

Looking Back at Smallpox

Mike Bray¹ and Mark Buller²

¹Biodefense Clinical Research Branch, Office of Clinical Research, Office of the Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and ²Department of Molecular Microbiology and Immunology, Saint Louis University, St. Louis, Missouri

Smallpox apparently arose through transfer of variola virus to humans from another animal species. By causing a brief infection that required close contact for transmission and engendered solid immunity, the agent was always vulnerable to simple isolation measures. The high replicative fidelity of the viral DNA polymerase limited variola's ability to adapt to humans and preserved orthopoxviral antigenic cross-reactivity, so that vaccinia vaccination protected against smallpox. Host-derived genes encoding immunomodulatory proteins helped shelter viral replication from innate immune responses. Examination of clinical variants suggests that severity of illness was usually determined by host responses during the incubation period. Control of viral replication was aided by early postexposure vaccination and might be strengthened by additional immunological interventions. Massive inflammatory responses were responsible for major features of illness. Some patients with high levels of circulating virus developed hemorrhagic disease resembling septic shock. Continued study of virus-host interactions is needed to defend against genetically modified agents.

The triumph of global smallpox eradication has led to a bitter irony: the ensuing worldwide cessation of vaccination has rendered most of today's population susceptible to infection and has made variola virus, the agent of smallpox, a potential bioterrorist weapon [1, 2]. However, progress in virology and immunology since the time of eradication may help to counter the threat of a deliberate reintroduction of the disease.

This article looks back at smallpox from our current scientific vantage point. We first describe orthopoxvirus evolution and acquisition of host genes, then we discuss the "dermatotropism" of variola virus, the biological basis of localized and disseminated infections, and the origin and history of smallpox. After a discussion of smallpox pathogenesis, we then note how improved understanding of the disease is leading to new forms of prophylaxis and therapy.

ANCIENT PATHOGENS

Poxviruses are the largest and most complex viruses that infect humans. The ability of these double-stranded DNA viruses to

replicate in the cell cytoplasm with little help from the nucleus and their present worldwide distribution among mammals, birds, reptiles, and insects suggest that they descended from organisms that infected early forms of life [3]. The co-evolution of poxviruses with vertebrates has resulted in the formation of distinct chordopoxviral genera (figure 1A), whose members differ in their ability to cause disease in various animal species. Thus, avipoxviruses infect birds but cannot replicate in humans, whereas humans are the only host of molluscum contagiosum virus (MCV).

Variola virus belongs to the genus *Orthopoxvirus*, the members of which cause skin lesions in mammals (table 1 and figure 1B). The prototype orthopoxvirus, vaccinia, is the current smallpox vaccine. Some orthopoxviruses, such as variola, infect only a single species (the maintenance host). Others, such as cowpox and monkeypox viruses, are maintained in one or a few host species but may cause localized or disseminated disease if transferred to other animals. Variola may have been introduced to humans through such a cross-species transfer from a host that has since become extinct. It is closest in DNA sequence to camelpox virus, which causes smallpox-like disease in camels; both viruses are apparently descended from a recent common ancestor [4]. Camelpox remains enzootic in southwest Asia, and epidemiologic considerations suggest that smallpox arose in the same region. Because variola virus causes a short-term infection that induces lasting immunity in those who

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Reprints or correspondence: Dr. Mike Bray, Biodefense Clinical Research Branch, OCR/OD/NIAID/NIH, Bethesda, MD 20892 (mbray@niaid.nih.gov).

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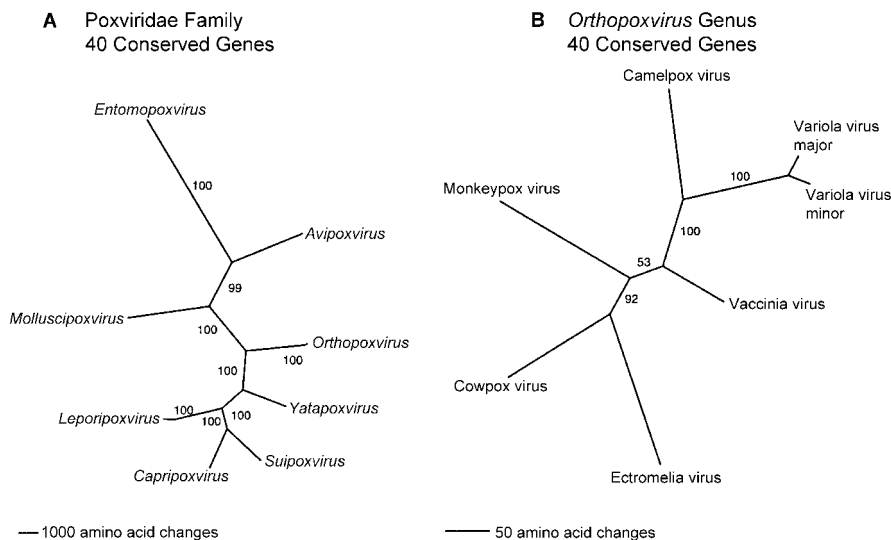


Figure 1. A, Evolutionary tree of the poxvirus family. The entomopoxviruses only infect insects. All other genera belong to the chordopoxvirus subfamily, consisting of viruses that infect vertebrates. Humans are the only host of molluscum contagiosum virus but can be infected with a number of members of the *Orthopoxvirus* genus. Insufficient data is available to chart the location of the *Parapoxvirus* genus. B, Evolutionary tree of the genus *Orthopoxvirus*. The representative species of variola major and minor (the Bangladesh and Garcia isolates, respectively) are very closely related. Their nearest relative is camelpox virus. (Both figures courtesy of Elliot Lefkowitz.)

recover, its survival requires a constant supply of naive hosts—a condition first satisfied when humans aggregated in cities in the ancient Near East [5]. By contrast, MCV persists in the skin for many months and can survive among much smaller groups; it probably infected our remote primate ancestors [6].

Variola shares many basic features with other orthopoxviruses. Its linear genome contains some 200 genes; those in the central region encode proteins involved in replication or the virion structure. The highly accurate poxviral DNA polymerase has conserved the sequences of these genes among all orthopoxviruses. The flanking regions contain genes encoding proteins that modify the intra- and extracellular environment in ways that favor viral replication and spread. They were apparently acquired from vertebrate hosts through random recombination, after which natural selection retained and modified those genes that improved viral fitness [7]. This ability to take up additional DNA has been exploited to make recombinant orthopoxviruses in the laboratory. No orthopoxvirus has acquired new host genes in the recent past. Instead, the evolution of distinct viral species has apparently involved the inactivation or loss of genes from a larger repertoire possessed by an ancestral virus. Cowpox virus encodes the largest number of host-derived genes of any orthopoxvirus and may be closest to that progenitor.

Many host-derived genes encode “immunomodulatory” proteins that block innate antiviral responses (table 2) [8]. Some act intracellularly to prevent the induction of programmed cell death, whereas others are modified cell-surface receptors that are secreted into the extracellular fluid, where they bind to

chemokines, cytokines, and other immune mediators. Several immunomodulatory proteins block the action of Th1, but none target Th2 cytokines, which is consistent with the critical role of cell-mediated immune responses in the resolution of primary poxvirus infections [8–11]. Orthopoxviruses do not encode cytokines or chemokines; however, the parapoxvirus orf has acquired a host IL-10 gene that may be responsible for its ability to cause repeated infections in sheep [12]. Recombinant vaccinia and mousepox viruses encoding Th1 cytokines are attenuated for mice, whereas those incorporating an IL-4 gene show enhanced pathogenicity, suggesting that a modified variola virus encoding a Th2 cytokine would have increased virulence for humans [8–11, 13].

LOCALIZED AND DISSEMINATED DISEASE

To understand the biological behavior of variola virus, one must answer 2 questions: why are the lesions of smallpox largely limited to the skin and oropharyngeal mucosa, and what determines the virus’s ability to cause disseminated disease? We are closer to answering the first question than the second. Although variola virus was recovered from many different tissues in patients with smallpox, infection of squamous epithelium appears to be essential to the virus’s “survival strategy.” The viral genome encodes a secreted homologue of epidermal growth factor, which stimulates keratinocyte proliferation, providing a favorable environment for viral spread. Variola virus grows better at 35°C than at higher temperatures, suggesting that the cooler environment of the skin enhanced replication

Table 1. Representative members of the orthopoxvirus family and the animal species from which they have been isolated.

Virus	Maintenance host	Other naturally susceptible species	Human disease
Variola	Humans	None	Disseminated
Monkeypox	Probably rodents	Humans, nonhuman primates, other large animals	Disseminated
Camelpox	Camels	None	None
Cowpox	Rodents	Humans, many small and large mammals	Localized
Vaccinia	Unknown (horses?)	Humans, cattle, horses	Localized

NOTE. Monkeypox virus causes a smallpoxlike illness in humans but is not closely related to variola, whereas camelpox virus, variola's closest relative, does not cause disease in humans. Vaccinia, the smallpox vaccine virus, was long believed to be identical to cowpox virus but is actually a distinct species that may be related to horsepox virus (figure 1B).

[14]. In addition, each infected cell produces 2 different kinds of virions. The majority (intracellular mature virions) (figure 2) remain within necrotic cells and are shed in skin debris or saliva droplets, where they serve as sources of infection. A small percentage acquire an additional membrane and are transported to the cell surface. These extracellular enveloped virions are responsible for cell-to-cell spread and may participate in systemic dissemination. The expanding skin lesion is protected against host immune responses by the battery of virus-encoded immunomodulatory proteins (table 2).

A number of factors determine whether an orthopoxvirus causes localized or disseminated infection in a given host. The route of infection is important, because variola causes severe systemic illness when inhaled, but it usually produces milder disease when inoculated into the skin. Host immune status is critical, because vaccinia virus can cause diffuse infection in persons with atopic dermatitis and progressive disease in those with cell-mediated immunodeficiency [15]. At the cellular level, differences in the affinity of viral immunomodulatory proteins for their target molecules in various animal species may affect the ability of a virus to suppress host immune responses. Thus, variola does not cause skin lesions when inoculated into animals other than humans and nonhuman primates, possibly because it cannot prevent apoptosis of infected cells [5]. Cowpox virus, by contrast, can cause lethal disseminated disease when it spreads from rodents to domestic cats or to elephants, lions, or other animals in zoos and circuses [16]. Such outbreaks "burn out" quickly because of a lack of new susceptible hosts. However, as noted above, humans were apparently sufficiently numerous in the ancient Near East for a cross-species transfer of variola virus to initiate continuous transmission.

THE RISE AND FALL OF SMALLPOX

Once established as a human disease, smallpox persisted in endemic form in large cities, where it was principally a disease of children. Epidemics occurred when travellers carried the agent to outlying populations that lacked immunity, but the disease soon died out in areas of low population density. Be-

cause smallpox transmission almost always took place during face-to-face contact through inhalation of virus-containing saliva droplets, the disease spread more slowly than influenza or measles [5, 17, 18]. However, under proper conditions of air flow and humidity, a patient with a severe cough could spread virus over longer distances [5].

Clinical descriptions indicate that smallpox always had a high case-fatality rate until around the end of the 19th Century, when a more benign form of the disease, with a similar rash but much lower mortality rate, appeared in the Western Hemisphere. Less lethal types of smallpox were also noted in Africa, where they may have existed for some time [5]. These milder variants are now designated "variola minor," in contrast to the traditional "variola major." The genetic changes responsible for attenuation have not been identified. The appearance of variola minor may represent a stage in variola's adaptation to its human host. Because the attenuated viruses induced immunity to variola major, they might eventually have driven severe smallpox out of existence, had all not succumbed to global eradication.

The first immunization procedure was variolation, in which material from pustules or scabs was inoculated into the skin. A small percentage of recipients developed full-blown infection, but the rest experienced a less severe form of disease with a transient skin rash, resembling "modified" smallpox, suggesting that inoculation induced protective immune responses more rapidly than inhalation [18]. The great breakthrough came when Edward Jenner discovered that infection with a more benign orthopoxvirus prevented illness on subsequent variolation and protected against naturally transmitted smallpox. He believed that vaccination conferred lifelong immunity, but the passage of time showed that it generally provided solid protection for no more than 10 years, although repeated inoculation could restore immunity [5, 18]. Persons exposed to smallpox decades after vaccination often developed a milder, "modified" form of the disease. These observations are consistent with recent findings that vaccinia-specific memory T cells persist for ≥ 50 years after vaccination [19, 20].

By the middle of the 20th Century, smallpox had been driven out of Europe and North America. Occasional reintroductions

Table 2. Host processes targeted by orthopoxviruses.

Site of action, virus-encoded protein	Host process targeted	Vaccinia ^a	Variola	Cowpox
Intracellular				
Serine proteinase inhibitor	Programmed cell death	crmA/SPI2	+	+
dsRNA BP	IFN response	E3L	+	+
eIF2 α homologue	Protein synthesis	K3L	+	+
Extracellular				
Epidermal growth factor homologue	Keratinocyte proliferation	C11R	+	+
TNF- α BP	Inflammatory response	—	+	+
IFN- γ BP	Th1 response	B8R	+	+
IFN- α/β BP	Type I IFN response	B18R	+	+
IL-1 β BP	Inflammatory response	B15R	—	+
IL-18 BP	Th1 response	B16R	+	+
Chemokine BP	Recruitment of neutrophils and macrophages	P35	+	+
Complement control protein	Lysis of virions and infected cells	C3L	+	+

NOTE. Virus species differ in the number of functional immunomodulatory proteins encoded in their genomes; for example, cowpox virus encodes 4 different TNF- α -binding proteins, whereas variola encodes 1 and vaccinia none. Similarly, variola differs from the others in lacking a functional IL-1 β -binding protein (BP). crmA, cytokine response modifier A; dsRNA, double-stranded RNA; SPI, serine proteinase inhibitor; +, functional gene present; —, functional gene absent.

^a Gene location(s) in vaccinia virus, the reference species.

produced small outbreaks that were usually limited to family members of the index case and unvaccinated hospital workers and were quickly contained once the disease was recognized [5, 17, 21]. Smallpox remained endemic in the “third world,” where instead of causing explosive outbreaks, it spread in somewhat leisurely fashion among the partially vaccinated populations of cities and more densely populated rural areas. The final elimination of the virus from these regions was initially thought to require nationwide programs of mass vaccination. However, it was eventually realized that, because smallpox patients were easy to recognize, the disease could be eradicated far more efficiently by tracking down individual chains of transmission, isolating infected individuals, and vaccinating their contacts. This “ring vaccination” strategy led surprisingly quickly to elimination of the disease in 1977.

Several factors facilitated the eradication effort. The highly accurate orthopoxviral DNA polymerase minimized antigenic variation of variola virus and made vaccination effective at all times and places. Because variola had no animal reservoir and did not cause persistent or latent infection, acutely ill patients were the only source of infection. Isolation was an effective control measure, because the virus did not appear in the saliva until after the onset of illness, oropharyngeal ulceration developed at the same time as the easily recognizable rash, and droplet transmission required close contact.

CLINICAL VARIANTS

Variola virus caused several different patterns of illness as it spread from person to person, ranging from the “classic” ves-

iculopustular exanthem to rapidly lethal disease lacking the typical rash, indicating that these clinical variants were determined by host factors. Roughly 90% of unvaccinated individuals developed “ordinary” smallpox, characterized by a 10–14-day incubation period, a 2–3-day flulike prodrome, and a centrifugally distributed rash (figure 3) [5, 18, 22]. Nearly one-half of these patients had “discrete” disease, in which pocks were sufficiently few in number to remain separated by normal skin. The rest developed more severe illness, in which a larger number of lesions became confluent in some areas. In either case, no new pocks formed after approximately the end of the first week of illness, roughly coinciding with the appearance of a specific antibody response [5]. Prompt postexposure vaccination reduced the incidence of severe disease [5, 18, 22].

The mortality rate for ordinary smallpox varied with the number of skin lesions, ranging from ~10% for discrete disease to \geq 30% for confluent disease [5, 18, 22]. In fatal cases, fever and hypotension progressed to steadily worsening shock, but as Fenner et al. [5] noted, “the absence of specific lesions anywhere except in the skin and mucous membranes” (p. 139) meant that a cause of death could seldom be identified. Bacterial superinfection did not appear to play an important role, because the introduction of antibiotic therapy did not reduce the mortality rate [5]. Bronchopneumonia was common in severely ill patients but was generally a terminal phenomenon. In fact, Dixon [18] observed that “respiratory complications [were] rarer in smallpox than in most other acute exanthemata” (p. 93). The death of patients with ordinary smallpox was therefore often attributed to “toxemia”—a conclusion consistent with modern concepts of sepsis and septic shock (see Pathogenesis).

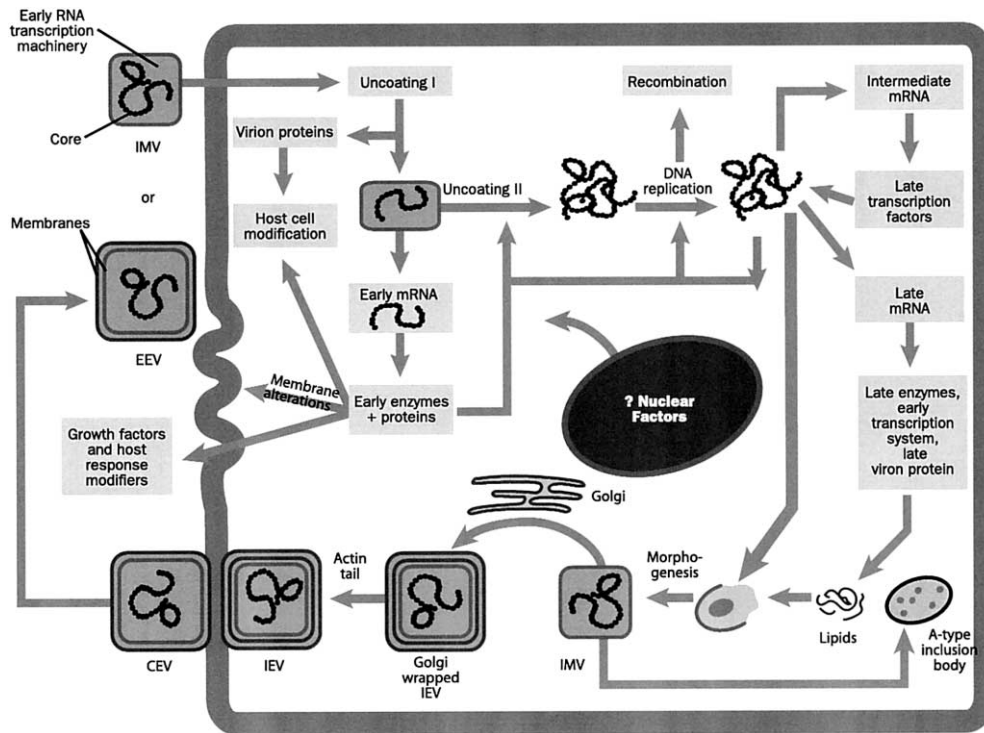


Figure 2. The poxviral replication cycle. A specific cell-surface receptor has not been identified. Infection begins with virion binding and fusion with the cell membrane (*top left*), followed by release of the viral core into the cytoplasm. Enzymes and factors carried within the core promptly initiate transcription. Early gene products include the epidermal growth factor homologue and many immunomodulatory proteins, plus enzymes and other products required for duplication of the genome and production of new virions. Most virions remain in the cytoplasm as intracellular mature virions (IMVs) and end up encased within the protein matrix of scabs. The remainder acquire an additional envelope (intracellular enveloped virions [IEVs]) and are shunted to the cell membrane. These cell surface-associated enveloped virions (CEVs) are responsible for the cell-to-cell spread of virus, whereas extracellular enveloped virions (EEVs) may participate in systemic dissemination. Virus- and host-encoded proteins on the surface of CEV and EEV protect them against complement activation.

A small percentage of patients with smallpox developed highly lethal forms of illness lacking the typical vesiculopustular rash [5, 18, 22]. The most common was “early hemorrhagic” disease, which was seen most often in adults, especially pregnant women. Patients became severely ill after a somewhat shortened incubation period, developed cutaneous and mucosal hemorrhages, quickly went into shock, and often died after a 4–5-day-long illness (figure 3). The “late” form of hemorrhagic disease, in which bleeding begins after vesicles have formed, was also associated with pregnancy, suggesting that the 2 syndromes were variants of a single process. Patients with hemorrhagic smallpox had a high levels of circulating virus at presentation that continued through death; they produced little or no specific antibodies [5, 22–25]. Blood samples showed clotting factor consumption and fibrinolysis, which is consistent with disseminated intravascular coagulation [23–25]. Hemorrhagic patients were not unusually infectious, but failure to recognize the disease and institute isolation measures sometimes led to spread of infection in hospitals.

The other highly lethal variant, “flat” smallpox, was more common in children. Patients developed a maculopapular rash

that failed to progress to vesicles and pustules and instead formed soft, spreading lesions that were barely raised above the surrounding skin. The rash was accompanied by severe illness, which had some hemorrhagic features but which progressed less rapidly than early hemorrhagic smallpox [5, 22].

PATHOGENESIS

Explanations of the sequence of events between the inhalation of virus-containing saliva droplets and the development of a rash have traditionally been based on studies of mousepox [5]. Inhalation of virus presumably initiated foci of mucosal infection in the upper airway but did not cause symptoms or demonstrable lesions. The mousepox model suggests that replication at the point of entry was followed by infection of mononuclear phagocytic cells in regional lymph nodes, possibly with further spread through the bloodstream to similar cells in the liver, spleen, and other tissues. The incubation period ended when the release of inflammatory mediators from infected cells caused fever and other symptoms, and the spread of virus—either within infected monocytes or as free virions—to capil-

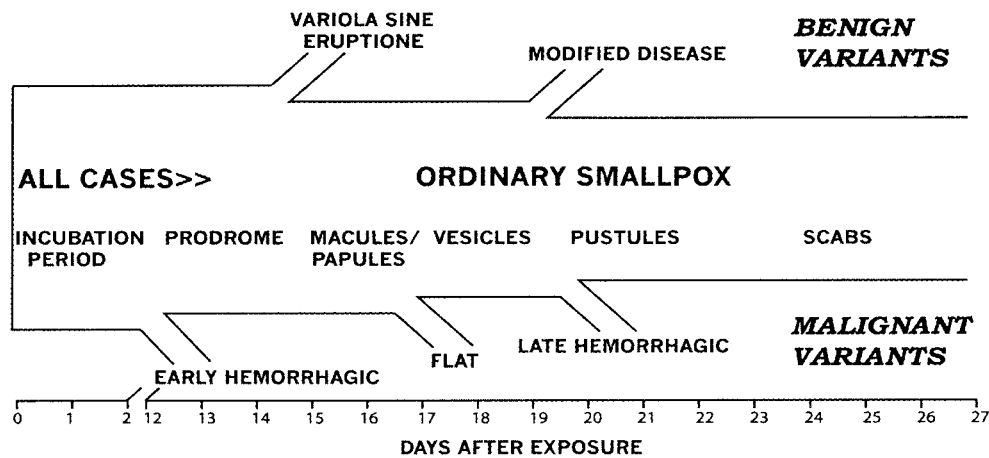


Figure 3. Progression of disease in smallpox (*variola major*). Approximately 90% of unvaccinated persons develop “ordinary” disease, for which the time sequence of skin lesion development is shown across the center of the diagram. The remainder develop malignant variants, in which normal development of skin pocks fails to take place (see Clinical Variants). Individuals who are partially immune as a result of earlier vaccination may develop benign variants of smallpox—either a febrile illness that completely lacks a rash (“*variola sine eruptione*”) or one that is accompanied by a small number of vesiculopustular lesions that evolve and heal more quickly than those of ordinary disease (“modified smallpox”).

larities in the skin and mucous membranes initiated the rash [5]. This “secondary viremia” is the only phase of the process that was actually demonstrated in patients with smallpox.

After reaching the skin, the virus spread cell-to-cell through its mid- and basal layers, causing expanding zones of necrosis that formed vesicles (figure 3). In the nonkeratinized squamous epithelium of the oropharynx, the same process produced ulcerated lesions. Recent studies of other orthopoxvirus infections have indicated that the release of proinflammatory mediators from infected cells caused dilatation and increased permeability of local blood vessels and the infiltration of neutrophils, macrophages, and lymphocytes, producing pustules with surrounding erythema and edema, and that cell-mediated immune responses were required for lesion resolution [3, 8–11]. Because this sequence of events resembles that which follows the inoculation of vaccinia virus into the skin, the severe disease of ordinary smallpox may be thought of as a greatly magnified form of the mild illness that often accompanies vaccination.

The clinical manifestations of most forms of smallpox can be explained by 2 concepts. First, disease severity was determined by the ability of host responses to limit viral replication during the incubation period, as reflected in the level of the secondary viremia; these responses could be strengthened and accelerated by postexposure vaccination. Second, once viral dissemination had occurred, many features of severe illness, including hypotension and coagulopathy, were the result of host inflammatory responses. In severe cases, the release of cytokines, chemokines, and other mediators into the bloodstream caused vascular dysfunction, coagulopathy, and multiorgan failure, resembling septic shock [5, 18, 22–26]. Differences in host responses thus produced a spectrum of disease, ranging from

mild illness with few or no lesions in persons with partial immunity, to rapidly fatal hemorrhagic disease when failure to control replication led to high viremia, coagulopathy, and intractable shock.

Ordinary smallpox lay between those 2 extremes. Many unvaccinated persons with discrete disease appear to have developed <1000 skin pocks, suggesting that the number of infectious particles transported through the bloodstream was quite small (or else that skin infection was very inefficient) and that viremia was quickly halted by developing immune responses. Postexposure vaccination was probably most effective in preventing illness in persons who would have developed discrete disease had they been left untreated. The higher mortality rate for confluent smallpox can be attributed to the greater inflammatory response associated with an increased number of virus-infected cells.

The similarity between host responses in smallpox and septic shock is supported by autopsy studies, which found that dermal blood vessels in both ordinary and hemorrhagic smallpox showed dilatation, endothelial cell swelling, and the adhesion and extravasation of leukocytes, consistent with effects of inflammatory mediators [5, 27]. The underlying immune defect in hemorrhagic smallpox is unknown; it may have combined genetic predisposition with pregnancy-associated changes in immune function. It is interesting to note that hemorrhagic disease may also occur in pregnant women infected with varicella or herpes simplex virus. Severe coagulopathy is also a feature of viral hemorrhagic fevers and of meningococcal and other gram-negative bacterial infections, suggesting that these illnesses elicit similar host responses [26, 28, 29].

The notion that the outcome of infection is determined by

events during the incubation period applies less well in the case of flat smallpox, in which skin lesions failed to mature into pustules. Fenner et al. [5] attributed the condition to deficient cell-mediated immunity but noted the lack of pathologic studies to support this conjecture. The clinical presentation appears to be consistent with a defect in cutaneous immunity that limited recruitment of inflammatory cells to sites of infection. One may therefore speculate that flat smallpox occurred in the setting of atopic dermatitis, which is most common in children, and in which an inherent bias towards Th2 responses predisposes affected persons to severe cutaneous viral infections [15, 30].

PROPHYLAXIS AND THERAPY

Current approaches to the prevention of smallpox are based on the antigenic cross-reactivity between vaccinia and variola viruses. Concern about the safety of vaccination for immunocompromised persons has led to the development of attenuated forms of vaccinia virus, such as modified vaccinia Ankara (MVA), which lack genes encoding various immunomodulatory proteins, such as the secreted IFN- α/β and IFN- γ binding proteins, limiting the agent's ability to overcome host immune defenses [31].

No effective treatment for smallpox had been found by the time of eradication. Recent efforts to develop antiviral therapies have targeted the poxviral DNA polymerase. The viral enzyme is much more susceptible than its cellular counterpart to inhibition by the nucleotide analogue cidofovir, which is licensed for treatment of cytomegalovirus infections. The drug's 3-day intracellular half-life enables a single dose to exert a prolonged effect. Cidofovir is active in vitro against variola specimens from widely dispersed epidemics during the middle of the 20th Century [32]. Attempts to create cidofovir-resistant orthopoxviruses by passage in the presence of the drug found that resistance was associated with loss of virulence [33]. Studies involving mice suggest that delivery of aerosolized cidofovir to the respiratory tract could protect against variola infection and that systemic delivery of the drug before disease onset could prevent fatal illness [34, 35]. Orally available forms of cidofovir are currently under development [36]. The relative timing of postexposure vaccination and antiviral therapy would need to be considered, so as not to impair vaccine efficacy, but mouse experiments indicate that treatment does not block the effect of simultaneous vaccination [35] (M. Buller, unpublished data); further studies are indicated. Because patients with hemorrhagic or flat smallpox do not control viral replication, antiviral therapy may be the only way to achieve survival.

If the outcome of variola infection is largely determined by host responses during the incubation period, then interventions to bolster those defenses may help to prevent or mitigate illness.

One such treatment, postexposure vaccination, has been in use for >200 years. It may be possible to increase the potency of this measure by administering neutralizing antibodies, a Th1 cytokine, or other immunomodulator during the incubation period. These or other countermeasures may be required if the threat of a modified variola virus encoding a Th2 cytokine ever becomes a reality. Improved understanding of the role of host responses in the generation of severe illness may also lead to improved forms of therapy [25].

CONCLUSION

Looking back at smallpox, we can now see that the disease was the result of a cross-species transfer of virus that was not followed by successful adaptation of the agent to its new host. By causing a brief infection that required close contact for transmission and engendered solid immunity, variola's survival was always endangered by simple isolation measures. Ultimately, it was the high replicative fidelity of the orthopoxviral DNA polymerase that made global eradication possible, by preventing variola from evolving a more successful "survival strategy" and by preserving antigenic cross-reactivity, so that vaccinia inoculation protected against variola. Jenner recognized the inherent vulnerability of smallpox when he predicted that vaccination would seal its fate, but further cultural developments, plus a remarkable combination of science and altruism, were required before his prophecy could be fulfilled.

Looking to the future, the view is murkier and rather troubling. Global eradication did not eliminate smallpox as a threat to human health. Even if all existing stocks of variola virus were to be destroyed, current laboratory capabilities would permit determined bioterrorists to construct a similar agent, perhaps with enhanced virulence, that could be released at times and places of their choosing. Against such a combination of science and malevolence, humanity's best efforts will again be required to develop effective countermeasures. Only by continuing to study how poxviruses cause disease can we maintain our defenses against these ancient pathogens.

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