Chapter 24

SMALLPOX AND RELATED ORTHOPOXVIRUSES

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INTRODUCTION

Variola virus, the causative agent of smallpox, is one of the most significant bioterrorist threat agents. During the 20th century, smallpox was estimated to have caused more than 500 million human deaths.[1] 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 with no animal reservoirs to reintroduce the virus into the human population. The impact of a potential smallpox virus attack in the human population would be more catastrophic now than in previous outbreaks. The pace of viral spread would be accelerated since most vaccination programs were abandoned worldwide in the 1970s, the prevalence of immune-suppressed individuals with human immunodeficiency virus (HIV) infections and those undergoing chemotherapy for various cancers have grown, and human mobility including intercontinental air travel has increased.

Variola virus is stable, highly infectious via the aerosol route, highly transmissible from infected to susceptible persons, and has a relatively long asymptomatic incubation period making contact tracing difficult.[3] Mathematical models of the reintroduction of variola 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 a potential attack.[5] However, the current licensed 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.

Revelations in the 1990s that the former Soviet Union produced ton quantities of variola virus as a strategic weapon and conducted open air testing of aerosolized variola on Vozrozhdeniya Island in the Aral Sea have increased the plausibility of variola being used as a bioterrorism agent.[3,7] Considerable investment is being made in biopreparedness measures against smallpox and related orthopoxviruses, including emergency response plans for mass immunization and quarantine, as well as development of improved countermeasures such as new vaccines and antiviral drugs.[8,9] 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 the monkeypox virus was weaponized by the former Soviet Union.[10] The monkeypox virus was first isolated from cynomolgus macaques in Denmark in 1958; however, scientific interest did not increase until the 1970s when it was demonstrated that monkeypox virus can cause lethal infection in humans.[11,12] A dramatic increase in monkeypox virus incidence has occurred in the Democratic Republic of the Congo (DRC) over the past 30 years following the cessation of routine smallpox vaccination, and current estimates state that approximately 50% of the DRC general population is not protected against either the monkeypox virus or variola virus.[13] 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 DRC, little morbidity was associated with infection.[14,15] Consequently, more than 50 human infections were documented in the United States, thus demonstrating the public health importance of this agent and its potential bioterrorist threat.[14,15]

AGENT CHARACTERISTICS

Classification

Poxviruses infect most vertebrates and invertebrates, causing various 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 consists of numerous genetically similar pathogens capable of causing disease in humans including variola virus (Figure 24-1), monkeypox virus, cowpox virus, and vaccinia virus.[16] Members of the *Orthopoxvirus* genus are mostly zoonotic pathogens (Table 24-1) that are antigenically similar.[17] Antigenic similarity was vital to the smallpox eradication in 1980 and remains a key component for the protection of military personnel, healthcare workers, and researchers who are likely to come into contact with orthopoxviruses.[9,18]

Morphology

Orthopoxviruses are oval, brick-shaped particles with a geometrically corrugated outer surface (Figure 24-2). Their size ranges from 220 nm to 450 nm long and 140 nm to 260 nm wide.[18] The outer envelope consists

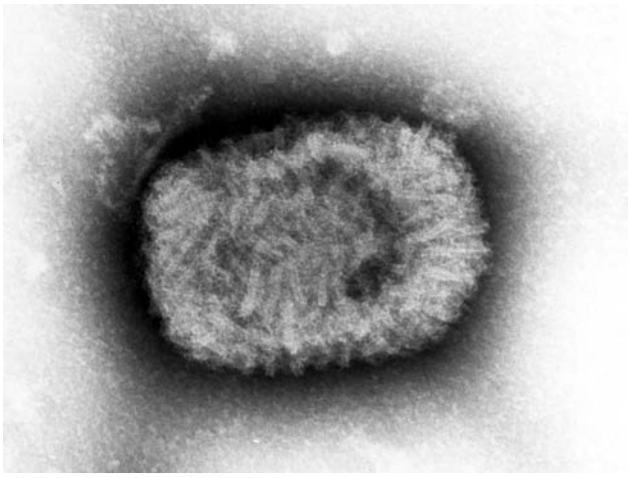

Figure 24-1. A transmission electron micrograph of a tissue section containing variola viruses.
Photograph: Courtesy of FA Murphy, University of Texas Medical Branch, Galveston, Texas.

of a lipoprotein layer embedded with surface tubules enclosing a core described as biconcave. 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.[19] Between the palisade layer and the outer envelope are two oval masses known as the lateral bodies.

Two forms of orthopoxviruses result from the replication cycle: intracellular mature virion (IMV) and enveloped virion (EV). EV can be further categorized as intracellular enveloped virion (IEV), cell-associated enveloped virion (CEV), and extracellular enveloped virion (EEV). IMV, CEV, and EEV are fully infectious, whereas the ability of IEV to promote productive infection in a naïve cell has not been demonstrated.[18,19] These virus forms will be discussed in greater detail in a later section.

Entry

Entry of orthopoxviruses into permissive cells has been extensively studied and, depending on the methodology used, these studies have produced conflicting results. Electron microscopy has suggested that the attachment of enveloped virions (EEV and CEV) to the cell surface results in the disruption of the envelope and exposes the IMV particle that subsequently binds to the cell surface. In this model, the outer membrane of the IMV particle fuses with the plasma membrane of the cell, releasing the viral core into the cell.[20]

Endocytosis has been suggested as an alternative model of orthopoxvirus entry using video microscopy of fluorescently labeled virus particles. IMV and enveloped virions (CEV and EEV) bind to the cell surface and, following a complex series of signaling events, are internalized by endocytosis. For enveloped particles, it is predicted that low pH and/or exposure to glycosaminoglycans results in the disruption of the envelope and exposure of the IMV particle. The outer membrane of the IMV then fuses with the endosomal membrane, releasing the viral core into the cell cytoplasm.[21]

It is likely that both suggested mechanisms of entry are used by orthopoxviruses and that the method used is dependent on the virus strain and the target cell type. Regardless of how the virus enters a given cell, the final step in entry is the initiation of early gene transcription that is followed by core uncoating and replication.

TABLE 24-1

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

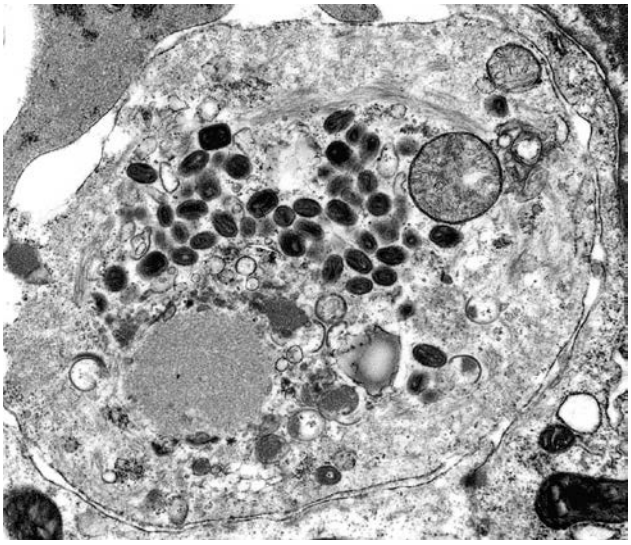

Figure 24-2. Thin section of smallpox virus growing in the cytoplasm of an infected chick embryo cell. Intracellular mature virions (brick-shaped) and immature virions (spherical) are visible. Magnification is approximately × 25,000. Photograph: Courtesy of FA Murphy, University of Texas Medical Branch, Galveston, Texas.

Replication

Orthopoxvirus genomes are linear, double-stranded DNA approximately 200 kb long. The genomes encode between 176 and 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, while 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. Early transcripts encode proteins involved in the modulation of the host immune response as well as the DNA polymerase and other enzymes that promote the replication of the viral genome. Uncoating of the core exposes viral DNA and 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 24-3).[18,19,21,22]

Nascent viral genomes are used as the templates for the production of intermediate transcripts that encode factors that promote the production of late transcripts. The late transcripts are translated into structural and nonstructural proteins of the virions.[19]

Morphogenesis and Egress

Late (structural and nonstructural) 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 the brick-shaped IMV. IMVs, which represent the majority of virus particles produced during infection, are fully infectious; however, their release from the cell is dependent on cell lysis. A small subset of IMV particles gains two additional membranes at either the trans-Golgi network or early endosomes to become IEVs. IEVs migrate to the cell surface via microtubules where their outermost membrane fuses with the cell membrane to form CEVs. CEVs induce the polymerization of actin to form filaments that promote the direct transfer of CEVs to adjacent cells. If CEVs become dissociated from the cell membranes, they become 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 host cells and immune processses.[18,23] Furthermore, the genomes of many poxvirus species also encode intracellular immune modulators, thus providing a broad and complex mechanism through which these viruses can subvert host immune responses that would be deleterious for infection. Interestingly, cowpox virus, which predominantly causes localized skin lesions in healthy humans, possesses the largest genome (~220 kbp) of the orthopoxvirus species and thus may contain the greatest number of immunomodulatory components to escape host immunity. In light of this, it is unsurprising that cowpox virus is also believed to have the broadest host range species of the orthopoxviruses.[24] 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.[19]

Significant efforts have been made to dissect the molecular mechanisms that orthopoxviruses use to modulate host cell signaling networks.[25–27] Investigations such as these provide important information regarding viral pathogenesis and may provide the identities of therapeutic targets for the development of novel orthopoxvirus specific antivirals.

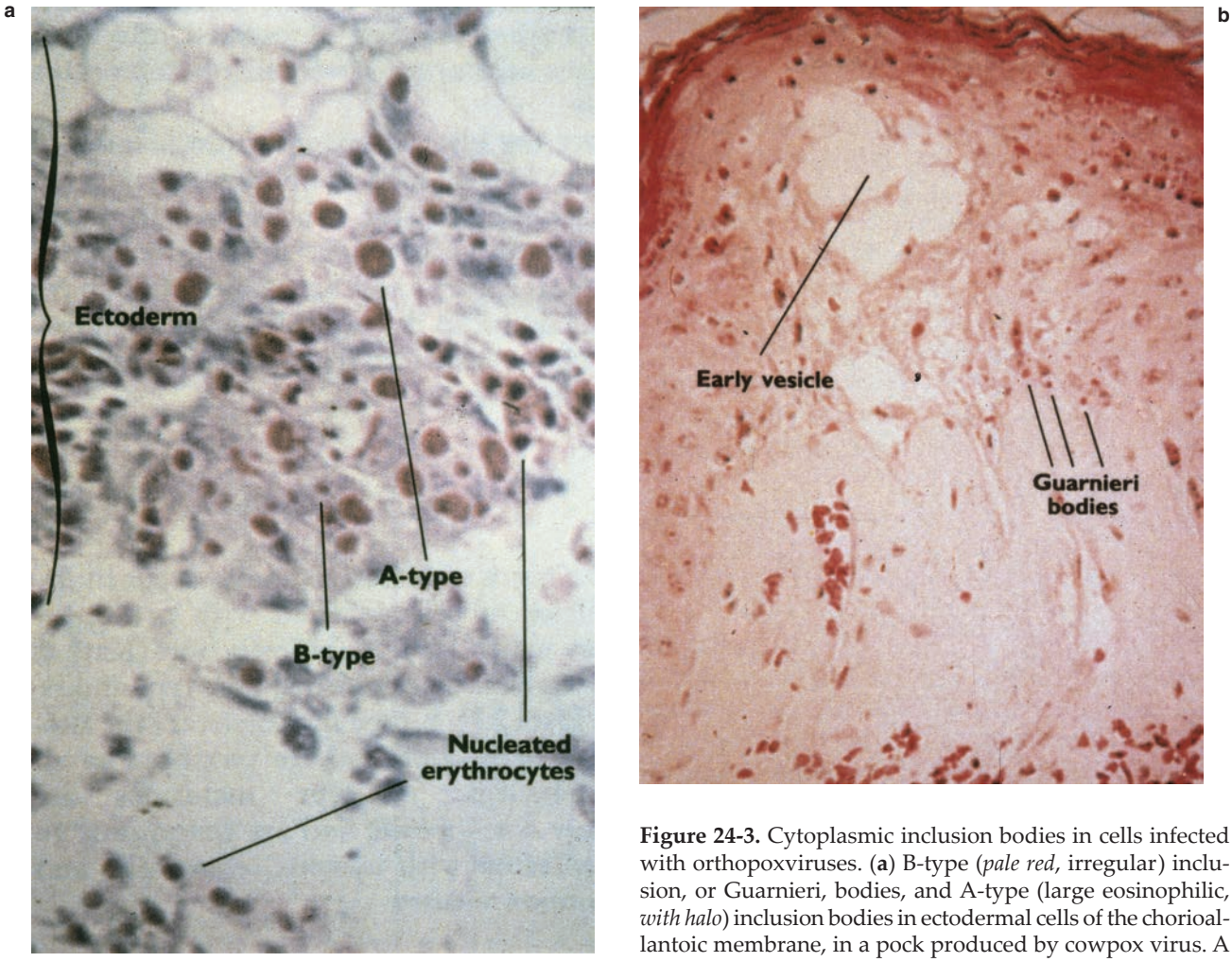

Figure 24-3. Cytoplasmic inclusion bodies in cells infected with orthopoxviruses. (**a**) B-type (*pale red*, irregular) inclusion, or Guarnieri, bodies, and A-type (large eosinophilic, *with halo*) inclusion bodies in ectodermal cells of the chorioallantoic membrane, in a pock produced by cowpox virus. 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.

Phylogenetic Relationships

The evolutionary relationships within the *Orthopoxvirus* genus have been facilitated by the recent availability of complete DNA sequences for more than 30 species. Phylogenetic analysis (Figure 24-4) reveals that at the sequence level, any two virus species contain at least 96% homogeny, variola is more closely related to camelpox and taterapox (isolated from rodents) viruses than any other members of the genus, and vaccinia is most closely related to cowpox virus strain GRI-90.[28–31] 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. 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.

When a phylogeny is developed based on gene content instead of genetic sequence, the clustering is slightly different. In the gene content phylogeny, all cowpox virus strains belonging to the same clade are distinctly divergent from vaccinia virus, and camelpox virus is in the same clade as monkeypox virus instead of variola virus.[30] Variola virus, monkeypox virus, and camelpox virus can all be traced back to a common branching point that is divergent from the less pathogenic orthopoxviruses vaccinia virus and

cowpox virus. The discrepancies that exist between the sequence-based and gene content-based phylogenies can predominantly be traced to the variable terminal ends of the genome.[30] Most of the proteins encoded at the terminal ends are known to be involved in virus:host interaction (immunomodulation, virulence, host range restriction).[19] Variola virus, which is strictly a human pathogen, has the fewest genes at its terminal ends whereas cowpox virus, which is capable of causing disease in numerous mammalian species, has the greatest number of genes at its terminal ends. It has been postulated that, through genetic reduction, variola virus has maintained only the genes essential to productive infection in humans. By minimizing the number of genes with potential overlapping or antagonistic functions, it is thought that variola virus became a highly adapted and efficient pathogen. In contrast, cowpox virus is capable of infecting a wide variety of mammalian species (including humans) but is not highly pathogenic in any of those species. The large number of genes at its terminal ends improves the host range of cowpox virus but decreases its overall efficiency. Regardless of phylogeny, all orthopoxviruses are antigenically similar allowing for cross protection.[30]

Viral neutralizing epitopes are associated with structural proteins encoded by genes located in the central conserved region of the viral genome, and they are present on the surface of IMVs as well as on the envelope of EEVs and CEVs.[32–34] Given the conserved nature of these proteins, epitopes are relatively uniform for all members of the genus *Orthopoxvirus*. Cross protection allowed for the development of the vaccine that eradicated smallpox, and it continues to be a fundamental key to the development of vaccinia based countermeasures against orthopoxviruses.[35–37]

Pathogenesis

Most knowledge about smallpox pathogenesis is inferred from animal studies of vaccinia in mice, mousepox, rabbitpox, monkeypox, and from vaccinia in humans.[16,38–40] Studies using nonhuman primates infected with variola corroborate these findings and lend further insight into human smallpox and monkeypox infections; however, certain deficiencies exist with the model.[41] The production of a clinical syndrome in cynomolgus macaques that resembles human smallpox requires intravenous infection (an unnatural route)

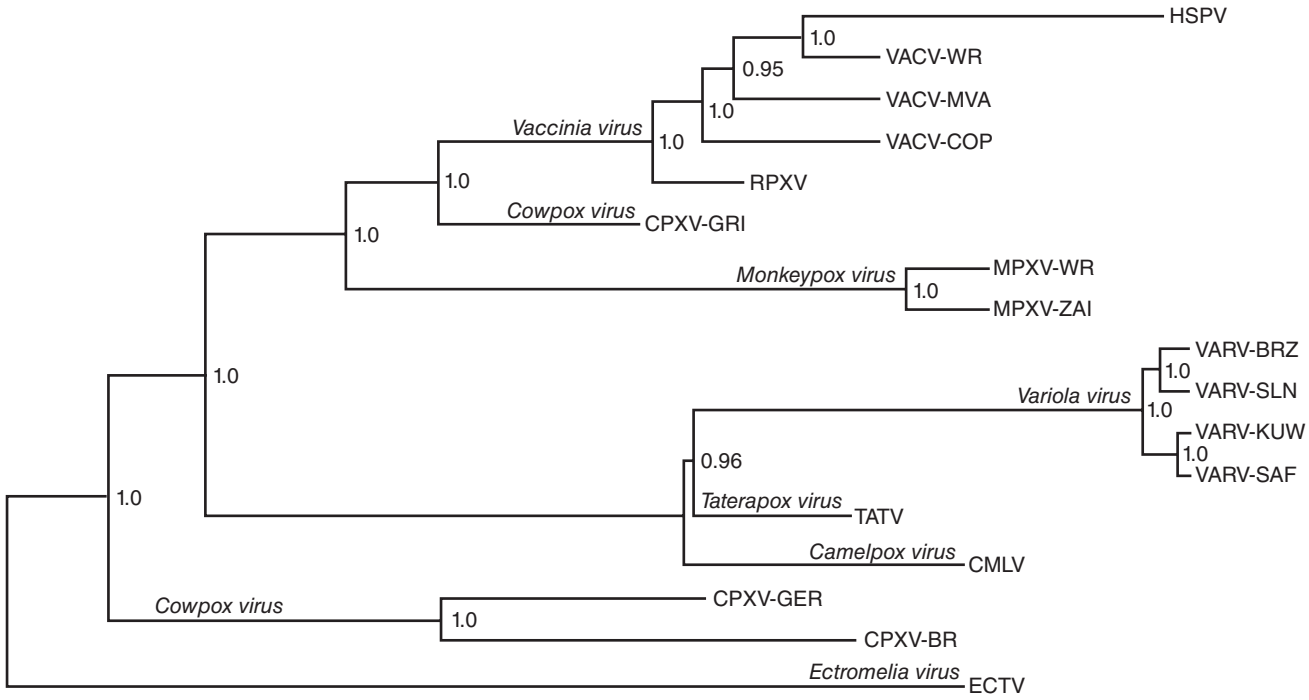

Figure 24-4. Orthopoxvirus gene sequence phylogenetic tree. Species names are noted on the branch line that separates different clades. Specific virus strains are indicated at the terminal point of the line. The numerical values represent the confidence predication using Bayesian inference for each clade. Reproduced (in accordance with the creative commons attribution license associated with open access articles [http://creativecommons.org/licenses/by/3.0]) from Hendrickson RC, Wang C, Hatcher EL, Lefkowitz EJ. Orthopoxvirus genome evolution: the role of gene loss. *Viruses*. 2010;2:1933–1967.

with an extremely high dose of inoculum; and even then, mortality in this model is inconsistent.[41] In both natural and experimental aerosol infections, the virus is introduced via the respiratory tract, where it first seeds the mucous membranes. Although it is unclear whether alveolar macrophages are responsible for the transport of virus, the first round of replication occurs in the lung draining lymph nodes, followed by a transient viremia, which seeds tissues, especially those of the reticuloendothelial system, including regional lymphatics, spleen, and tonsils.[42,43] A second, brief viremia transports the virus to the skin and visceral tissues immediately before the prodromal phase. In humans, the prodrome is characterized by an abrupt onset of headache, backache, fever, and a sore throat as a result from viral replication in the oral mucosa. Characteristic skin lesions develop with a centrifugal distribution and synchronous progression following viral invasion of the capillary epithelium of the dermal layer. The virus may also be present in urine and conjunctival secretions.[43] At death, most visceral tissues contain massive virus concentrations.

In a review of all pathology reports published in English over the past 200 years,[44] 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 may have caused a degree of compromise, but liver failure was not usually the proximate cause of death.

Much of the human pathogenesis of smallpox remains a mystery because of the limited tools that were available when it was an endemic disease. Comparisons between the limited clinical and pathological data from human smallpox victims with the pathophysiology of disease from monkeypox and variola nonhuman primate infection models suggest a role for the dysregulation of immune responses responsible for 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 likely associated with organ dysfunction and multisystem failure. Immunohistochemistry staining confirmed the distribution of viral antigen, while electron microscopy demonstrated evidence of replicating virus, which correlated with pathology observed in the lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive system, and liver. Apoptosis, particularly within the T-cell population, was a prominent observation in lymphoid tissues, although the cause of this widespread apoptosis remains unknown. However, the strong production of proinflammatory cytokines is due at least in part to the upregulation of various proapoptotic genes. The strong upregulation of cytokines may have also 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. However, in human populations, the occurrence of hemorrhagic smallpox was approximately 1% to 3% of the total cases observed.[2,23,39,44,45]

From these recent studies of variola and monkeypox virus infection in nonhuman primates, the "toxemia" described by clinicians for human smallpox may be fundamentally related to the processes underlying septic shock.[2,46] 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.[47]

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.[48–50] During the American Civil War, allegations were made about the use of variola as a biological weapon, although no definite evidence existed.[51,52] In the years leading up to and during World War II, the Japanese military explored

weaponization of variola 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 variola virus on a continuing basis well into the 1980s.[10,53] The former Soviet Union also conducted open air testing of weaponized variola and demonstrated that infectious virus could infect humans 15 km downwind.[7]

Although declared stocks of variola 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, Novosibirsk Oblast, 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.[53] The probability that such stocks exists 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 resulting from waning immunity following vaccinations in the distant past and immunosuppression as a result of 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.[54] The disease can be 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 ($TCID_{50}$).[39,55–57] Monkeypox virus, like variola, is relatively stable and can resist desiccation in both heat and cold.[58] Monkeypox virus also grows 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.[40] Furthermore, monkeypox virus may have already been weaponized by the former Soviet military.[10]

Cowpox and buffalopox produce limited cutaneous disease in humans in natural infection.[17] Buffalopox, like cattlepox, may be essentially identical to vaccinia.[59] 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.[40,60–64] Aerosol infection has the potential to produce more pronounced pulmonary disease.[40,56,65] In addition, all orthopoxviruses share a significant amount of homology with variola and monkeypox.[29] If the critical virulence factors for systemic human disease were determined, then cowpox, buffalopox, or other orthopoxviruses could potentially be genetically modified to express these critical factors. When designed as a weapon and delivered by aerosol, these viruses could have a 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.[66] Camelpox virus is a close relative of variola virus; the major difference between camelpox virus and variola major virus strain Bangladesh-1975 genomes is four additional insertions, elongated inverted terminal repeats, and a small area of gene rearrangement present in camelpox virus.[28] As with other orthopoxviruses, slight modifications in the camelpox virus genome may 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 is now technically feasible to create infectious variola using an oligonucleotide synthesizer, analogous to the recent demonstration for creation of the much simpler polio virus.[31,67,68]

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.[69,70] Whether these results represent findings unique to the virus–host model used or reflect a more general premise of enhanced virulence is unclear.[71,72] 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.[73] Variola virus is infectious by aerosol, but natural airborne spread other than among close contacts is unusual.[3,74,75] Approximately 30% of susceptible contacts became infected during the era of endemic smallpox,[76] 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 variola virus can be spread through airborne dissemination in conditions of low relative humidity.[77] 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 virus transmission were greatly increased. Indirect transmission via contaminated bedding or other fomites was infrequent.[78] Some people in close contact with patients harbored virus in their throats without developing disease and may have been a means of secondary transmission.[43,79]

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 a primary viremia.[2] The incubation period of smallpox averages 12 days (range 9–14 days). Those in contact with infected patients were quarantined for a minimum of 16 to 17 days following exposure to ensure an adequate window of time had passed without the appearance of symptoms.[2] During normal smallpox disease, variola virus was sporadically recovered from the blood, but not nearly at the levels detected in patients with hemorrhagic smallpox.[80] After replication in regional lymph nodes virus disseminated systemically to other lymphoid tissues, spleen, liver, bone marrow, and the lungs, and created a secondary viremia. Clinical manifestations began acutely with malaise, fever, rigors, vomiting, headache, and backache; 15% of patients developed delirium. Approximately 10% of light-skinned patients exhibited an observable erythematous rash during this phase. After 2 to 3 more days, an exanthem appeared concomitantly with a discrete rash about the face, hands, and forearms. Given 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.[81] These respiratory secretions were the most significant but not the sole means of virus transmission. Following subsequent eruptions on the lower extremities, the rash spreads centrally to the trunk. Lesions quickly progressed from macules to papules and eventually to pustular (umbilicated) vesicles, and were more abundant on the extremities and face (Figure 24-5). This centrifugal distribution of lesions is an important diagnostic feature, typical of smallpox disease. In contrast to the lesions seen in varicella (chickenpox), 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 upon healing. Although variola titers in the throat, conjunctiva, and urine diminish with time, virus can readily be recovered from scabs throughout convalescence.[2,82] 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 and variola minor. 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. Analysis conducted on human viral isolates indicated a distinct evolution of three virus clades (A, B, C) associated with geographical distribution and case fatality rates.[31]

Although isolates from clade C, found predominantly in Asia, were associated with an overall higher case fatality rate compared to A and B, virus evolution was present, as evidenced by the attenuated strains detected in Africa. 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.[83] 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.[84] 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.[85] Three-quarters of variola major cases were designated classic or ordinary type (Figure 24-5). 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.

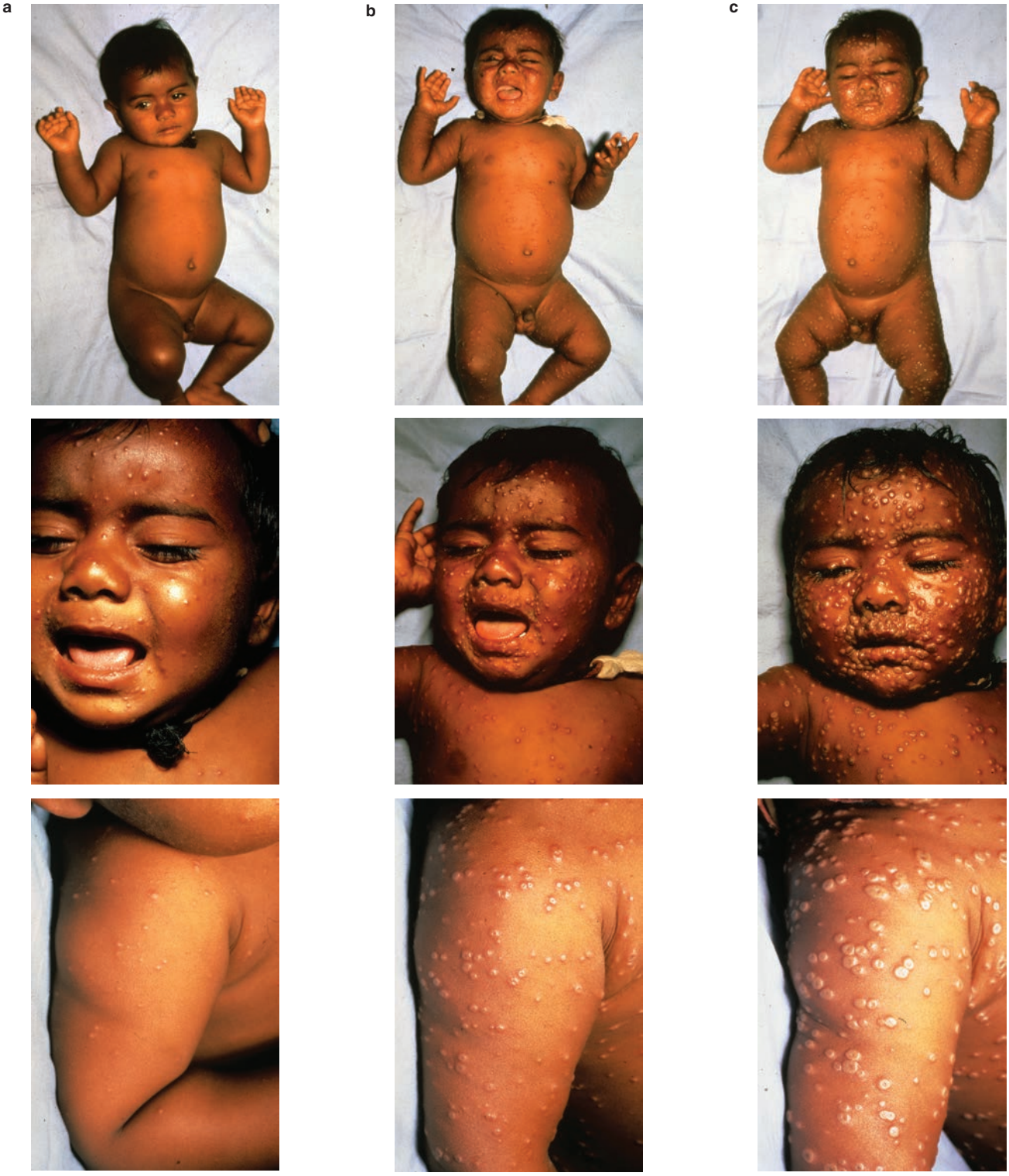

Figure 24-5. This series of photographs illustrates the evolution of skin lesions in an unvaccinated infant with the classic form of variola major. (**a**) The third day of rash shows synchronous eruption of skin lesions; some are becoming vesiculated. (**b**) On the fifth day of rash, almost all papules 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.

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, and focal skin lesions that did not resemble the classical variola exanthem (Figure 24-6). This syndrome caused 66% mortality in vaccinated patients and 95% mortality in unvaccinated patients.[85] Fewer than 3% of smallpox patients developed hemorrhagic-type smallpox, which was accompanied by extensive petechiae (Figure 24-7), mucosal hemorrhage, and intense toxemia; death usually occurred before typical pox lesions developed.[86] However, on occasion hemorrhagic smallpox also occurred as a late stage complication of classical smallpox disease. Both hemorrhagic-type and flat-type smallpox may have indicated underlying immunodeficiency; hemorrhagic forms occurred more commonly in pregnant women and young children.[87] The modified type, which occurred typically (but not exclusively) in previously vaccinated individuals, was characterized by moderation of constitutional symptoms and typically reduced numbers of and a 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.[85] In actuality, the manifestations of variola infection fell along a spectrum, and classification was 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 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.[88] 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 less than 1% of cases. Disease during pregnancy precipitated high perinatal mortality, and congenital infection was also recognized.[2]

Partial immunity through 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.[89] 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 and up to several decades in some individuals.[34,45,90]

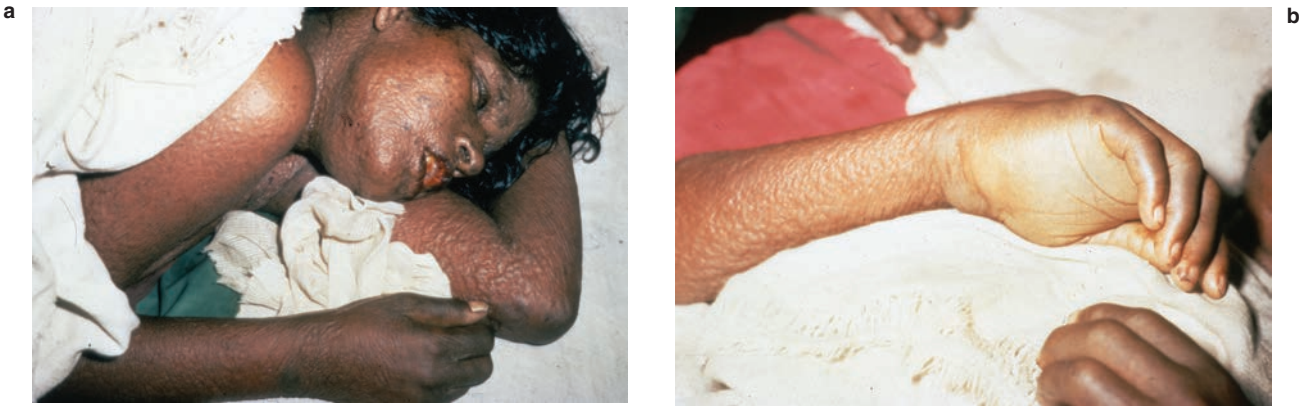

Figure 24-6. Flat-type smallpox in an unvaccinated woman on the sixth day of rash. Extensive flat lesions (**a** and **b**) and systemic toxicity with fatal outcome were typical. Reproduced with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 33. Photographs by F Dekking.

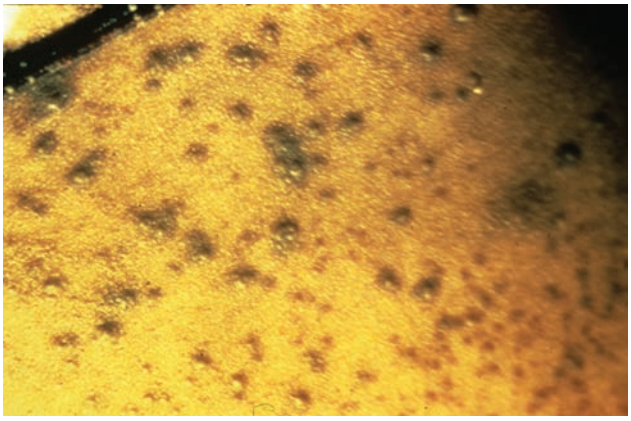

Figure 24-7. Early hemorrhagic-type smallpox with cutaneous signs of hemorrhagic diathesis. Death usually occurred before the complete evolution of pox lesions. Reproduced with permission from Herrlich A, Munz E, Rodenwaldt E. *Die pocken; Erreger, Epidemiologie und klinisches Bild*. 2nd ed. Stuttgart, 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.

Monkeypox

The clinical features of human monkeypox are classically described as being similar to those of smallpox.[91] 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.[91,92] Lymphadenopathy is a common finding that differentiates monkeypox from smallpox. This has been documented in up to 83% of unvaccinated persons with monkeypox and 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.[91] In a series of 282 patients (of which 250 were not vaccinated), the exanthema first appeared somewhere other than the face in 18% of the unvaccinated patients; 31% of vaccinated patients had pleomorphic or "cropping" appearance of rash lesions, and 9.4% had centripetal distribution.[93] All of these features were inconsistent with a mimic of smallpox. Patients in the 2003 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.[94,95] A surveillance study conducted from 2005 to 2007 in the DRC laboratory confirmed 760 cases, which suggests a 20-fold increase in human monkeypox incidence since the 1980s in the same health zone.[13]

Monkeypox virus can be subdivided into two distinct clades that are genetically, clinically, and geographically distinct. The Congo Basin monkeypox virus clade has associated case fatality rates of approximately 10% in nonvaccinated individuals,[96] as opposed to the reduced pathogenicity and transmissibility of the West African clade of monkeypox (which caused the US outbreak).[57,97] Comparative infection models in nonhuman primates, mice, prairie dogs, and ground squirrels have all demonstrated greater lethality or morbidity associated with Congo Basin monkeypox virus infection as compared to West African monkeypox virus.[98–101] A *sine eruptione* form of monkeypox has not been described, but the number of serologically diagnosed infections without consistent rash illness suggests it is a possibility.[102] A hemorrhagic form of human monkeypox has not been documented.[103,104]

Complications of monkeypox are more common in unvaccinated persons and children.[96] During intensive surveillance in the DRC 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.[105] Encephalitis was detected in at least one monkeypox case in the DRC and in one of the cases in the US outbreak of 2003.[93,95] 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.[91,105–107] 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.[54] The low fatality rates in certain outbreaks were influenced by the lack of proper laboratory confirmation to exclude cases of varicella virus from monkeypox virus infections. It is believed no fatalities occurred among the 78 suspected

cases in the 2003 US monkeypox outbreak, at least in part because the less virulent West African strain was responsible.[94] The presence of comorbid illnesses, such as measles, malaria, or diarrheal disease, may have a significant impact on mortality in children.[96] 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.[17] Baxby, Bennett, and Getty reviewed 54 cases of cowpox infection with a detailed discussion of clinical manifestations.[108] 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.[108,109] Three of these four described cases included a history of atopic dermatitis, indicating a risk of increased severity of disease analogous to vaccinia.

Buffalopox and cattlepox infections in humans have not been extensively described but have been observed in areas of Brazil. Limited data suggest human infection usually occurs on the hands and consists of inflamed and painful pustular lesions progressing through a Jennerian evolution.[110–112] Regional lymphadenopathy and fever can accompany local disease.[112]

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 website (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.

Specimen Collection and Handling

Collection of appropriate specimens is paramount for accurate laboratory diagnosis of orthopoxvirus infection. Ideally, cutaneous tissue (from skin lesions) and blood are sent for diagnostic testing, with other samples being sent at the request of public health officials or experts in the field.[104] Detailed instructions for specimen collection can be found in the Department of Defense Smallpox Response Plan (http://www.bt.cdc.gov/agent/smallpox/responseplan/index.asp) or on the CDC website (http://www.cdc.gov/ncidod/monkeypox/index.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 serum separator tube (which is then centrifuged to separate serum) and in an 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 (specimens potentially containing variola virus must be handled in biosafety level 4).[113]

Many phenotypic and genotypic methods involving virological, immunological, and molecular approaches have been used to identify orthopoxviruses.

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 24-3]) in tissue samples under light microscopy. These cytoplasmic inclusions are hematoxylinophilic, stain reddish purple with Giemsa stain, and contain Feulgen-positive material.[114] Electron microscopy reveals the unmistakable brick-like morphology of orthopoxviruses in thin sections of infected materials.

Microscopy alone cannot differentiate members of the genus *Orthopoxvirus*, yet the epidemiological setting can suggest which species is involved. The orthopoxviruses with pathogenicity in 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 cells and additional monkey kidney cell lines, A549 cells, 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.[115–117] For example, immunogold staining prior to electron microscopy permits a 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] Current common approaches include enzyme-linked immunosorbent assay (ELISA), plaque reduction neutralization test (PRNT), and immunofluorescence microscopy. Although these assays are proficient at demonstrating the presence of an orthopoxvirus infection, antigenic similarity that results in serologic cross-reactivity makes species differentiation extremely difficult.

ELISAs and PRNTs can be used to detect orthopoxvirus antibodies in a serum sample; however, data interpretation is different. ELISA assays measure the total amount of antibody present in a given serum sample, and they can measure both immunoglobulin M (IgM) and IgG antibodies, allowing for the identification of markers for both active and prior infection. Since IgM antibodies 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.[95] More recently, a combination of T-cell measurements and a novel IgG ELISA were used to enhance epidemiological follow-up studies to this outbreak.[118,119] It has also been suggested that, by using linear peptides as antigens, species-specific orthopoxvirus ELISA assays can be developed. Although ELISAs can be sensitive, quick, and easy to perform, they do not provide information as to antibody functionality and their ability to neutralize orthopoxviruses in vitro, or any inferred protective immunity. To determine the neutralizing (ie, inferred protective immunity) antibody titer, a PRNT must be performed. The theoretical protective immunity value for serum antibody levels against variola virus is greater than or equal to 1:40 as determined by PRNT.[120] PRNT cannot differentiate between IgM and IgG antibodies, and orthopoxvirus species-specific PRNT assays have not been developed.

Similar to ELISAs, immunofluorescence microscopy has been used to detect IgM in acute infection directed against cowpox.[109] The technique used is similar to ELISA except that a fluorescent tag attached to the detection antibody allows visual, colorimetric observation of orthopoxvirus antibodies. Immunofluorescence microscopy is not a quantitative assay and only allows determinations of presence versus absence. Quantification can be performed using a fluorescence plate reader. Similar to ELISA, this assay will not provide information on protective antibody levels.

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 and microarray genotyping also provide high levels of specificity and when combined with PCR, these approaches can offer high levels of sensitivity.[121–123]

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. Numerous commercial nucleic acid purification methods are available 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 and 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 uses PCR-amplified regions of the genome separated on agarose gels by electrophoresis, where 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.[117,124–126]

Large fragment (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.

Real-time PCR methods provide exquisite levels of sensitivity and specificity.[127] 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, CA), 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, Foster City, CA), Smart Cycler (Cepheid, Synntvale, CA), LightCycler (Roche Diagnostics Corporation, Indianapolis, IN), MJ Opticon (Bio-Rad, Hercules, CA), RotorGene (Corbett Life Science, Sydney, Australia), RAPID (Idaho Technology, Salt Lake City, UT), 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. 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.[127–130]

Real-time PCR was one of the diagnostic techniques used in the investigation of the 2003 US monkeypox outbreak.[95] It has also been used during monkeypox active disease surveillance studies in the DRC, identifying 760 new human cases of monkeypox between November 2005 and November 2007.[13] Because of its sensitivity, rapidity, and ease of use, 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.[83] During a smallpox outbreak in Boston in 1752, more than 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.[131]

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 caused 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.[83] 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.[83] 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. The risk of bioterrorism prompted smallpox vaccination in at-risk military personnel and civilian healthcare workers to be resumed in 2003.[132,133]

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 roughly 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.[134,135] 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 more than 450,000 military vaccinees.[132] In this 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.[133] Live (replicating) vaccinia immunization has also been used as postexposure prophylaxis and is believed effective if administered within 4 days of exposure. As a potential replacement for vaccine strategies used during the eradication campaign, a new vaccine was prepared in massive quantities (>300 million doses) by selection of plaque-purified progeny virus from the New York Board of Health strain (Dryvax, Wyeth Laboratories, Marietta, PA), which was amplified in VERO cell cultures. This vaccine is of greater purity and free of adventitious agents in comparison with its predecessor, which was prepared on calf skin. Phase I safety and immunogenicity trials for ACAM2000 indicate greater than 95% take rates and adverse events comparable to those of the predecessor vaccine.[136] ACAM2000 was approved for use in 2007 by the Food and Drug Administration (FDA), effectively replacing Dryvax as the vaccine of choice for licensed use. Additionally, this vaccine is currently being maintained as part of the Strategic National Stockpile.

ACAM2000 is no less reactogenic than Dryvax; therefore, vaccination remains limited to groups at the greatest risk of contracting orthopoxviruses.

Vaccination is performed with a bifurcated needle onto which the reconstituted vaccinia preparation has been drawn, using 15 jabs 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 a 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." The 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.

The immunization 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.[137] 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 were immunized with vaccinia and challenged with monkeypox virus, Edghill-Smith et al showed that vaccinia specific B-cells are critical for protection.[138] Antibody depletion of B-cells (but not CD4+ or CD8+ T-cells) abrogated vaccinia-induced protection. Edghill-Smith et al have also shown that simian immunodeficiency virus compromised monkeys could withstand monkeypox infection 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 < 300 mm.[138]

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 more than 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.[139] 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.[140] Surprisingly, a single dose of MVA also protected when a 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.

Aside from live and attenuated virus vaccines, a number of other approaches are being investigated. Most of these vaccine strategies involve the use of viral DNA or viral protein(s). Prime-boost strategies (ie, an initial vaccination with a DNA-based vaccine followed by a protein-based vaccine) have also been used. All of these strategies have shown some or significant promise in animal models; however, none of these have been approved by the FDA and are not currently being used as investigational new drugs.[141]

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 importation 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 more than 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.[142] Multiple vaccinations are thought to produce more long-lasting immunity. Vaccination has been effective in preventing disease in 95% of vaccinees.[143] Vaccination was also shown to prevent or substantially reduce the severity of infection when given as a 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);
- 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 also 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.[144]

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, generalized vaccinia, eczema vaccinatum, progressive vaccinia, postvaccinial central nervous system disease, and fetal vaccinia.[6,145]

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 (ViraA, King Pharmaceuticals, Bristol, TN) is often recommended, although the FDA does not specifically approve the treatment of ocular vaccinia for either of these drugs. Most published experience is with use of vidarabine, but this drug is no longer manufactured.[146]

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, which affects individuals who are age 2 or older, 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,[66] symptomatic cases of myopericarditis were reported, for a rate of 1.2 per 10,000. 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 12,000 vaccinees.[131] 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.[147,148]

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 the 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 human infections with other orthopoxviruses.[2] Despite a lack of hard evidence in humans, postexposure vaccination is likely efficacious against other orthopoxviruses, and during the 2003 US monkeypox outbreak the CDC recommended vaccination of potentially exposed persons.[93]

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

The introduction of monkeypox virus to the United States, the endemic nature of monkeypox virus in certain regions of Africa, and the continued threat of an act of bioterrorism with monkeypox or variola indicate the need for anti-*Orthopoxvirus* therapeutic drugs. In addition, a therapeutic would be useful for the treatment of adverse events associated with vaccination. The only FDA approved antiviral drug available for treating orthopoxviruses is cidofovir. However, it is approved for treatment of cytomegalovirus in HIV patients so it can only be offered for treatment of orthopoxvirus infections under emergency use protocols maintained by both the Department of Health and Human Services and the Department of Defense.[149]

The elaborate replication strategy of poxviruses offers a number of potential targets for therapeutic intervention.[150] Initial studies to identify effective antiviral agents for orthopoxviruses tested drugs developed for other viruses that share similar molecular targets.[149] The effort to discover effective drugs against DNA

viruses initially focused on treatment of herpesvirus 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. Acyclovir failed to inhibit cytomegalovirus 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.[151] Cidofovir is licensed for treatment of cytomegalovirus-associated retinitis under the trade name Vistide (Gilead Sciences Inc, Foster City, CA), and inhibits the cytomegalovirus DNA polymerase, a target shared with the poxviruses. Cidofovir has been demonstrated to protect nonhuman primates against severe disease in both the monkeypox and variola nonhuman primate models, when administered within 48 hours of intravenous or respiratory exposure to the virus.[152–157] 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 or every other day cidofovir administration would not significantly increase the healthcare burden. Cidofovir has been associated with nephrotoxicity; therefore, careful attention to fluid management is important and patient hydration and coadministration of probenecid is required.

Cidofovir requires bolus dosing to allow drug entry into cells by pinocytosis; however, bolus dosing results in transiently high concentrations in the kidney. For this reason, an oral formulation with lower toxicity is more desirable. CMX001 is a lipid conjugate of cidofovir with a 1-0-hexadecyl-oxypro-pyl (HDP) covalently linked to the nucleotide analogue. Attachment of the lipid moiety allows CMX001 to be taken up into cells through lysophosphatidylcholine (LPC) uptake pathways,[158] which results in lower toxicity and increased bioavailability. This formulation dramatically reduced transient drug levels in the kidney and eliminated nephrotoxicity in toxicology studies using mice.[159] CMX001 has also demonstrated protection in mouse and rabbit models of orthopoxvirus infection. Demonstrating efficacy of CMX001 in nonhuman primates is not possible because of the higher oxidative metabolism in monkeys. However, both cidofovir and CMX001 produce the same antiviral product in vivo (cidofovir diphosphate), allowing cidofovir to be used as a surrogate. Increased oxidative metabolism is not observed in humans. Although an oral formulation of cidofovir is not yet available for human use, it is in phase I/II clinical trials and is used under emergency authorization for the treatment of systemic adenovirus infections of pediatric hematopoietic stem cell transplant recipients.[160]

An alternative approach to identifying compounds with anti-*Orthopoxvirus* activity was the use of a high-throughput screen using vaccinia and cowpox virus. More than 300,000 compounds were evaluated and several potent lead structures were identified for optimization and evaluation against vaccinia, monkeypox, and variola viruses.[161] 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(1*H*)-yl)-benzamide] was identified and is under advanced development by the Biomedical Advanced Research and Development Authority. 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 against monkeypox, variola, cowpox, vaccinia, and ectromelia in vivo.[161] The viral target of ST-246 is the F13L (homologue) gene product p37. This viral phospholipase plays a critical role in egress of viral particles from the host cell and the inhibition of this process with ST-246 reduced extracellular virus by 10-fold.[161] ST-246 has demonstrated efficacy in multiple animal models of orthopoxvirus infection both prophylactically and therapeutically, and more importantly, it has prevented morbidity and mortality against monkeypox virus and prevented mortality from variola virus in nonhuman primates.[162] In addition, the compound was well tolerated by human subjects after daily oral administration for 21 consecutive days.[163] ST-246 has been placed in the Strategic National Stockpile, but FDA has not approved it.

Cidofovir, ST-246, CMX001, and VIG have been used successfully in combination to treat the adverse effects of vaccination. In a recent case, vaccinia virus mutants resistant to ST-246 were isolated from a marine who developed progressive vaccinia following vaccination and was subsequently treated with ST-246, CMX001, and VIG.[164] The patient recovered, but it highlighted the importance of combination therapy; and as stated previously, VIG is not in development as an antiviral against monkeypox or variola virus.

Myriad alternative approaches to orthopoxvirus treatment, such as interferon mimetics, interferon beta, RNAi, mixantrone, and terameprocol (to name a recent few), are under investigation. These compounds have shown varying degrees of success in a wide range of test systems; however, none of them has reached the stage of clinical development for use as an anti-*Orthopoxvirus* treatment.[157,165–170]

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, it is believed that the Soviets continued developing smallpox for biowarfare into the 1980s. The Soviet Union is dissolved and its offensive program has been 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. Since 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 orthopoxvirus 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 such as ST-246 and CMX001 for treatment of orthopoxvirus infections and safer vaccines. There is still no approved treatment for smallpox; however, FDA approved ACAM2000 as a smallpox vaccine in 2007. MVA, although not FDA approved, is placed in the Strategic National Stockpile.

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