox are more common in unvaccinated persons and children.[85] During intensive surveillance in the Democratic Republic of the Congo between 1980 and 1986, secondary bacterial superinfection of the skin was the most common complication (19.2% of unvaccinated patients), followed by pulmonary distress/pneumonia (11.6% of unvaccinated patients), vomiting/diarrhea/dehydration (6.8% of unvaccinated patients), and keratitis (4.4% of unvaccinated patients). With the exception of keratitis, the incidence of these complications in vaccinated persons was at least 3-fold less. Alopecia has been noted in

some cases.[86] Encephalitis was detected in at least one monkeypox case in the Democratic Republic of the Congo and in one of the cases in the US outbreak of 2003.[79,81] As in smallpox, permanent pitted scars are often left after scabs separate.

Severity of disease and death is related to age and vaccination status, with younger unvaccinated children faring worse.[77,86-88] The case fatality rate in Africa varied in different outbreaks and periods of increased surveillance. The fatality rate was 17% from 1970 through 1979, 10% from 1981 through 1986, and 1.5% from 1996 through 1997.[40] No fatalities occurred among 78 suspected cases in the recent US outbreak.[80] The presence of comorbid illnesses, such as measles, malaria, or diarrheal disease, may have a significant impact on mortality in children.[85] Cause of death in monkeypox is not universally clear, although 19 of 33 fatalities in one series of patients involved pulmonary distress or bronchopneumonia, suggesting superimposed bacterial pneumonia.

Other Orthopoxviruses Infecting Humans

Cowpox is primarily a localized, cutaneous disease.[45] Baxby, Bennett, and Getty reviewed 54 cases of cowpox infection with a detailed discussion of clinical manifestations.[89] Disease usually consists of single pock-like lesions on the hands or face, although multiple lesions are seen in roughly one quarter of cases. Typical lesions progress from macule to papule to vesicle to pustule to dark eschar, with a hemorrhagic base being common in the late vesicular stage. Progression from macule to eschar is slow, often evolving over 2 to 3 weeks. Local edema, induration, and inflammation are common and can be pronounced. Lesions are painful and are accompanied by regional lymphadenopathy. Complete healing and scab separation usually occur within 6 to 8 weeks of onset, but may take 12 weeks or longer. A majority of patients experience some constitutional symptoms before the eschar stage.

The majority of human cowpox infections are self-limited and without complication. Ocular involvement, including the cornea, can occur, but it usually resolves without permanent damage. A few severe generalized cowpox infections have been reported, including one fatality.[89,90] Three of these four described cases included a history of atopic dermatitis, indicating a risk of increased severity of disease analogous to vaccinia.

Buffalopox infection in humans has not been extensively described. Limited data suggest that human infection usually occurs on the hands and consists of inflamed and painful pustular lesions progressing through a Jennerian evolution.[91-93] Regional lymphadenopathy and fever can accompany local disease.[93]

DIAGNOSIS

Clinical Diagnosis

The clinical presentation of smallpox is similar to many vesicular and pustular rash illnesses, including varicella, herpes simplex, drug reactions, and erythema multiforme. Although the index of suspicion for an eradicated disease may be low, the failure to recognize a case of smallpox could result in the exposure of hospital contacts and the seeding of an outbreak. The Smallpox Diagnosis and Evaluation page on the CDC Web site (http://www.bt.cdc.gov/agent/smallpox/diagnosis) is an essential resource to assist a clinician in evaluating a febrile patient presenting with a rash. This site contains an algorithm to quickly determine the likelihood of clinical smallpox and a standardized worksheet to classify the risk of smallpox using the CDC criteria.

Laboratory Diagnosis

Collection of appropriate specimens is paramount for accurate laboratory diagnosis of *Orthopoxvirus* infection. For virological diagnosis, specimens from skin lesions are most important, because when viremia does occur in *Orthopoxvirus* infections, it is an early phenomenon.[2] Ideally, cutaneous tissue and blood are sent for diagnostic testing, with other samples being sent at the request of public health officials or experts in the field.[84] Detailed instructions for specimen collection can be found in the Department of Defense Smallpox Response Plan (http://www.bt.cdc.gov/agent/smallpox/response-plan/index.asp) or on the CDC Web site (http://www.cdc.gov/ncidod/monkeypox/diagspecimens.htm). Briefly, vesicles or pustules should be unroofed, the detached vesicle skin sent in a dry tube, and the base of the lesion scraped to make a touch-prep on a glass slide. Biopsy specimens should be split (if possible) and sent in formalin and in a dry tube. If scabs are collected, two scabs should be sent in a dry tube. Dacron or polyester swabs should be used for oropharyngeal swabs and transported in dry tubes. Blood should be collected in a marble-topped or yellow-topped serum separator tube (which is then centrifuged to separate serum) and in a purple-topped anticoagulant tube for whole blood. Clinical

specimens potentially containing orthopoxviruses other than variola virus, including monkeypox virus, may be handled in a biosafety level 2 using biosafety level 3 practices.[94]

Many phenotypic and genotypical methods involving virological, immunological, and molecular approaches have been used to identify *Orthopoxvirus*.

Phenotypic Diagnosis

In the past, a presumptive diagnosis of orthopoxviruses required a laboratory with capabilities and expertise in viral diagnostics. Microscopists with experience in poxvirus infections can often recognize the characteristic inclusion bodies (Guarnieri bodies, corresponding to B-type poxvirus inclusions [see Figure 11-3]) in tissue samples under light microscopy. These cytoplasmic inclusions are hematoxylinophilic, stain reddish purple with Giemsa stain, and contain Feulgen-positive material.[95] Microscopy alone cannot differentiate members of the *Orthopoxvirus* genus, yet the epidemiological setting can suggest which species is involved. The orthopoxviruses with pathogenicity for humans (with the exception of molluscum contagiosum) can be grown on the chorioallantoic membranes of 12-day-old embryonated chicken eggs, where they form characteristic pocks. These viruses also grow readily in easily obtained cell cultures, including VERO,[96] other monkey kidney cell lines, A549, and others. Variola could characteristically be differentiated from other viruses by a strict temperature cut-off at 39°C. Methods for isolation and identification of individual virus species have been reviewed.[97,98] Electron microscopy reveals the unmistakable brick-like morphology of orthopoxviruses in thin sections of infected materials. Immunogold stains permit more precise identification to the species level.

Immunodiagnosis

Serologic testing for anti-*Orthopoxvirus* antibodies is an old technique, and various assays were used extensively in the study of smallpox.[2] However, significant serologic cross-reactivity exists between all the *Orthopoxvirus* species; therefore, species differentiation is not possible with conventional serologic assays. Techniques developed in the 1980s to detect monkeypox-specific antibodies are complex and considered unreliable by some experts.[82,99] Although complement-fixation tests detect antibodies that disappear within 12 months of infection, other traditional techniques, such as immunofluorescence assay, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), hemagglutination-inhibition and neutralization assay, detect immunoglobulin (IgG) antibodies that are persistent. Thus, differentiating antibodies due to acute infection from antibodies resulting from prior vaccination can be difficult with single specimens.

Immunofluorescence assays and ELISAs have been used to detect IgM in acute infection directed against cowpox and monkeypox, respectively.[90,99] Because IgM seems to disappear within 6 months, IgM ELISAs can be used to detect recent infections when virus detection is not possible after lesions have healed and scabs have separated. In the investigation of the 2003 US monkeypox outbreak, the CDC relied on anti-*Orthopoxvirus* IgG and IgM ELISAs for serologic diagnosis.[81] More recently, a combination of T-cell measurements and a novel IgG ELISA was used to enhance epidemiological follow-up studies to this outbreak.[100,101]

Nucleic Acid Diagnosis

The molecular diagnostic approaches, including DNA sequencing, polymerase chain reaction (PCR), restriction fragment-length polymorphism (RFLP), real-time PCR, and microarrays, are more sensitive and specific than the conventional virological and immunological approaches. Of these techniques, sequencing provides the highest level of specificity for species or strain identification, but current sequencing techniques are not yet as practical as rapid diagnostic tools in most laboratories. RFLP analysis[102,103] and microarray genotyping[104] also provide high levels of specificity, and when combined with PCR, these approaches can offer high levels of sensitivity. Real-time PCR methods provide exquisite levels of sensitivity and specificity.[105] The basic concept behind real-time PCR is the measurement, by fluorescence detection, of the amount of nucleic acids produced during every cycle of the PCR. Several detection chemistries, such as the intercalating dyes (SYBR Green, Applied Biosystems, Foster City, Calif), Hydrolysis probes (5′ nuclease or Taqman, Minor Groove Binding Proteins [MGBP]), Hybridization probes (Fluorescence Resonance Energy Transfer [FRET]) and molecular beacons, are used. There are several commercially available instruments for real-time PCR, such as the ABI—7900 (Applied Biosystems), Smart Cycler (Cepheid, Synntvale, Calif), LightCycler (Roche Diagnostics Corporation, Indianapolis, Ind), MJ Opticon (Bio-Rad, Hercules, Calif), RotorGene (Corbett Life Science, Sydney, Australia); RAPID (Idaho Technology, Salt Lake City, Utah); and others. When combined with portable analytical platforms such as the Smart Cycler or LightCycler, real-time PCR systems can be readily deployed to field sites for rapid testing.

Successful performance of PCR-based diagnostics requires extraction of DNA from body fluid and tissue samples, careful design of oligonucleotide primers and probes, and optimization of amplification and detection conditions. There are numerous commercial nucleic acid purification methods for various sample types, which involve cell lysis and protein denaturation followed by DNA precipitation or fractionation by reversible binding to an affinity matrix. Selection of appropriate primers, probes, and optimization of assay conditions require knowledge of genome sequences and molecular biology techniques.

One of the basic techniques used in PCR-based diagnostics is gel analysis, in which PCR-amplified regions of the genome are separated on agarose gels by electrophoresis, and the amplicon sizes are used to identify the sample. Several PCR gel-analysis assays have been used to identify cowpox, monkeypox, vaccinia, and variola viruses from clinical specimens.[98,106-108]

Large fragment PCR-RFLP (LPCR-RFLP) analysis requires amplifying large DNA fragments with high-fidelity DNA polymerase enzymes. The amplified LPCR products are purified on agarose gels and digested with a restriction enzyme. The digested DNA fragments are then electrophoresed on polyacrylamide gels for a constant period at constant voltage and stained with ethidium bromide. The restriction pattern is then visualized and photographed with a digital camera. The positions for all DNA fragments in each restriction pattern are determined and digitized by appropriate fingerprinting software. From this pattern, a similarity coefficient is calculated for every pair of restriction patterns and used as an index for species differentiation.

Recently developed real-time PCR assays, which can be performed in a few hours, can test clinical specimens for all orthopoxviruses or for specific species such as vaccinia, variola, or monkeypox.[105,109-111] Real-time PCR was one of the diagnostic techniques used in the investigation of the 2003 US monkeypox outbreak.[81] Because of its sensitivity, rapidity, and ease, real-time PCR will likely become the primary method of preliminary diagnosis of *Orthopoxvirus* infection, with isolation and growth in a high-level containment laboratory reserved for confirmation.

MEDICAL MANAGEMENT

Prophylaxis

Vaccination

History. Attempts to use infected material to induce immunity to smallpox date to the first millennium; the Chinese used scabs or pus collected from mild smallpox cases to infect recipients usually via insertion of bamboo splinters into the nasal mucosa. This procedure produced disease in a controlled situation that was typically milder than naturally occurring disease and allowed for isolation or controlled exposure of nonimmune individuals. The practice spread to India and from there to Istanbul, where Europeans encountered it in the early 18th century. In Europe the inoculation of the skin with infected pock material was later referred to as variolation to distinguish the procedure from vaccination. Inducing immunity using variola-contaminated materials had been known to the British Royal Medical Society through Joseph Lister's reports from China as early as 1700, but the procedure was not practiced until Lady Mary Wortley Montagu, wife of the British ambassador to Turkey, introduced it to British society. Lady Montagu, who had been badly disfigured from smallpox, had her son inoculated in Constantinople in 1717 and subsequently arranged for surgeon Charles Maitland to inoculate her daughter in 1722. In the British American colonies, Cotton Mather of Boston persuaded Dr Zabdiel Boylston to conduct variolation on 224 people in 1721 after reading about inoculation in a Royal Medical Society publication.[70] During a smallpox outbreak in Boston in 1752, over 2,000 persons underwent variolation, resulting in a 90% reduction in mortality among the population immunized. During the Revolutionary War, the Canadian Campaign failed largely because the American reinforcements contracted smallpox. Continued problems with recurring smallpox epidemics among recruits to the Continental Army resulted in a directive in 1779 for variolation of all new recruits. General Washington, who had undergone variolation himself as a young man, was the first military commander to order immunization of his forces.[112]

The practice of variolation, which was never widely accepted, was outlawed at times because many of those inoculated developed grave clinical illness. Variolation often caused a 1% to 2% mortality rate, and the individuals who died had the potential to transmit natural smallpox. Edward Jenner overcame problems of inoculation with variola by capitalizing on the long-held observation that milkmaids had clear complexions (without smallpox scars), presumably because they had had cowpox, which causes milder disease in humans. Folklore maintained that human infection with cowpox conferred lifelong immunity to smallpox. In 1796 Jenner scientifically demonstrated

that inoculation with material obtained from a milkmaid's cowpox lesions would result in immunity and protection from infection with smallpox when introduced by inoculation. Jenner published his findings in 1798, and in 1801 he reported that 100,000 persons had been vaccinated in England. By the 1820s vaccination had become widespread throughout Britain and much of Europe. Although derivation of current vaccinia strains is uncertain, it is not a form of cowpox, and because Jenner lost his original material used for vaccination, the specific source of current vaccinia strains remains unknown.[70] The United States began regulating production of the vaccine in 1925. Since then, the New York City Board of Health strain of vaccinia has been used as the primary US vaccine strain. The WHO global vaccination program eventually led to smallpox eradication, with the last serially transmitted smallpox case reported in 1977. Routine vaccination of children in the United States ceased in 1971, and vaccination of hospital workers ceased in 1976. Vaccination of military personnel was continued because of Cold War concerns about its intentional use but eventually halted in 1989. Because of the risk of bioterrorism, smallpox vaccination in at-risk military personnel and civilian healthcare workers was resumed in 2003.[113,114]

During the WHO global eradication program, most of the human population received vaccinia virus by scarification. Although there were multiple manufacturers worldwide, and vaccine lots varied with respect to potency and purity, almost all vaccinia administered was derived from one of two lineages, the New York Board of Health and Lister strains.[2] Live vaccinia virus suspension was placed as a drop on the skin or drawn up by capillary action between the tines of a bifurcated needle; the nominal dose of live vaccinia was about 10^5 virions. Usually, primary vaccination is uneventful; following introduction into the skin, the virus replicates in basal layer keratinocytes, spreads cell-to-cell, and leads to discrete vesicle formation. Within a week, the vesicle evolves into a pustule surrounded by inflammatory tissue. This lesion scabs over within 10 to 14 days; eventually, the scab is shed. Vaccinees in the global campaign often experienced tender axillary lymph nodes, fever, and malaise for brief periods. Occasionally, however, complications arose with varying degrees of severity. Accidental transfer of vaccinia from the inoculation site was common, but of little consequence unless transferred to the eye. Generalized vaccinia, which involved systemic spread of the virus and eruption of multiple pocks at distant sites, was more serious; in individuals with eczema or atopic dermatitis, however, it sometimes led to extensive inflammation and secondary bacterial infection. More serious, life-threatening complications arose in vaccinees with defects in cell-mediated immunity; the vaccination site frequently enlarged to form an ulcer, secondary ulcers appeared, and the infection cleared slowly or not at all. The most serious event was postvaccinial encephalitis. Although rare, this condition was frequently fatal. Death occurred in approximately one in one million primary vaccinations.[115,116] Adverse events may be more frequent and severe if mass immunization were to be resumed in an unscreened general population that now includes transplant recipients on immunosuppressive drugs, HIV-infected individuals, and geriatric patients.

Recent Vaccination Campaigns. The requirement that any alternative vaccine must not be inferior to live vaccinia sets a high standard. The successful immunization or "take rate" has been greater than 95%, both historically and in a more recent series of over 450,000 military vaccinees.[113] In this recent series, one case of encephalitis and 37 cases of myopericarditis were documented in a prescreened, healthy, young adult population. Although the incidence of myopericarditis was below the historical average and the cases were mild, this adverse event contributed to the general reluctance of the civilian healthcare population to accept vaccination.[114] A potential replacement vaccinia was prepared in massive quantities (> 300 million doses) by selection of plaque-purified progeny virus from the New York Board of Health strain, which was amplified in VERO cell cultures. This vaccine is more purified and free of adventitious agents in comparison with its predecessor, which was prepared on calf skin. Phase I safety and immunogenicity trials for ACAM 2000 indicate greater than 95% take rates and adverse events comparable to those of live vaccinia.[117] Historically, live (replicating) vaccinia immunization has also been used as postexposure prophylaxis and is believed effective if administered within 4 days of exposure.

The recent immunization of modest numbers of military and civilian individuals has provided an opportunity to study the nature of adverse events using modern tools of immunology. A strong association was established between adverse events and increased systemic cytokines, in particular, IFN-γ, tumor necrosis factor-α, interleukin-5, and interleukin-10.[118] Some researchers have speculated that cardiac events, although rare, may be related to dramatic alterations in cytokine profiles.

Protective immunity elicited by live vaccinia is thought to depend on a combination of humoral and cellular immune responses. Using a monkey model in which animals are immunized with vaccinia and challenged with monkeypox, Edghill-Smith has shown that vaccinia-specific B cells are critical for protection.[119] Antibody depletion of B cells, but not $CD4^+$ or $CD8^+$ T cells, abrogated vaccinia-induced protection. Edghill-Smith has also shown that simian-immunodeficiency-virus–

compromised monkeys could withstand vaccinia if it was preceded by a dose of nonreplicating Modified Vaccinia Ankara (MVA) strain vaccinia, but they were not protected against monkeypox challenge when their $CD4^+$ T-cell counts were less than 300 mm.[3.]

MVA is an alternative vaccine that has promise as a nonreplicating immunogen. MVA, which was used in Germany in the later stages of global eradication, was shown to be safe and immunogenic, but its protective efficacy has not been established in humans. MVA was generated by over 500 serial passages in chick embryo fibroblasts, which resulted in multiple deletions and mutations and an inability to replicate efficiently in human and most other mammalian cells.[120] Ultrastructural examination of purified MVA reveals that most of the particles are enveloped; the host restriction occurs at a late stage of maturation. The presence of enveloped particles is believed to be important to the elicitation of protective immunity. Experimentally, MVA was demonstrated to protect monkeys against a monkeypox virus challenge, after one or two doses of MVA or MVA followed by Dryvax (Wyeth Laboratories, Marietta, Pa).[121] Surprisingly, a single dose of MVA also protected when challenge followed immunization by as little as 10 days, although protection was not absolute; a modest number of pocks and a low-level viremia occurred in the MVA recipients following challenge. Rhesus monkeys were used in a similar intravenous challenge model to evaluate a DNA vaccine strategy, a combination of four genes (L1R, A27L, A33R, and B5R) with promising results.[122]

The smallpox vaccine used in the United States is Dried, Calf Lymph Type (Dryvax), a live-virus preparation of the New York Board of Health vaccinia strain prepared from calf lymph. The calf lymph is purified, concentrated, and lyophilized. The diluent for the vaccine contains 50% glycerin and 0.25% phenol in US Pharmacopeia sterile water, with no more than 200 bacterial organisms per milliliter in the reconstituted product (Polymyxin B sulfate, dihydrostreptomycin sulfate, chlortetracycline hydrochloride, and neomycin sulfate are used in the processing of the vaccine, and therefore small amounts of these antibiotics may be present in the final product).

Vaccination is performed with a bifurcated needle onto which the reconstituted vaccinia preparation has been drawn, using three intradermal jabs for immunologically naïve individuals (new vaccinees) or 15 jabs for prevaccinated individuals, with enough strength to produce a visible trace of bleeding. The resulting vaccination lesion is then kept covered with a nonadherent and nonimpervious dressing. Care must be taken to prevent inadvertent inoculation of the vaccinee or others. In primary vaccinees, a papule forms within 5 days, developing into a vesicle on the 5th or 6th day postvaccination, which signifies a major reaction, or take. The vesicle subsequently becomes pustular, swelling subsides, and a crust forms, which comes off in 14 to 21 days. At the height of the primary reaction, known as the Jennerian response, regional lymphadenopathy usually occurs, which may be accompanied by systemic manifestations of fever and malaise. Primary vaccination with vaccine at potency of 100 million pock-forming units per milliliter elicits a 97% response rate both by major reaction and neutralizing antibody response. Allergic sensitization to viral proteins can persist so that the appearance of a papule and redness may occur within 24 hours of revaccination, with vesicles occasionally developing within 24 to 48 hours. This allergic response peaks within 3 days and does not constitute a "major reaction or take." Immunological response occurring after 3 days is an accelerated but otherwise similar appearance of papule, vesicle, and/or pustule to that seen in the primary vaccination response. Revaccination is considered successful if a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion (scar or ulcer) is present on examination at 6 to 8 days after revaccination.

Outcome. Successful smallpox vaccination provides high-level immunity for the majority of recipients for 3 to 5 years followed by decreasing immunity. In Mack's review of importations cases in Europe from 1950 through 1972, he provided epidemiological evidence of some relative protection from death, if not from disease severity, in individuals who had been immunized over 20 years before exposure. However, for the older population in particular, vaccination within 10 years of exposure did not prevent all cases but did prevent some smallpox deaths.[123] Multiple vaccinations are thought to produce more long-lasting immunity. Vaccination has been effective in preventing disease in 95% of vaccinees.[124] Vaccination also was shown to prevent or substantially lessen the severity of infection when given as secondary prophylaxis within a few days of exposure.[2]

Contraindications. Smallpox vaccination is contraindicated in the preoutbreak setting for individuals with the following conditions or those having close contact with individuals with the following conditions:

- a history of atopic dermatitis (eczema);
- active acute, chronic, or exfoliative skin conditions that disrupt the epidermis;
- pregnancy or the possibility of becoming pregnant; or
- a compromised immune system as a consequence of HIV infection, acquired immunodeficiency syndrome, autoimmune disorders, cancer, radiation treatment, immunosuppressive therapy, or other immunodeficiencies.

Additional relative contraindications for potential vaccinees, but not close contacts, are smallpox vaccine-component allergies, moderate or severe acute intercurrent infections, topical ophthalmologic steroid medications, age younger than 18, and maternal breastfeeding. A history of Darier's disease and household contact with active disease are contraindications for vaccination.[6]

Adverse Events. Vaccinia can be transmitted from a vaccinee's unhealed vaccination site to other persons by close contact and the same adverse events as with intentional vaccination can result. To avoid inadvertent transmission, vaccinees should wash their hands with soap and water or use antiseptic hand rubs immediately after touching the vaccination site and after dressing changes. Vaccinia-contaminated dressings should be placed in sealed plastic bags and disposed in household trash.[125]

Adverse reactions to smallpox vaccination are diagnosed by a clinical examination. Most reactions can be managed with observation and supportive measures. Self-limited reactions include fever, headache, fatigue, myalgia, chills, local skin reactions, nonspecific rashes, erythema multiforme, lymphadenopathy, and pain at the vaccination site. Adverse reactions that require further evaluation and possible therapeutic intervention include inadvertent inoculation involving the eye,[126] generalized vaccinia, eczema vaccinatum, progressive vaccinia, postvaccinial central nervous system disease, and fetal vaccinia.[6]

Inadvertent inoculation generally results in a condition that is self-limited unless it involves the eye or eyelid, which requires an ophthalmologist's evaluation. Topical treatment with trifluridine (Viroptic, Glaxo/Smith/Kline, Brentford, Middlesex, United Kingdom) or vidarabine (Vira-A, King Pharmaceuticals, Bristol, Tenn) is often recommended, although treatment of ocular vaccinia is not specifically approved by the Food and Drug Administration for either of these drugs. Most published experience is with use of vidarabine, but this drug is no longer manufactured.[127]

Generalized vaccinia is characterized by a disseminated maculopapular or vesicular rash, frequently on an erythematous base and typically occurring 6 to 9 days after primary vaccination. Treatment with vaccinia immune globulin (VIG) is restricted to those who are systemically ill or have an immunocompromising condition or recurrent disease that can last up to a year. Contact precautions should be used to prevent further transmission and nosocomial infection.[6]

Eczema vaccinatum occurs in individuals with a history of atopic dermatitis, regardless of current disease activity, and can be a papular, vesicular, or pustular rash. This rash may be generalized, or localized with involvement anywhere on the body, with a predilection for areas of previous atopic dermatitis lesions. Mortality ranges from 17% to 30% and is reduced by use of VIG. Contact precautions should be used to prevent further transmission and nosocomial infection.[6]

Progressive vaccinia is a rare, severe, and often fatal complication of vaccination that occurs in individuals with immunodeficiency conditions and is characterized by painless progressive necrosis at the vaccination site with or without metastases to distant sites. This condition carries a high mortality rate; therefore, progressive vaccinia should be aggressively treated with VIG, intensive monitoring, and tertiary medical center level support. Persons with the following conditions are at the highest risk:

- congenital or acquired immunodeficiencies;
- HIV infection/acquired immunodeficiency syndrome;
- cancer;
- autoimmune disease;
- immunosuppressive therapy; or
- organ transplant.

Anecdotal experience has shown that despite treatment with VIG, individuals with cell-mediated immunity defects have a poorer prognosis than those with humoral defects. Infection control measures should include contact and respiratory precautions to prevent transmission and nosocomial infection.[6]

Central nervous system disease, which includes postvaccinial encephalopathy and postvaccinial encephalomyelitis, occurs rarely after smallpox vaccination. Postvaccinial encephalopathy occurs more frequently, typically affects infants and children younger than age 2, and reflects vascular damage to the central nervous system. Symptoms that typically occur 6 to 10 days postvaccination include seizures, hemiplegia, aphasia, and transient amnesia. Histopathologic findings include cerebral edema, lymphocytic meningeal inflammation, ganglion degeneration, and perivascular hemorrhage. Patients with postvaccinial encephalopathy who survive can be left with cerebral impairment and hemiplegia. Postvaccinial encephalomyelitis affects individuals who are age 2 or older and is characterized by abrupt onset of fever, vomiting, malaise, and anorexia occurring approximately 11 to 15 days postvaccination. Symptoms progress to amnesia, confusion, disorientation, restlessness, delirium, drowsiness, and seizures. The cerebral spinal fluid has normal chemistries and cell count. Histopathology findings include demyelization and microglial proliferation in demyelinated areas, with lymphocytic infiltration but without significant edema. The cause for central nervous system disease

is unknown, and no specific therapy exists. Therefore, intervention is limited to anticonvulsant therapy and intensive supportive care. Fetal vaccinia, which results from vaccinial transmission from mother to fetus, is a rare but serious complication of smallpox vaccination during or immediately before pregnancy.[6]

In the Department of Defense 2002–2003 vaccination program involving 540,824 vaccinees, 67 symptomatic cases of myopericarditis were reported, for a rate of 1.2 per 100,000. Mean time from vaccination to evaluation for myopericarditis was 10.4 days, with a range of 3 to 25 days. Reports of myocarditis in vaccinees in 2003 raised concerns of carditis and cardiac deaths in individuals undergoing smallpox vaccination. That year, 21 cases of myo/pericarditis of 36,217 vaccinees were reported, with 19 (90%) occurring in revaccinees. The median age of those affected was 48, and they were predominantly women. Eleven of the individuals were hospitalized, but there were no fatalities. Of the 540,824 total vaccinees over the 2 years, 449,198 were military personnel (the rest were civilians), and of these there were 37 cases, for an occurrence rate of 1 per 120,000 vaccinees.[112] Ischemic cardiac events including fatalities have also been reported as a consequence of the use of vaccinia vaccine (Dryvax) during the campaign. Although no clear association has been found, history of ischemic heart disease and significant cardiac risk pose relative contraindications for smallpox vaccination. Consequently, individuals with a history of myocarditis, pericarditis, or ischemic heart disease should refrain from vaccination.[128,129]

Smallpox Biothreat Policy. In a smallpox release from a bioterrorist event, individuals would be vaccinated according to the current national policy, which recommends initial vaccination of higher risk groups (individuals directly exposed to the release and those with close contact to smallpox patients) and medical and emergency transport personnel. Vaccination of the general population would then be extended in concentric rings around the initial cases to impede the spread. There are no absolute contraindications to vaccination for individuals with high-risk exposure to smallpox. Persons at greatest risk of complications of vaccination are those for whom smallpox infection poses the greatest risk. If relative contraindications exist for an individual, the risks must be weighed against the risk of a potentially fatal smallpox infection.

Postexposure prophylaxis with vaccine offers protection against smallpox but is untried in other *Orthopoxvirus* diseases.[2] Despite a lack of hard evidence, postexposure vaccination is likely efficacious against other orthopoxviruses, and during the 2003 US monkeypox outbreak the CDC recommended vaccination of potentially exposed persons.[80]

Treatment

Passive Immunization

VIG is available from the CDC as an investigational new drug in two formulations, intramuscular and intravenous. VIG may be beneficial in treating some of the adverse effects associated with vaccination. VIG has no proven benefit in smallpox treatment, and its efficacy in treatment of monkeypox infections is unknown. Monoclonal antibodies have been shown to be beneficial in animal models under certain conditions, but this concept has not yet been sufficiently developed for efficacy testing in humans.

Antiviral Drugs

Antiviral drugs would be useful for treatment of orthopoxviral diseases including smallpox and monkeypox, as well as adverse effects associated with vaccination. The only antiviral drug available for treating orthopoxviruses is cidofovir, which may be offered under emergency use protocols maintained by both the Department of Health and Human Services and the Department of Defense.

The elaborate replication strategy of poxviruses offers a number of potential targets for therapeutic intervention.[130] Although inhibition of viral replication may be necessary to halt the pathogenic disease course, it may not be sufficient—it may also be necessary to reverse the effects of the mounting damage that increasingly appears to be the result of a cytokine storm, which accounts for the "toxicity" of systemic orthopoxvirus infection.[29] In this regard, cytokine antagonists developed to treat bacterial sepsis and other conditions may play a role in effective management of smallpox- and monkeypox-infected patients.

Initial studies to identify effective antiviral agents for orthopoxviruses tested drugs developed for other viruses that share molecular targets with poxviruses.[131] The effort to discover effective drugs against DNA viruses initially focused on treatment of herpesviruses infections. The discovery of acyclovir led to practical therapy and a better understanding of the importance of viral and cellular enzymes involved in phosphorylation of acyclovir to acyclovir triphosphate, the active chemical entity. The failure of acyclovir to inhibit cytomegalovirus was because, unlike the thymidine kinase of herpes simplex, cytomegalovirus thymidine kinase lacked the appropriate specificity, which was overcome by synthesis of a series of phosphorylated analogues using a stable phosphonate bond. The most promising candidate using this approach was cidofovir, which is a dCMP analog.[132] Cidofovir is licensed

for treatment of cytomegalovirus-associated retinitis under the trade name Vistide (Gilead Sciences Inc, Foster City, Calif), and may inhibit the cytomegalovirus DNA polymerase, a target shared with the poxviruses. Cidofovir also may inhibit the activity of the proofreading exonuclease, leading to error-prone DNA synthesis during poxvirus replication. Cidofovir has been demonstrated to protect monkeys against severe disease in both the monkeypox and authentic smallpox primate models, when administered within 48 hours of intravenous exposure to the virus.[133] Although the drug formulation used in these studies has been criticized for requiring intravenous administration, patients with advanced disease would already be receiving intravenous fluids as part of their supportive care, and once weekly cidofovir administration would not significantly increase the healthcare burden. Because cidofovir has been associated with nephrotoxicity, primarily in dehydrated patients, careful attention to fluid management is important, and patient hydration and coadministration of probenecid is required.

Oral formulations of cidofovir analogues with better bioavailability and lower toxicity, designed to overcome the lack of an active transport pathway for unmodified cidofovir into cells, are under development.[134] Cidofovir requires bolus dosing to allow drug entry into cells by pinocytosis; however, bolus dosing results in transiently high concentrations in the kidney. The primary design paradigm for oral formulations is the creation of a lipid mimic that allows drugs to enter cells via the chylomicron pathway.[135] This formulation dramatically reduced transient drug levels in the kidney and eliminated nephrotoxicity in toxicology studies using mice. However, an oral formulation of cidofovir is not available for human use.

The first drug used to empirically treat progressive vaccinia and smallpox was Marboran, a compound of the class of N-aminomethyl-isatin-beta-thiosemicarbazones. As with most early treatment strategies, controlled clinical trials were not reported, and recent studies show that Marboran was only capable of inhibiting replication by 80% at maximum tolerated concentration in VERO cells.[136] Through combinatorial chemistry, potent and more selective compounds have now been discovered and are in preliminary testing.[137] A number of essential viral enzymes have been targeted using a homology-based bioinformatics approach, such as that used to develop a structural model of vaccinia virus I7L proteinase. A unique chemical library of 51,000 compounds was computationally queried to identify potential active site inhibitors.[138] A subset of compounds was assayed for toxicity and ability to inhibit vaccinia replication, and a family was identified with 50% minimal inhibitory concentrations of 3 to 12 μM. Alternatively, a high-throughput screening approach using cowpox virus evaluated a collection of over 250,000 compounds and identified several potent lead structures for optimization and evaluation against vaccinia, monkeypox, and variola viruses. From this effort ST-246 {4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide} was identified and is under development. ST-246 is both potent (EC_{50} = 0.010 μM), selective (CC_{50} > 40 mM), and active against multiple orthopoxviruses, including monkeypox, camelpox, cowpox, ectromelia (mousepox), vaccinia, and variola viruses in vitro and monkeypox, variola, cowpox, vaccinia, and ectromelia in vivo.

Alternative approaches include peptide mimetics of IFN-γ that play a direct role in the activation of STAT 1 alpha transcription factor.[139] These mimetics do not act through recognition of the extracellular domain of the IFN-γ receptor; rather, they bind to the cytoplasmic domain of the receptor chain and thereby initiate the cellular signaling. The authors hypothesize that mimetics would bypass the poxvirus virulence factor B8R protein that binds the intact IFN-γ and would prevent interaction with its receptor. Experimentally, these mimetics, but not intact IFN-γ, inhibited replication of vaccinia in BSC-40 cells. Thus these mimetics can avoid the B8R virulence factor and have potential activity against poxviruses in vivo.

Gleevec (Novartis Pharmaceuticals Corporation, East Hanover, NJ), a drug licensed for use in chronic myeloid leukemia, has been shown to block the egress of vaccinia virus from infected cells.[140] Smallpox virus includes an epidermal-growth-factor–like domain that targets human Erb-1, inducing tyrosine phosphorylation of certain host cell substrates, thereby facilitating viral replication. Poxviruses migrate to the cell membrane via the polymerization of actin tails to produce EEV, which facilitates viral dissemination. The authors reason that low molecular weight inhibitors of Erb-1 kinases might function as antiviral agents. CI-1033, one such inhibitor, blocked variola replication in BSC-40 and Vero cells, primarily at the level of secondary viral spreading. CI-1033 protected mice exposed to a lethal vaccinia challenge via the aerosol route. In conjunction with a monoclonal antibody directed against L1R, CI-1033 cleared the mice's lungs of virus within 8 days. Gleevec is also a small molecule that inhibits the Abl-1 family of tyrosine kinases, thereby inhibiting the release of EEV from infected cells. Gleevec inhibited the vaccinia virus spread from the mouse peritoneum to the ovaries and protected the mice from all lethal intranasal challenge. The advantage of Gleevec over other tyrosine kinase inhibitors such as CI-1033 is that it is already approved for human use. The potential

success of Gleevec suggests that strategies that block key host signaling pathways have merit and augment the approaches that target classical viral replication enzymes. An alternative approach to inhibiting the polymerization of actin, which in turn inhibits the propulsion of viral particles along actin filaments toward the cell membrane, is small interfering RNA directed against the Arp2/3[141] complex.

ST-246 is a new drug that is orally available and is currently in phase I human safety studies. Based on activity in multiple small animal models, oral ST-246 was evaluated in a recent USAMRIID study with a variola virus-cynomolgus monkey model of classical smallpox that closely resembles human disease.[142] The placebo group demonstrated typical disease with greater than 1,250 pox lesions and 33% mortality. Oral gavage with ST-246 began 24 hours after infection, when bone marrow, spleen, some lymph nodes, and liver have greater than 10^8 genomes per gram and all tissues have 10^4 to 10^6 per gram, eliminated disease as judged by complete lack of lesion formation, the best predictor of smallpox disease severity in man, and no significant clinical or laboratory findings. Virus levels in blood did not increase over pretreatment levels (10^6/mL), and virus was cleared in 6 days versus 16 days for placebo based on historical data. ST-246 was next evaluated using the authors' monkeypox virus-cynomolgus monkey model of classical smallpox that also closely resembles human disease. The placebo-treated group demonstrated typical disease with greater than 1,500 pox lesions and 100% mortality. Oral gavage treatment with ST-246 began 1 day after infection, when bone marrow, spleen, some lymph nodes, and liver have greater than 10^7 genomes per gram and all tissues have 10^5 to 10^6 per gram, and eliminated disease as judged by complete lack of lesion formation and no significant clinical or laboratory findings. Virus levels in blood did not increase over pretreatment levels and virus was cleared in 4 days versus 16 days for placebo or intravenous cidofovir based on historical data. Oral gavage treatment with ST-246 began 3 days after infection, when bone marrow, spleen, some lymph nodes, and liver have greater than 10^8 genomes per gram and all tissues have greater than 10^6 per gram, eliminated disease as judged by complete lack of lesion formation in 2 of 3 monkeys and less than 5% of control lesions in 1 of 3 that did not progress, and no significant clinical or laboratory findings resulted. Virus levels in blood did not increase over pretreatment levels and virus was cleared in 6 days versus 16 days for placebo. ST-246 has been granted fast track investigational new drug status and has not shown toxicity in phase I human single oral dosing at 2,000 mg and is now in repeated dosing studies.[142]

Lastly, treatment strategies may be developed to target the toxemia or clinical manifestations of smallpox. In particular, modulation of the systemic immune response to orthopox infection, specifically the prevention of organ damage caused by vascular leakage and fibrin deposition, may provide a useful therapeutic target. Uncontrolled or inappropriate immune responses can contribute to multiple organ failure and death; in this respect the "toxemia" associated with fatal orthopox infections resembles severe sepsis. Several treatment strategies for targeting the manifestations of septic shock,[143] such as activated protein C and inhibitors of the tissue factor pathway,[144] are under consideration for testing in the nonhuman primate model for smallpox.

SUMMARY

Smallpox no longer causes human disease thanks to the dedicated efforts of public health officials who participated in the WHO smallpox eradication program. Although the former Soviet Union participated in the eradication program, recent revelations have shown that the Soviets continued developing smallpox for biowarfare into the 1980s. The Soviet Union is dissolved and its offensive program dismantled, but the institutions and technology that developed this and other offensive weapons systems remain. Because the submission and destruction of smallpox virus stores was a voluntary program, it cannot be ascertained with certainty that smallpox viruses do not exist outside US and Russian storage facilities. Because the sequence of several variola isolates is known to a high degree of certainty, it is technically possible to generate viable virus either by modification of a closely related virus such as camelpox or chemical synthesis using increasingly powerful automated equipment.

The potential threat from smallpox specifically and orthopox infections in general will expand as the technology to create these viruses becomes increasingly available in laboratories around the world. Furthermore, scientists have been successful in making orthopoxviruses more virulent through genetic manipulation. The biodefense community has made considerable progress in developing new drugs for treatment of orthopoxvirus infections and safer vaccines; however, much work remains. There is still no approved treatment for smallpox, and the new safer vaccines remain unlicensed and unavailable.

REFERENCES

1. Tucker JB. *Scourge: The Once and Future Threat of Smallpox*. New York, NY: Atlantic Monthly Press; 2001.

2. Fenner F, Henderson DA, Arita I, Jezek A, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988.

3. Henderson DA, Inglesby TV, Bartlett JG, et al. Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA.* 1999;281:2127–2137.

4. Ferguson NM, Keeling MJ, Edmunds WJ, et al. Planning for smallpox outbreaks. *Nature.* 2003;425:681–685.

5. Kaplan EH, Craft DL, Wein LM. Emergency response to a smallpox attack: the case for mass vaccination. *Proc Natl Acad Sci U S A.* 2002;99:10935–10940.

6. Cono J, Casey CG, Bell DM. Smallpox vaccination and adverse reactions. *MMWR Recomm Rep.* 2003;52:1–28.

7. Zelicoff AP. An epidemiological analysis of the 1971 smallpox outbreak in Aralsk, Kazakhstan. *Crit Rev Microbiol.* 2003;29:97–108.

8. Centers for Disease Control and Prevention. *Smallpox Response Plan and Guidelines (version 3.0), 9/21/02 Guide A. Surveillance, Contract Tracing, and Epidemiological Investigation Guidelines.* Atlanta, Ga: CDC; 2002.

9. LeDuc JW, Jahrling PB. Strengthening national preparedness for smallpox: an update. *Emerg Infect Dis.* 2001;7:155–157.

10. Alibek K. *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World—Told From the Inside By the Man Who Ran It.* New York, NY: Random House; 1999.

11. Charatan F. US doctors investigate more than 50 possible cases of monkeypox. *BMJ.* 2003;326:1350.

12. Reed KD, Melski JW, Graham MB, et al. The detection of monkeypox in humans in the Western Hemisphere. *N Engl J Med.* 2004;350:342–350.

13. Gubser C, Smith GL. The sequence of camelpox virus shows it is most closely related to variola virus, the cause of smallpox. *J Gen Virol.* 2002;83:855–872.

14. Gubser C, Hue S, Kellam P, Smith GL. Poxvirus genomes: a phylogenetic analysis. *J Gen Virol.* 2004;85:105–117.

15. Baxby D. The surface antigens of orthopoxviruses detected by cross-neutralization tests on cross-absorbed antisera. *J Gen Virol.* 1982;58:251–262.

16. Czerny CP, Johann S, Holzle L, Meyer H. Epitope detection in the envelope of intracellular naked orthopox viruses and identification of encoding genes. *Virology.* 1994;200:764–777.

17. Vanderplasschen A, Hollinshead M, Smith GL. Antibodies against vaccinia virus do not neutralize extracellular enveloped virus but prevent virus release from infected cells and comet formation. *J Gen Virol.* 1997;78:2041–2048.

18. Viner KM, Isaacs SN. Activity of vaccinia virus-neutralizing antibody in the sera of smallpox vaccinees. *Microbes Infect.* 2005;7:579–583.

19. Payne LG. Significance of extracellular enveloped virus in the in vitro and in vivo dissemination of vaccinia. *J Gen Virol.* 1980;50:89–100.

20. Kaplan C, Benson PF, Butler NR. Immunogenicity of ultraviolet-irradiated, non-infectious, vaccinia-virus vaccine in infants and young children. *Lancet.* 1965;191:573–574.

21. Moss B. Poxviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, et al, eds. *Fields Virology*. 4th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001: 2849–2883.

22. Smith GL, Vanderplasschen A, Law M. The formation and function of extracellular enveloped vaccinia virus. *J Gen Virol.* 2002;83:2915–2931.

23. Johnston JB, McFadden G. Poxvirus immunomodulatory strategies: current perspectives. *J Virol.* 2003;77:6093–6100.

24. McFadden G. Poxvirus tropism. *Nat Rev Microbiol.* 2005;3:201–213.

25. Fenner F. The clinical features and pathogenesis of mousepox (infectious ectromelia of mice). *J Pathol Bacteriol.* 1948;60:529–552.

26. Buller RM, Palumbo GJ. Poxvirus pathogenesis. *Microbiol Rev.* 1991;55:80–122.

27. Wenner HA, Macasaet FD, Kamitsuka PS, Kidd P. Monkey pox. I. Clinical, virologic and immunologic studies. *Am J Epidemiol.* 1968;87:551–566.

28. Zaucha GM, Jahrling PB, Geisbert TW, Swearengen JR, Hensley L. The pathology of experimental aerosolized monkeypox virus infection in cynomolgus monkeys (Macaca fascicularis). *Lab Invest.* 2001;81:1581–1600.

29. Jahrling PB, Hensley LE, Martinez MJ, et al. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. *Proc Natl Acad Sci U S A.* 2004;101:15196–15200.

30. Sarkar JK, Mitra AC, Mukherjee MK, De SK, Mazumdar DG. Virus excretion in smallpox. I. Excretion in throat, urine, and conjunctiva of patients. *Bull World Health Organ.* 1973;48:517–522.

31. Martin DB. The cause of death in smallpox: an examination of the pathology record. *Mil Med.* 2002;167:546–551.

32. Levi M, de Jonge E, van der Poll T. Sepsis and disseminated intravascular coagulation. *J Thromb.* 2003;16:43–47.

33. Moss B, Shisler JL. Immunology 101 at poxvirus U: immune evasion genes. *Semin Immunol.* 2001;13:59–66.

34. Heagerty J. *Four Centuries of Medical History in Canada.* Vol 1. Toronto, Ontario, Canada: MacMillan; 1928.

35. Parkman F. *The Conspiracy of Pontiac.* Vol 2. Boston, Mass: Little, Brown and Company; 1969.

36. Stearn E, Stearn A. *The Effect of Smallpox on the Destiny of the Amerindian.* Boston, Mass: Bruce Humphries; 1945.

37. Kean RGH. *Inside the Confederate Government.* New York, NY: Oxford University Press; 1957.

38. Steiner P. *Disease in the Civil War: Natural Biological Warfare, 1861–1865.* Springfield, Ill: Charles C Thomas; 1968.

39. Miller J, Engelberg S, Broad W. *Germs. Biological Weapons and America's Secret War.* New York, NY: Simon and Schuster Inc; 2001.

40. Heymann DL, Szczeniowski M, Esteves K. Re-emergence of monkeypox in Africa: a review of the past six years. *Br Med Bull.* 1998;54:693–702.

41. World Health Organization. *Technical Advisory Group on Human Monkeypox. Report of a WHO Meeting.* Geneva, Switzerland: WHO; 1999. WHO/CDS/CSR/APH/99.5.

42. Hahon N, Mc GM. Air-borne infectivity of the variola-vaccinia group of poxviruses for the cynomolgus monkey, Macaca irus. *J Infect Dis.* 1961;109:294–298.

43. Chen N, Li G, Liszewski MK, et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology.* 2005; 340:46–63.

44. Cho CT, Wenner HA. Monkeypox virus. *Bacteriol Rev.* 1973;37:1–18.

45. Esposito JJ, Fenner F. Poxviruses. In: Knipe DM, Howley PM, Griffin DE, et al, eds. *Fields Virology*. 4th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001:2885–2921.

46. Damaso CR, Esposito JJ, Condit RC, Moussatche N. An emergent poxvirus from humans and cattle in Rio de Janeiro State: Cantagalo virus may derive from Brazilian smallpox vaccine. *Virology.* 2000;277:439–449.

47. Roberts JF, Coffee G, Creel SM, et al. Haemorrhagic smallpox. I. Preliminary haematological studies. *Bull World Health Organ.* 1965;33:607–613.

48. Lancaster MC, Boulter EA, Westwood JC, Randles J. Experimental respiratory infection with poxviruses. II. Pathological studies. *Br J Exp Pathol*. 1966;47:466–471.

49. Westwood JC, Boulter EA, Bowen ET, Maber HB. Experimental respiratory infection with poxviruses. I. Clinical virological and epidemiological studies. *Br J Exp Pathol.* 1966;47:453–465.

50. Mims CA. Aspects of the pathogenesis of virus diseases. *Bacteriol Rev.* 1964;28:30–71.

51. Martinez MJ, Bray MP, Huggins JW. A mouse model of aerosol-transmitted orthopoxviral disease: morphology of experimental aerosol-transmitted orthopoxviral disease in a cowpox virus-BALB/c mouse system. *Arch Pathol Lab Med.* 2000;124:362–377.

52. Hahon N. Smallpox and related poxvirus infections in the simian host. *Bacteriol Rev.* 1961;25:459–476.

53. Jezek Z, Kriz B, Rothbauer V. Camelpox and its risk to the human population. *J Hyg Epidemiol Microbiol Immunol.* 1983;27:29–42.

54. Cello J, Paul AV, Wimmer E. Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science.* 2002;297:1016–1018.

55. Jackson RJ, Ramsay AJ, Christensen CD, Beaton S, Hall DF, Ramshaw IA. Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *J Virol.* 2001;75:1205–1210.

56. Robbins SJ, Jackson RJ, Fenner F, et al. The efficacy of cidofovir treatment of mice infected with ectromelia (mousepox) virus encoding interleukin-4. *Antiviral Res.* 2005;66:1–7.

57. Mullbacher A, Lobigs M. Creation of killer poxvirus could have been predicted. *J Virol.* 2001;75:8353–8355.

58. Stanford MM, McFadden G. The 'supervirus'? Lessons from IL-4-expressing poxviruses. *Trends Immunol.* 2005;26:339–345.

59. Huq F. Effect of temperature and relative humidity on variola virus in crusts. *Bull World Health Organ.* 1976;54:710–712.

60. Meiklejohn G, Kempe CH, Downie AW, Berge TO, St Vincent L, Rao AR. Air sampling to recover variola virus in the environment of a smallpox hospital. *Bull World Health Organ.* 1961;25:63–67.

61. Downie AW, Meiklejohn M, St Vincent L, Rao AR, Sundara Babu BV, Kempe CH. The recovery of smallpox virus from patients and their environment in a smallpox hospital. *Bull World Health Organ.* 1965;33:615–622.

62. Foege WH, Millar JD, Henderson DA. Smallpox eradication in West and Central Africa. *Bull World Health Organ.* 1975;52:209–222.

63. Wehrle PF, Posch J, Richter KH, Henderson DA. An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. *Bull World Health Organ.* 1970;43:669–679.

64. Maccallum FO, McDonald JR. Survival of variola virus in raw cotton. *Bull World Health Organ.* 1957;16:247–254.

65. Sarkar JK, Mitra AC, Mukherjee MK, De SK. Virus excretion in smallpox. 2. Excretion in the throats of household contacts. *Bull World Health Organ.* 1973;48:523–527.

66. Sarkar JK, Mitra AC, Mukherjee MK, De SK, Mazumdar DG. Virus excretion in smallpox. 1. Excretion in the throat, urine, and conjunctiva of patients. *Bull World Health Organ.* 1973;48:517–522.

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

68. Downie AW, St Vincent L, Meiklejohn G, et al. Studies on the virus content of mouth washings in the acute phase of smallpox. *Bull World Health Organ.* 1961;25:49–53.

69. Mitra AC, Sarkar JK, Mukherjee MK. Virus content of smallpox scabs. *Bull World Health Organ.* 1974;51:106–107.

70. Dixon CW. *Smallpox*. London, England: Churchill; 1962.

71. Rao AR. *Smallpox.* Bombay, India: Kothari Book Depot; 1972.

72. Downie AW, Fedson DS, Saint Vincent L, Rao AR, Kempe CH. Haemorrhagic smallpox. *J Hyg (Lond).* 1969;67:619–629.

73. Rao AR, Prahlad I, Swaminathan M, Lakshmi A. Pregnancy and smallpox. *J Indian Med Assoc.* 1963;40:353–363.

74. Gupta SK, Srivastava TP. Roentgen features of skeletal involvement in smallpox. *Australas Radiol.* 1973;17:205–211.

75. Rao AR. Haemorrhagic smallpox: a study of 240 cases. *J Indian Med Assoc.* 1964;43:224–229.

76. Downie AW, Saint Vincent L, Goldstein L, Rao AR, Kempe CH. Antibody response in non-haemorrhagic smallpox patients. *J Hyg (Lond).* 1969;67:609–618.

77. Jezek Z, Gromyko AI, Szczeniowski MV. Human monkeypox. *J Hyg Epidemiol Microbiol Immunol.* 1983;27:13–28.

78. Jezek Z, Marennikova SS, Mutumbo M, Nakano JH, Paluku KM, Szczeniowski M. Human monkeypox: a study of 2,510 contacts of 214 patients. *J Infect Dis.* 1986;154:551–555.

79. Jezek Z, Szczeniowski M, Paluku KM, Mutombo M. Human monkeypox: clinical features of 282 patients. *J Infect Dis.* 1987;156:293–298.

80. Updated Interim CDC Guidance for Use of Smallpox Vaccine, Cidofovir, and Vaccinia Immune Globulin (VIG) for Prevention and Treatment in the Setting of an Outbreak of Monkeypox Infections. June 25, 2003. Available at: http://www.cdc.gov/ncidod/monkeypox/treatmentguidelines.htm. Accessed April 25, 2007.

81. Sejvar JJ, Chowdary Y, Schomogyi M, et al. Human monkeypox infection: a family cluster in the midwestern United States. *J Infect Dis.* 2004;190:1833–1840.

82. Jezek Z, Fenner F. *Human Monkeypox.* Vol 17. Basel, Switzerland: Karger; 1988.

83. Di Giulio DB, Eckburg PB. Human monkeypox. *Lancet Infect Dis.* 2004;4:199.

84. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. *Lancet Infect Dis.* 2004;4:15–25.

85. Jezek Z, Grab B, Paluku KM, Szczeniowski MV. Human monkeypox: disease pattern, incidence and attack rates in a rural area of northern Zaire. *Trop Geogr Med.* 1988;40:73–83.

86. Hutin YJ, Williams RJ, Malfait P, et al. Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. *Emerg Infect Dis.* 2001;7:434–438.

87. Jezek Z, Nakano JH, Arita I, Mutombo M, Szczeniowski M, Dunn C. Serological survey for human monkeypox infections in a selected population in Zaire. *J Trop Med Hyg.* 1987;90:31–38.

88. Meyer H, Perrichot M, Stemmler M, et al. Outbreaks of disease suspected of being due to human monkeypox virus infection in the Democratic Republic of Congo in 2001. *J Clin Microbiol.* 2002;40:2919–2921.

89. Baxby D, Bennett M, Getty B. Human cowpox 1969-93: a review based on 54 cases. *Br J Dermatol.* 1994;131:598–607.

90. Pelkonen PM, Tarvainen K, Hynninen A, et al. Cowpox with severe generalized eruption, Finland. *Emerg Infect Dis.* 2003;9:1458–1461.

91. Lal SM, Singh IP. Buffalopox–a review. *Trop Anim Health Prod.* 1977;9:107–112.

92. Baxby D, Hill BJ. Characteristics of a new poxvirus isolated from Indian buffaloes. *Arch Gesamte Virusforsch.* 1971;35:70–79.

93. Wariyar KG. Variola in buffaloes. *Indian Vet J*. 1937;14:169–170.

94. US Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.* Washington, DC: US Government Printing Office; 2007.

95. Kato S, Cutting W. A study of the inclusion bodies of rabbit myxoma and fibroma virus and a consideration of the relationship between all pox virus inclusion bodies. *Stanford Med Bull.* 1959;17:34–45.

96. Artenstein AW, Johnson C, Marbury TC, et al. A novel, cell culture-derived smallpox vaccine in vaccinia-naive adults. *Vaccine.* 2005;23:3301–3309.

97. Damon IK, Esposito JJ. Poxvirus infections in humans. In: Murray PR, Jorgensen JH, Yolken RH, Baron EJ, Pfaller MA, eds. *Manual of Clinical Microbiology*. 8th ed. Washington, DC: American Society for Microbiology Press; 2003.

98. Meyer H, Damon IK, Esposito JJ. Orthopoxvirus diagnostics. *Methods Mol Biol.* 2004;269:119–134.

99. Karem KL, Reynolds M, Braden Z, et al. Characterization of acute-phase humoral immunity to monkeypox: use of immunoglobulin M enzyme-linked immunosorbent assay for detection of monkeypox infection during the 2003 North American outbreak. *Clin Diagn Lab Immunol.* 2005;12:867–872.

100. Hammarlund E, Lewis MW, Carter SV, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. *Nat Med.* 2005;11:1005–1111.

101. Slifka M, Hammarlund E. Monkeypox outbreak diagnostics and implications for vaccine protective effect. *Nat Med.* 2006;12:496–497.

102. Loparev VN, Massung RF, Esposito JJ, Meyer H. Detection and differentiation of old world orthopoxviruses: restriction fragment length polymorphism of the crmB gene region. *J Clin Microbiol.* 2001;39:94–100.

103. Ibrahim MS, Mellott JD. Orthopoxviruses: monkeypox, cowpox, vaccinia, camelpox, mousepox. In: Fuchs J, Podda M, eds. *Encyclopedia of Medical Genomics and Proteomics.* New York, NY: Marcel Dekker, Inc; 2005: 947–952.

104. Lapa S, Mikheev M, Shchelkunov S, et al. Species-level identification of orthopoxviruses with an oligonucleotide microchip. *J Clin Microbiol.* 2002;40:753–757.

105. Ibrahim MS, Kulesh DA, Saleh SS, et al. Real-time PCR assay to detect smallpox virus. *J Clin Microbiol.* 2003;41:3835–3839.

106. Ropp SL, Jin Q, Knight JC, Massung RF, Esposito JJ. PCR strategy for identification and differentiation of smallpox and other orthopoxviruses. *J Clin Microbiol.* 1995;33:2069–2076.

107. Schupp P, Pfeffer M, Meyer H, Burck G, Kolmel K, Neumann C. Cowpox virus in a 12-year-old boy: rapid identification by an orthopoxvirus-specific polymerase chain reaction. *Br J Dermatol.* 2001;145:146–150.

108. Dhar AD, Werchniak AE, Li Y, et al. Tanapox infection in a college student. *N Engl J Med.* 2004;350:361–366.

109. Espy MJ, Cockerill IF III, Meyer RF, et al. Detection of smallpox virus DNA by LightCycler PCR. *J Clin Microbiol.* 2002;40:1985–1988.

110. Egan C, Kelly CD, Rush-Wilson K, et al. Laboratory-confirmed transmission of vaccinia virus infection through sexual contact with a military vaccinee. *J Clin Microbiol.* 2004;42:5409–5411.

111. Kulesh DA, Baker RO, Loveless BM, et al. Smallpox and pan-orthopox virus detection by real-time 3′-minor groove binder TaqMan assays on the roche LightCycler and the Cepheid smart Cycler platforms. *J Clin Microbiol.* 2004;42:601–609.

112. Fenn EA. *Pox Americana: The Great Smallpox Epidemic of 1775–82.* New York, NY: Hill & Wang; 2001.

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

114. Yih WK, Lieu TA, Rego VH, et al. Attitudes of healthcare workers in US hospitals regarding smallpox vaccination. *BMC Public Health.* 2003;3:20.

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

116. Lane JM, Goldstein J. Adverse events occurring after smallpox vaccination. *Semin Pediatr Infect Dis.* 2003;14:189–195.

117. Greenberg RN, Kennedy JS, Clanton DJ, et al. Safety and immunogenicity of new cell-cultured smallpox vaccine compared with calf-lymph derived vaccine: a blind, single-centre, randomised controlled trial. *Lancet.* 2005;365:398–409.

118. Rock MT, Yoder SM, Talbot TR, Edwards KM, Crowe JE Jr. Adverse events after smallpox immunizations are associated with alterations in systemic cytokine levels. *J Infect Dis.* 2004;189:1401–1410.

119. Edghill-Smith Y, Golding H, Manischewitz J, et al. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. *Nat Med.* 2005;11:740–747.

120. Blanchard TJ, Alcami A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J Gen Virol.* 1998;79:1159–1167.

121. Earl PL, Americo JL, Wyatt LS, et al. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature.* 2004;428:182–185.

122. Hooper JW, Thompson E, Wilhelmsen C, et al. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. *J Virol.* 2004;78:4433–4443.

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

124. Centers for Disease Control and Prevention. Smallpox fact sheet: Vaccine overview.

125. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Recomm Rep.* 2001;50:1–25.

126. Lewis FM, Chernak E, Goldman E, et al. Ocular vaccinia infection in laboratory worker, Philadelphia, 2004. *Emerg Infect Dis.* 2006;12:134–137.

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

128. Centers for Disease Control and Prevention. Update: cardiac-related events during the civilian smallpox vaccination program–United States, 2003. *MMWR Morb Mortal Wkly Rep.* 2003;52:492–496.

129. Halsell JS, Riddle JR, Atwood JE, et al. Myopericarditis following smallpox vaccination among vaccinia-naive US military personnel. *JAMA.* 2003;289:3283–3289.

130. Harrison SC, Alberts B, Ehrenfeld E, et al. Discovery of antivirals against smallpox. *Proc Natl Acad Sci U S A.* 2004;101:11178–11192.

131. Prichard MN, Kern ER. Orthopoxvirus targets for the development of antiviral therapies. *Curr Drug Targets Infect Disord.* 2005;5:17–28.

132. Magee WC, Hostetler KY, Evans DH. Mechanism of inhibition of vaccinia virus DNA polymerase by cidofovir diphosphate. *Antimicrob Agents Chemother.* 2005;49:3153–3162.

133. Jahrling PB, Huggins JW. Orthopoxviruses. In: Swearengen JR, ed. *Biodefense: Research Methodology and Animal Models.* Boca Raton, Fla: CRC Press; 2005.

134. Raulin J. Development in lipid drugs. *Mini Rev Med Chem.* 2005;5:489–498.

135. Painter GR, Hostetler KY. Design and development of oral drugs for the prophylaxis and treatment of smallpox infection. *Trends Biotechnol.* 2004;22:423–427.

136. Huggins JW. Unpublished observation, 1996.

137. Pirrung MC, Pansare SV, Sarma KD, Keith KA, Kern ER. Combinatorial optimization of isatin-beta-thiosemicarbazones as anti-poxvirus agents. *J Med Chem.* 2005;48:3045–3050.

138. Byrd CM, Bolken TC, Mjalli AM, et al. New class of orthopoxvirus antiviral drugs that block viral maturation. *J Virol.* 2004;78:12147–12156.

139. Ahmed CM, Burkhart MA, Subramaniam PS, Mujtaba MG, Johnson HM. Peptide mimetics of gamma interferon possess antiviral properties against vaccinia virus and other viruses in the presence of poxvirus B8R protein. *J Virol.* 2005;79:5632–5639.

140. Yang H, Kim SK, Kim M, et al. Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction. *J Clin Invest.* 2005;115:379–387.

141. Komano J, Miyauchi K, Matsuda Z, Yamamoto N. Inhibiting the Arp2/3 complex limits infection of both intracellular mature vaccinia virus and primate lentiviruses. *Mol Biol Cell.* 2004;15:5197–5207.

142. Huggins JW. Unpublished communication, 2007.

143. Levi M, de Jonge E, van der Poll T. New treatment strategies for disseminated intravascular coagulation based on current understanding of the pathophysiology. *Ann Med.* 2004;36:41–49.

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