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Smallpox vaccines for biodefense: need and feasibility

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Smallpox, eradicated as a cause of natural disease through an intensive global effort in the later part of the 20th Century, has resurfaced as a possible agent of bioterrorism. For this reason, there is renewed interest in smallpox vaccines. Live vaccinia virus, an orthopoxvirus related to smallpox, has a long and successful clinical track record as an effective smallpox vaccine; however, its use is associated with uncommon yet serious adverse events. This has led to a surge of recent research into newer-generation smallpox vaccines with improved safety profiles and retained efficacy. This article will review the history of smallpox vaccines, assess the status of newer-generation vaccines and examine the overall risk-versus-benefit profile of smallpox vaccination.

Keywords: bioterrorism • myopericarditis • smallpox • smallpox vaccine • vaccine safety • vaccinia

Vaccines against infectious diseases, ranked first among the ten greatest public-health achievements of the 20th Century [1], have arguably resulted in greater benefits to the health of mankind than any other cultural, social or scientific advances. Their implementation has eradicated scourges of nature and controlled a host of lethal, communicable diseases, allowing generations of children to survive, unscathed, into adulthood. Perhaps more than any other, the smallpox vaccine provides the most compelling illustration of vaccination's success. The impact of smallpox on human history is well documented and has been the subject of numerous textbooks, works of literature, objects of art and theses regarding the rise and fall of civilizations [2,3]. The eradication of smallpox and its theoretical resurgence as an agent of bioterrorism illuminate a number of controversial issues engendered by vaccines: safety, public acceptance and risk versus benefit are chief among them. This article will review the genealogy of smallpox vaccines and discuss their potential use in the arena of biodefense.

Brief history of smallpox vaccination

The history of vaccination, from a scientific standpoint, is traditionally dated from the publication, in 1798, of Edward Jenner's landmark experiments with cowpox, in which he inoculated a neighbor's boy with purulent material from a milkmaid's hand lesion in Berkeley, UK [4]. The boy, 8-year-old James Phipps, was subsequently shown to be protected against a smallpox challenge. In many ways, smallpox represented a natural choice for the earliest explorations into systematic vaccination because of its historical position as the greatest disease scourge of mankind.

It was commonly observed, as early as ancient times, that survivors of smallpox were protected against further episodes of the disease. Toward that end, various forms of inoculating healthy individuals with powdered scabs or lesions from infected individuals were used in Africa, China, India and the Ottoman Empire before being introduced into Europe in the early 18th Century [5]. Such procedures were termed 'variolation', derived from *variola*, the Latin word meaning 'mark on the skin' and the scientific name for smallpox [5]. Lady Mary Wortley Montagu, the wife of the British Ambassador to Turkey, is credited with the introduction of variolation to England in 1721 [2]. The practice also spread to the New World, where it was adopted to abort smallpox epidemics and used by General George Washington in 1777 to inoculate all susceptible members of the Continental Army, in what became the first large-scale inoculation of a military force [6].

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Despite an observed mortality rate of 2–3%, variolation still offered better odds than the 15–30% mortality from naturally acquired smallpox, but because of the risks, alternative practices arose among rural agricultural societies. It was believed, although not necessarily widely known, that milkmaids who developed cowpox, generally a benign disease in humans manifested by pustular lesions on the hands or forearms following contact with infected cow udders, were protected against smallpox and failed to demonstrate cutaneous responses to variolation [2,7]. Jenner became the first to systematically study the hypothesis that cowpox infection protected against subsequent smallpox infection [8]. In *An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire and Known by the Name of Cow Pox*, Jenner described in detail the vaccination of ten individuals and an additional 17 who resisted variolation after acquiring natural cowpox infection [9]. While Jenner's work was met with initial skepticism, vaccination against smallpox using his cowpox product *in lieu* of variolation became widespread in the Western World by the early part of the 19th Century [2,10].

Smallpox was eradicated as a cause of natural human disease after an intensive global campaign in the 1960s and 1970s by the WHO and sponsoring countries using live vaccinia virus, a distinct species of orthopoxvirus that is of an unknown derivation, but is genetically related to Jenner's vaccine [3]. The last naturally acquired case of smallpox occurred in Somalia in 1977 [3]; the last known human case occurred in 1978 as a result of inadvertent laboratory exposure [3,11]. Despite this, smallpox vaccination continued to be administered selectively into the late 20th Century at which time it had become clear that the risks associated with smallpox vaccine outweighed any perceived benefits [12]. However, in December 2002 after more than a 12-year hiatus, the US Department of Defense (DoD) reinstituted large-scale military vaccination using live vaccinia to mitigate against the perceived threat of bioterrorism involving smallpox. Concurrently, a program of voluntary civilian healthcare worker vaccination was initiated by the US Department of Health and Human Services (DHHS). Since that time, in excess of 1.5 million individuals have been vaccinated in the military program. Approximately 39,000 individuals received the vaccine in the civilian program before it came to an end in late 2003 due to lack of participation.

Efficacy of smallpox vaccines

The historical premise underlying traditional smallpox vaccines, that of a localized infection with either variola or a cross-reactive orthopoxvirus leading to immune protection, was established long before Jenner published his treatise on vaccination in 1798 [13], and continues to guide the development of newer-generation smallpox vaccines (Table 1). First-generation smallpox vaccines comprising a variety of live vaccinia viruses were used for protection against smallpox yet were hampered by uncommon, potentially life-threatening adverse events that limited their use in the absence of substantial disease risk [14]. This, in concert with concerns regarding the threat of smallpox as a potential agent of bioterrorism has prompted recent efforts toward developing new vaccines with a focus on enhancing safety while maintaining efficacy.

First-generation smallpox vaccines possess a proven track record of clinical effectiveness, highlighted by their success in the global eradication campaign of the 1970s [3]. While immune determinants of protection against smallpox remain incompletely understood, the historical record provides ample data concerning a clinical correlate of protection in humans; observations from the use of variola, cowpox and vaccinia viruses document the direct relationship between a vaccine-associated major cutaneous reaction, or 'take', and protection against smallpox [3,14,15]. The protection appears to be of long duration and to correlate with the presence of neutralizing antibodies [3]. The cellular arm of the immune response is also known to have a significant role in containing vaccinia [12] and, by extrapolation, variola. Smallpox vaccination induces robust vaccinia-specific cytotoxic T lymphocytes (CTLs) and IFN-γ production by T cells in naive recipients, and these may correlate with neutralizing antibody responses [16].

The original production method of first-generation vaccines involved scarification of calf, sheep or water buffalo skin and viral isolation from skin scrapings containing pus, serum and extruded lymph [3,17]. The resultant liquid suspension of vaccine or 'wet' lymph contained viable bacteria, primarily skin commensals, which were minimized by the use of glycerol and, later, phenol in processing [3]. By the 1950s, liquid vaccine lymph preparations had largely been replaced by lyophilized preparations that enhanced preservation of vaccinia virus viability [3]. Vaccine production by animal scarification was abandoned more than 25 years ago and, because smallpox had been eradicated, essentially no first-generation vaccine has been manufactured since then. This led to the view in 2001 that the stockpiled supply was insufficient to cope with a potential large-scale bioterrorist threat. The stockpile consisted of lymph-derived vaccinia, mainly the last production lots of Dryvax®-brand smallpox vaccine, manufactured by Wyeth Laboratories using the New York City Board of Health (NYCBH) strain of vaccinia. Multiple studies have since demonstrated that existing stockpiles can be expanded by diluting the vaccine; lymph-derived, live vaccinia products retain surrogate clinical efficacy at tenfold dilutions in both vaccinia-naive and vaccinia-experienced subjects [17,18].

Second-generation smallpox vaccines (Table 1), in which full-strength vaccinia virus is grown in tissue culture rather than in the skin of large mammals, possess theoretical advantages conferred by this modern manufacturing technique: lowered risk of contamination by adventitious agents [19], viral genetic homogeneity and relative ease of large-scale, consistent production. ACAM1000, a clonal isolate derived from Dryvax and grown in human diploid lung cells (Medical Research Council [MCR]-5), demonstrates similar immunogenicity and cutaneous efficacy at comparable doses to the Dryvax gold standard in animal models, and demonstrates an improved safety profile in preclinical neurovirulence studies in suckling mice and rhesus macaques [20,21]. ACAM2000™, derived from the ACAM1000 master virus by three additional passages in Vero cells [22], has nearly identical biological characteristics to those of its progenitor in animals [23].

Table 1. Smallpox vaccines and vaccine candidates (2008).

Platform	Product	Parent strain	Rationale for its use
First-generation			
Lymph-derived vaccinia virus	Dryvax® (Wyeth)	NYCBH	Historical experience in the USA through the era of routine use
	Sanofi Pasteur smallpox vaccine (SPSV)	NYCBH	Produced in 1956–1957 and used in the USA program of that era; in frozen storage since
	Elstree-RIVM (master seed stock held at the National Institute of Public Health in The Netherlands [RIVM])	Lister	Historical experience in the Intensified Smallpox Eradication Programme
Second-generation			
Replication-competent tissue-cultured vaccinia virus	ACAM2000™ (Acambis): cloned virus grown in Vero cells	NYCBH	Defined manufacturing process; reduced theoretical risk of adventitious agents compared with lymph-derived vaccine; less neurovirulent in animal models
	Elstree-BN (Bavarian-Nordic)	Lister	Defined manufacturing process; reduced theoretical risk of adventitious agents compared with lymph-derived vaccine
Third-generation			
Replication-competent, highly attenuated vaccinia virus	LC16m8 vaccine: derived from 53 serial passages in rabbit kidney cells; temperature sensitive, small-plaque phenotype due to mutation in the *B5R* gene	Lister	Experience in more than 100,000 Japanese children between 1973 and 1975; better safety profile than traditional live vaccinia, less neurovirulent in animals but unproven clinical efficacy
Replication-deficient, highly attenuated vaccinia virus	MVA: derived from more than 570 serial passages in chicken embryo fibroblasts: IMVAMUNE (Bavarian-Nordic); TBC-MVA (Therion)	Ankara	Theoretically improved safety profile, especially for those in whom live vaccinia is contraindicated. Used in 120,000 primary vaccinees in Germany in 1970s but unproven clinical efficacy
	NYVAC (Sanofi-Pasteur): attenuated by the deletion of 18 open-reading frames from a plaque-cloned vaccinia isolate	Copenhagen	Theoretically improved safety profile, especially for those in whom live vaccinia is contraindicated
	dVV-L: derived from deletion of *UDG* gene needed for viral replication	Lister	Theoretically improved safety profile and can be manufactured in cell line that complements UDG deficiency, thus increased capacity for rapid production
Subunit vaccines	Recombinant proteins; plasmid DNA	Vaccinia viruses, different sources	Theoretically improved safety profile

MVA: Modified vaccinia Ankara; NYCBH: New York City Board of Health; UDG: Uracil DNA glycosylase.

Randomized Phase II and III clinical trials, in which nearly 1100 vaccinia-naive subjects were vaccinated with ACAM2000, demonstrated its noninferiority compared with Dryvax at similar vaccinia virus inocula, using cutaneous responses (i.e., takes) as an efficacy end point; ACAM2000 did not meet the noninferiority measure using geometric mean neutralizing antibody titers (GMT) on day 30 after vaccination as another efficacy end point [22,201]. In vaccinia-experienced subjects, ACAM2000 only met the noninferiority threshold for the GMT end point but not for cutaneous responses [201]. Nonetheless, in August 2007, ACAM2000 became the initial second-generation smallpox vaccine to be licensed for human use by the US FDA, leading to the delivery of 192.5 million doses to the US government for stockpiling purposes [202]. The vaccine received the following clinical indication: 'active immunization against smallpox disease for persons deemed to be at high risk for smallpox infection' [201]. ACAM2000 is not expected to be commercially distributed in the USA in order to minimize its use and, therefore, its risk [203]. CCSV, another second-generation vaccine grown in MRC-5 cells, compared favorably with Dryvax in a single-center study of 150 vaccinia-naive and 100 vaccinia-experienced subjects [24]. However, this agent was apparently 'deselected' by the manufacturer for further advancement.

Despite the theoretical advantages conferred by second-generation vaccines, they comprise replication-competent, virulent vaccinia viruses and, therefore, possess the potential for a number of uncommon but well-described serious adverse events

associated with first-generation smallpox vaccines [14]. Alternative candidates based on attenuated vaccinia strains, third-generation vaccines, may offer more favorable therapeutic ratios.

LC16m8, a replication-competent, highly attenuated vaccinia strain, derives from 53 serial passages of a Lister strain isolate in rabbit kidney cells [25]. LC16m8 appears to be less neurovirulent in animals than unattenuated Lister strain vaccinia [26,27]; its use in more than 100,000 Japanese children in the 1970s demonstrated take rates and neutralizing antibody responses similar to those of lymph-derived smallpox vaccines [27,28]. However, the vaccine was never formally field tested, as smallpox was no longer an epidemic threat in Japan at the time.

Recently, LC16m8 was shown to engender complete protection in both a rabbit model using intradermal rabbitpox challenge and a mouse model using aerosolized ectromelia (i.e., mousepox) virus [29]. In the mouse model, LC16m8-vaccinated animals developed higher vaccinia-specific neutralizing antibody titers, enhanced neutralization of intracellular mature virus (IMV) and comparable capacity to neutralize extracellular enveloped virus (EEV), compared with Dryvax-vaccinated animals [29]. The latter finding is reassuring in that the *B5R* gene, required for EEV formation, but deleted during the attenuation process in LC16m8, is a neutralizing antibody target. Additional data suggest that LC16m8 may be a safer alternative to unattenuated vaccine strains in immunocompromised hosts. While comparable protection is noted between LC16m8 and Dryvax in a BALB/c mouse vaccinia challenge model, LC16m8 is nonlethal to severe combined immunodeficiency mice [30,31]. Combined data from trials involving nearly 1700 vaccinia-naive subjects demonstrate 95% take rates and neutralizing antibody seroconversions with LC16m8 [32,33], similar to rates reported with first- and second-generation vaccines in naive individuals [22].

Modified vaccinia Ankara (MVA) strain, a replication-defective, highly attenuated vaccinia virus was initially used as a priming vaccine followed by first-generation smallpox vaccination in more than 120,000 primary vaccinees in Germany in the 1970s [34]. It is attenuated via 570 serial passages in chicken embryo fibroblasts leading to DNA deletions in approximately 15% of its genome, including genes related to host range and immune evasion; thus MVA is generally replication incompetent in mammalian cells [35]. It has been advanced as a third-generation alternative vaccine of potential utility in immunocompromised hosts in whom live vaccinia vaccines are generally contraindicated [36]. Theoretically though, MVA may regain the potential for growth in certain mammalian cell lines owing to reversions at the nucleotide level [35].

Unlike replication-competent vaccinia, MVA does not result in stereotypical neurovirulence upon intracerebral inoculation of suckling mice and may protect against subsequent intracerebral live vaccinia challenge [35]. Additionally, MVA is not associated with detectable viral replication in irradiated mice and rabbits and protects irradiated mice against live vaccinia challenge [35]. Immunosuppressed cynomolgus macaques demonstrate no significant clinical, hematological or pathological abnormalities following inoculation with high-dose MVA by multiple routes, although vaccinial genomes are detectable by PCR from tissues in the majority of macaques [37].

Modified vaccinia Ankara strain is immunogenic and protective in both normal and variably immunosuppressed mice [38,39]. However, animals clearly require multiple and higher doses of MVA to achieve comparable antibody titers to those induced by replication-competent vaccinia [38], and immunosuppressed macaques may fail to develop MVA-specific IgG responses despite high vaccine doses [37]. In comparisons of first-generation vaccinia virus, LC16m8 and MVA, the latter appears to be the least immunogenic, requiring 100-fold more virus to produce similar response levels [30].

Modified vaccinia Ankara strain protects cynomolgus macaques from lethal intravenous [40] or respiratory [41] monkeypox challenges. Such studies confirm data in mice that high-dose MVA or priming with MVA followed by vaccination with first-generation vaccinia virus is necessary to generate immune responses and protection analogous to those observed with replication-competent vaccinia virus alone [40–43]. In some cases MVA-immunized animals, while protected against lethal disease, develop pox lesions following viral challenge; thus, this product may not abrogate the transmission potential of orthopoxviruses.

In humans, MVA induces neutralizing antibodies in only 50% of naive subjects receiving a single dose; whereas 80% seroconvert after two doses [44]. The magnitude and duration of humoral immune responses are dose dependent; the proportion of subjects with neutralizing antibodies diminishes by at least half within 3 months following the second dose [44]. Vaccinia-experienced subjects demonstrate more rapid seroconversion or a boosting response and more durable antibody levels after a single dose of MVA [44]. When employed as a priming vaccine in vaccinia-naive subjects, MVA induces a 'modified-take skin reaction' with or without a vesicle upon Dryvax challenge 3 months later, similar to cutaneous responses observed in vaccinia-experienced subjects primed with MVA or administered Dryvax alone [45]. Priming with multiple doses of MVA decreases cutaneous viral shedding after Dryvax challenge in naive subjects. Neutralizing antibody titers are comparable among the vaccinated groups; higher vaccinia-specific $CD8^+$ CTLs are noted in those receiving multiple doses of MVA than in those administered one dose of MVA or Dryvax alone [45]. In summary, MVA modifies the cutaneous reactogenicity of live vaccinia without altering its immunogenicity, and multiple MVA priming doses may enhance immune responses to live vaccinia products.

Other attenuated, replication-defective vaccine candidates may show promise as priming agents in immunocompromised hosts. NYVAC, derived from the Copenhagen vaccine strain of vaccinia and attenuated by the deletion of 18 nonessential open reading frames [46,47], modulates the effects of Dryvax when used as a priming agent in immunodeficient rhesus macaques [48], yet fails to protect macaques with AIDS against a lethal, intravenous monkeypox challenge [49]. A replication-defective derivative of the Lister strain of vaccinia, bioengineered by deleting the gene encoding for an essential replication cycle enzyme, uracil-DNA-glycosylase [50], has similar preclinical characteristics to MVA, but is theoretically unable to revert to virulence because it only grows in permanent cell lines capable of complementing the enzyme deletion [50,51].

Subunit products are also under investigation as alternative smallpox vaccines. Limited preclinical data support the immunogenicity and protective effect of a vaccinia envelope protein, H3L, in BALB/c mice; passive transfer of H3L-neutralizing antibodies also appears protective [52]. Multiple immunizations with combinations of three outer membrane proteins of IMV (e.g., L1 and A27) and EEV (e.g., A33 and B5) or with combinations of the genes encoding these proteins, are protective in mice and macaque models [53,54]. The latter approach prevents viremia in immunized, challenged monkeys [54]. Animals primed with plasmid DNA encoding the four proteins, then boosted with the analogous proteins, survive lethal monkeypox challenge with significantly milder disease than those immunized with the proteins alone [55].

Safety of smallpox vaccines

Substantial volumes of safety data have accumulated on first-generation vaccines through the period of widespread smallpox vaccination, the intensified eradication program and posteradication vaccination exemplified by the recent US military and civilian healthcare worker programs. Surveillance data from the late 1960s in the USA showed serious complications of smallpox vaccination in approximately four per 100,000 individuals with an overall risk of death of one per million primary vaccinations [56–58]. The rate of serious adverse events may be strain related; a retrospective meta-analysis describes a sixfold increased risk of death with the Lister compared with the NYCBH strains [59].

Serious, albeit rare, complications of vaccination are well documented and occur with higher frequency in primary vaccinees or those with immunologic abnormalities (Table 2) [56,57,60]. Postvaccinial encephalitis, a rare disorder of the CNS that generally occurs in children younger than 5 years of age during the second week following vaccination, is associated with a high mortality rate and severe neurological impairment [14,61]. Other serious adverse events are associated with specific predispositions: progressive vaccinia, a frequently fatal complication of smallpox vaccination in immunocompromised hosts, involves regional and metastatic spread of vaccinia virus as a consequence of the inability to contain the localized infection; and eczema vaccinatum, characterized by extension of the local vaccinia infection to other cutaneous areas actively or remotely affected by atopic dermatitis [14,58].

A number of other complications of smallpox vaccination, including generalized vaccinia, congenital vaccinia, inadvertent inoculation and bacterial superinfection [3,58,63,64], are all potential causes of severe morbidity (or mortality in the case of congenital vaccinia) in vaccinees or their close contacts [14,58]. The incidence of serious adverse events expected in modern mass vaccinations using first-generation vaccinia viruses could potentially be significantly higher than historical levels due to a larger population of individuals with vaccine contraindications and a larger proportion of vaccinia-naive individuals in the population [65,66]. That this higher risk did not materialize in contemporary, posteradication programs was probably due to rigorous, risk-based contraindication screening and extensive education. In the setting of a smallpox outbreak, however, fewer exemptions might be granted. Thus, a major focus of newer vaccine approaches is to improve upon safety while maintaining efficacy.

Live vaccinia virus vaccines are also associated with a high incidence of local and systemic symptoms. The majority of vaccinia-naive subjects experience local symptoms related to the

Table 2. Noteworthy adverse events after smallpox vaccination, USA, December 2002–June 2004.

Event type	Events and rates among 628,414 DoD vaccinees*		Events and rates among 39,566 DHHS vaccinees		Historical rate per million vaccinees
	Events (n)	*Rate per million DoD vaccinees*	*Events (n)*	*Rate per million DHHS vaccinees*	
Moderate or serious					
Postvaccinial encephalitis	1	1.6	1	26	2.6–8.7‡
Acute myopericarditis	83§	132	21§	531	100
Eczema vaccinatum	0	0	0	0	2–35‡
Progressive vaccinia	0	0	0	0	1–7‡
Mild or temporary					
Generalized vaccinia, mild	40	64	3	77	45–212‡
Erythema multiforme major	1	1.6	0	0	NA
Inadvertent inoculation, self	73¶	116	24¶	607	606‡
Vaccinia transfer to contact	47	75	0	0	8–27‡

*Primarily composed of uniformed military personnel plus some DoD civilian employees; a minority of this total was healthcare workers.
‡Based on adolescent and adult smallpox vaccination from 1968 studies (both primary and revaccination).
§DoD events include four biopsy-confirmed, 73 probable and six suspected cases; DHHS events include none confirmed, five probable and 16 suspected cases.
¶DoD events include 59 inadvertent inoculations of the skin and 14 of the eye; DHHS events include 21 inadvertent inoculations of the skin and three of the eye.
DoD: Department of Defense; DHHS: Department of Health and Human Services; NA: Not available.
Data from [56,57,60–62,75,106].

vaccination site and as many as 40% experience mild-to-moderate constitutional symptoms, such as headache, myalgias, malaise or fever [14]. Data from both the Lister/Elstree [59,67] and the NYCBH strains [14,56,68] of vaccinia virus confirm the higher incidence of local and systemic adverse events in primary vaccinees, compared with revaccinees [36]. While immunogenicity and efficacy in primary vaccinees are apparently not affected by diluting unattenuated vaccinia viruses up to tenfold, fever, systemic symptom score and missed activities are significantly mitigated [69].

The rates of adverse events in the ongoing DoD vaccination program (Table 2) are below historically anticipated levels [70–72] for a number of reasons, including careful screening to exclude those at predictably higher risk, enhanced vaccine education, and a generally healthy population pool. Ten military subjects with undiagnosed HIV infection, all with $CD4^+$ counts above 280 cells/mm^3, were inadvertently vaccinated and tolerated the local vaccinia infection without untoward clinical sequelae [73]. In the concurrent DHHS program, seven cases involving the well-described, serious complications of smallpox vaccination were reported: one subject experienced suspected postvaccinial encephalitis; three had confirmed or suspected generalized vaccinia; and three subjects experienced ocular autoinoculation (Table 2) [62,74,75]. The relative dearth of 'expected' vaccine complications in these programs is probably multifactorial with more rigorous screening for contraindications than during the era of routine vaccine use, a lower overall denominator of vaccinees than during past routine vaccination, limiting vaccines to adults and possible reporting differences being the main reasons [76].

Cardiac complications of first-generation smallpox vaccines were reported, albeit infrequently, during the era of routine use decades ago. Five cases of myopericarditis were described in association with the NYCBH strain in the USA [75]; data from Finland and Australia involving non-NYCBH vaccinia strains support rates as high as one case per 10,000 vaccinees [77] and 1.6 per million [78], respectively. Up to 3% of Swedish military recruits were found to have nonspecific, asymptomatic T-wave changes on electrocardiogram following smallpox vaccination in the 1960s [79,80]. Nonetheless, a retrospective review of death certificates in New York (NY, USA) during a 4-month period in 1947 in which 6 million people were vaccinated against smallpox using the NYCBH strain failed to show a significant increase in cardiac deaths attributable to vaccination [81].

In the recent, posteradication vaccination programs, two forms of cardiac complications associated with smallpox vaccination were recognized: ischemic events and myopericarditis. The US military identified 24 subjects with ischemic events within 4 weeks of vaccination; the civilian program identified ten [62,74,75,82]. Of these, 19 experienced myocardial infarction, three of whom died. Both the military and civilian rates of ischemic events were within the range expected for an age-matched population, and all occurred in vaccinia-experienced individuals [82,83]. In addition, four cases of dilated cardiomyopathy in the military cohort and three cases in the civilian cohort, all but one in re-vaccinees, were recognized between 1 and 7 months after vaccination [75].

Despite the lack of a clear causal relationship between ischemic cardiac events and smallpox vaccination, the US CDC promulgated new recommendations regarding cardiac prescreening, surveillance and vaccine contraindications for pre-outbreak smallpox vaccination based on the temporal associations [63]. Vaccine deferral on the basis of known heart disease or multiple cardiac risk factors was not associated with a clear reduction in ischemic cardiac events [83].

The DoD identified 140 cases of myopericarditis during its first 2 years of the program, largely in male, Caucasian, primary vaccinees [75,84], representing a rate of approximately 1.2 per 10,000 – similar to the historical rates in Finnish conscripts [77]. The rate in the civilian DHHS vaccination program in which 21 cases were identified was similar if only probable cases were considered, but was approximately 5.5 per 10,000 [74] if both suspected and probable cases were included. Both rates were higher than age-matched, unvaccinated individuals and since cases cluster in the second week after vaccination, the appropriate conclusion is that primary smallpox vaccination of adults using first-generation vaccinia is associated with a hitherto unrecognized, increased risk of myopericarditis.

Second-generation vaccines, ACAM2000 [22] and CCSV [24], show no significant differences in local or systemic adverse events compared with Dryvax. While none of the rare but well described, serious adverse events related to smallpox vaccines have been noted with these newer vaccines to date, small sample sizes preclude a relative risk determination. Seven out of 2983 (0.2%) vaccinia-naive subjects who received ACAM2000 and three out of 868 (0.3%) who received Dryvax during recent Phase II and III trials were identified as cases of suspected vaccine-induced myopericarditis [17,22,201]. These rates extrapolate to approximately fivefold higher than those noted in the DoD and DHHS efforts, possibly as a result of rigorous, active surveillance for cardiac complications informed by the findings of these posteradication vaccination programs, although the distinction between suspected and confirmed cases needs to be taken into account [22,63].

Although no statistically significant differences were observed in the rates of myopericarditis between those who received ACAM2000 versus Dryvax, the Phase III trials of ACAM2000 were prematurely terminated on this basis. Since myopericarditis cases have occurred in subjects who had received first- or second-generation vaccines, this complication appears to be directly or indirectly related to vaccinia virus and unlikely to be related to an adventitious agent introduced in the processing of lymph. The higher incidence of myopericarditis observed in both treatment groups in the ACAM2000 studies, compared with the government-sponsored vaccination programs, probably results from active surveillance using routine assessments of cardiac symptoms, cardiac enzymes and electrocardiograms designed to identify asymptomatic individuals or cases involving only mild or transient symptoms.

The prototypical third-generation vaccines, LC16m8 and MVA, lack large-scale human safety evaluations. LC16m8 was noted to be well tolerated in both an open-label study involving 476 primary vaccinees and 552 revaccinees [32] and in comparison

with Dryvax in 153 vaccinia-naive volunteers; neither vaccinia-associated serious adverse events nor cardiovascular complications were noted, although planned cardiac evaluations were not performed [33]. In an open-label study, one primary vaccinee developed acute sensorineural deafness and one reported chest pain ascribed by the authors to musculoskeletal causes, with no further information provided [32].

MVA appears to be associated with dose-related, local reactions in the majority of recipients; these self-limited events have not led to discontinuation of subjects from Phase I studies [44]. In a small study of vaccinia-naive individuals with either a history of atopic dermatitis or with active atopic dermatitis, groups in which first-generation vaccinia vaccines are traditionally contraindicated, all subjects receiving MVA reported mild-to-moderate local reactogenicity but no serious adverse reactions [85]. MVA-primed subjects exhibit decreased reactogenicity and minimal systemic symptoms following Dryvax challenge compared with placebo-primed subjects, supporting a modulating effect of MVA in the context of safety, similar to that seen in efficacy studies. No vaccinia-associated serious adverse events or cardiac complications have been observed with MVA to date, although cardiac evaluations are uniformly lacking [45].

Feasibility & acceptability of smallpox vaccination

As smallpox is no longer a cause of naturally occurring disease in humans and there is no known animal reservoir for this pathogen in nature, any human case of smallpox occurring outside of a known laboratory exposure would be tantamount to bioterrorism [86]. Thus, any discussion of smallpox mitigation strategies, specifically pre-event or postevent vaccination, hinges on the concept of 'risk'. Risk refers to the likelihood that exposure to a hazard will lead to a negative consequence [87]. In the context of a smallpox threat, it is essential to consider both the probabilities of exposure and the potential range of consequences associated with the disease and with its vaccines in order to attempt to objectively gauge risk of this type. The probability of exposure to smallpox outside of a laboratory setting is believed to be low but not zero. It has been suggested that unreported smallpox stocks may have existed in the former Soviet Union; if this is accurate, the whereabouts of such viruses would not be known [88]. Since the exposure variable is dependent on the unpredictable tactics of terrorists, accurate, quantifiable risk appraisal is not possible [89]. However, the potential consequences of a bioterrorist attack using smallpox would be devastating.

Multiple characteristics of smallpox ensure that its deliberate reintroduction into the human population would be a global health catastrophe of profound dimensions. Smallpox is stable in aerosol form, raising the possibility of a large-scale attack; it has a low infective dose, requiring minimal viral inocula to cause productive infection in humans [3]; and case–fatality rates historically approached 30%. Morbidity from smallpox was substantial and included prolonged duration of illness, scarring of survivors, secondary soft-tissue infections and blindness [3]. Secondary attack rates among unvaccinated close contacts ranged from 37–88%, although these data derive from historical studies in developing countries and may not be analogous to current circumstances [3,86,90]. Additionally, much of the world's population is susceptible to smallpox due to the cessation of routine vaccination in the early 1970s and the absence of low-level, boosting exposures that would be expected if variola circulated naturally in the environment. Finally, other orthopoxviruses, such as monkeypox, may be pathogenic for humans in either naturally occurring outbreaks or bioterrorism scenarios and may be partially ameliorated by vaccinia immunity [88,91,92].

While improvements in medical care and infection-control procedures, and advances in health technology may mitigate some of the expected morbidity and mortality from smallpox in the 21st Century, they represent a double-edged sword. These same advancements have increased the prevalence of immunocompromised hosts, a population at higher risk of serious morbidity and mortality from smallpox. Similarly, the prevalence of atopic dermatitis in the population has increased markedly since the discontinuation of routine smallpox vaccination; up to 10% of adults and 30% of children in industrialized countries are now diagnosed with this disorder [93]. Live-virus smallpox vaccines are traditionally contraindicated in this population as well. Furthermore, mass casualties due to a smallpox outbreak could rapidly overwhelm healthcare resources.

It has been variably estimated that the number of deaths in the USA after implementation of mass vaccination, using first-generation smallpox vaccines presumably in response to a realized threat, would conservatively range between 125 and 500, accompanied by thousands of serious adverse events [65,66]. In a postevent setting, where the actual smallpox 'event' was realized anywhere throughout the world, the risks associated with the disease would probably far outweigh the potential risks associated with vaccination; thus the benefits of vaccination would favor its deployment, although the relative merits of various strategies, ranging from mass vaccination to a more targeted, ring vaccination approach, are debatable [66,94].

In an outbreak scenario, some combination of ring and mass vaccination would probably be implemented. Dilution studies that have expanded the existing supply of first-generation vaccines in concert with the licensure of a second-generation vaccine, ACAM2000 [202], have resulted in stockpiles of clinically effective vaccines sufficient to vaccinate the entire US population, serving as a fail-safe posture in the event of a biologic attack using smallpox.

In addition to the US stockpile, smallpox vaccine stockpiles are also being developed by other nations [95]. Recent experiences with posteradication vaccination and previous mass smallpox vaccination efforts [17,81] have demonstrated the feasibility of this approach. Japan has limited stockpiles of the attenuated LC16m8 vaccine, although more data would be needed to assure its efficacy and safety in individuals with vaccinia contraindications [95]. Many other nations have developed stockpiles of first- and, in some cases, second-generation vaccines; it is estimated that current capabilities would be sufficient to vaccinate approximately 10% of the world's population [95]. To assure vaccine availability to poorer nations and provide for a nimble response by the

international public-health community, the WHO has recently implemented a plan to develop a strategic smallpox vaccine stockpile of at least 200 million doses, largely derived from pledged donations from member countries and reminiscent of the WHO's efforts during the global smallpox eradication program of the 1970s [204].

By contrast, the concept of pre-event vaccination presents a more problematic analysis. Despite the relative dearth of serious adverse events in both the recent DoD and DHHS smallpox vaccination programs [62,75], there was a low but meaningful incidence of complications related to first-generation vaccines. While the risks can be mitigated via careful screening and exclusion of those in selected higher risk categories, they cannot be completely abrogated. For instance, it has been demonstrated that more than a third of subjects with atopic dermatitis or other vaccine contraindications were unrecognized using various screening strategies [96,97]. In a setting of a very low perceived risk of smallpox, are any levels of significant vaccine-related risks acceptable? Data from the civilian healthcare worker vaccination program of 2003 address this issue [62].

Multiple, detailed evaluations of the DHHS program have been reported elsewhere [62,74,82,98,99]. While there appears to be general agreement that many aspects of the program were instructive from an operational public health standpoint, it remains unclear whether the program achieved the stated goal of enhancing national biodefense preparedness [100]. Certainly the number of civilians actually vaccinated fell far short, approximately 8%, of the 500,000 target set at the program's inception [62]. However, this in and of itself does not necessarily constitute failure, to the extent that the program served as a pilot study to explore the feasibility, acquire experience and reveal hitherto unrecognized issues.

Perhaps the most instructive aspects of the posteradication DHHS smallpox vaccination program, however, relate to the acceptability of vaccination among the targeted civilian groups, largely healthcare workers and others potentially involved in the initial response to a bioterrorist event. Revelations from this experience should inform future vaccination programs in the arena of biodefense. One contemporary study that modeled various smallpox attack scenarios demonstrates that the risk associated with pre-event vaccination of healthcare workers generally outweighs the potential health benefits when the probability of a smallpox attack is less than 22%; in order for mass pre-event vaccination of the public to be beneficial, the probability of an attack would have to be significantly higher, above 47% [101].

Table 3. Risk-versus-benefit considerations for pre-event smallpox vaccination.

Risks/concerns	Benefit/mitigating factors	Unresolved issues	Ref.
• Probability of exposure to smallpox is difficult to reliably quantify, but is greater than zero – Weaponized viruses may be available – Growth and expansion of terrorist activities	• Currently available first- and second-generation vaccines are efficacious • Variola virus is difficult to manipulate in the laboratory • No known animal or natural reservoir of infection	• Second-generation vaccines demonstrate surrogate efficacy in humans; in the absence of a smallpox outbreak, their clinical effectiveness cannot be ascertained • Exposure to smallpox depends on the unpredictable acts of terrorists	[14,17,89]
• Reintroduction of smallpox into the human population would be potentially catastrophic, especially in settings with inadequate public health infrastructure – Variola virus can be aerosolized – Air-borne transmission has been documented – Mortality can exceed 30% – High secondary attack rates in some settings (e.g., healthcare environments) – Most people are either immunologically naive or were vaccinated decades ago – High transmission risk in healthcare settings – Genetic or molecular technology could be used to enhance virulence	• Effective vaccines are available • Postexposure vaccination, within 4 days, is protective • Aerosol transmission is not the predominant route • Improvements in supportive medical care may mitigate excess mortality • Remote vaccination may afford at least partial protection • Possible role for antivirals that were not available when smallpox occurred naturally	• Advances in medical care and health technology have also served to increase the prevalence of immunocompromised hosts and other subgroups in whom currently licensed smallpox vaccines are contraindicated • Medical resources may be overwhelmed by a large number of cases • Durability of vaccine-induced immunity is ill-defined in clinical settings	[3,89,107]
• Smallpox vaccines are associated with significant safety issues (Table 2) • Public acceptance will depend on scenario	• Post-eradication programs show that careful screening can minimize some serious adverse effects but may not avert myopericarditis	• Newer-generation vaccines may be associated with improved safety profiles, but currently there are insufficient data to demonstrate this	

In large part, healthcare workers and traditional first responders who declined voluntary smallpox vaccination determined that their personal risk associated with vaccination using first-generation smallpox vaccine outweighed the perceived risk of smallpox [102,103]. A number of additional factors contributed to the risk equation that ultimately limited the acceptability of pre-event smallpox vaccination in the 2003 setting: uncertainties regarding liability for vaccine-induced injury; sources of compensation and mechanisms of remedy for illness or injury related to vaccination; inadequate education concerning the risks and potential benefits of the program; the recognition of novel cardiac adverse events; and the lack of biological weapon caches in Iraq [104,105]. Individuals will generally act according to their personal perceptions of risk, but since it is inherently impossible to quantify the probability that a terrorist will release a biological weapon, the perceived risks associated with smallpox vaccination apparently dominated the equation in 2003.

Expert commentary

First-generation smallpox vaccines have a long, distinguished track record of effectiveness in the control and subsequent eradication of naturally occurring smallpox. However, their utility in the posteradication setting is limited by uncommon but serious adverse effects (Table 2). The incidence of some of the more notorious of these complications can be minimized by rigorous screening for known contraindications and site hygiene; others, such as myopericarditis, have not yet had clear precipitating factors identified. A significant proportion of the population would be excluded from receiving these vaccines in nonemergent scenarios.

New-generation smallpox vaccines, specifically second- (tissue culture-derived vaccinia) and third-generation (highly attenuated vaccinia) vaccines potentially have a similar efficacy to first-generation smallpox vaccines. Second-generation vaccines, as with first-generation ones, are associated with a significant risk of myopericarditis that substantially limits their utility in a pre-event setting. With the licensure of ACAM2000 and its substitution as the principal vaccine in the ongoing DoD program, the FDA has imposed a risk-minimization action plan that includes a myopericarditis case registry and Phase IV cohort study of military vaccinees to further characterize cardiac adverse events [203]. Third-generation products may possess improved safety profiles, but this has yet to be proven in adequately powered studies or experience with large numbers of vaccinees. Highly attenuated, replication-defective vaccinia MVA sacrifices degrees of immunogenicity and efficacy for its theoretically improved safety profile. For some third-generation products, multidose regimens limit their utility in outbreak settings.

The risk versus benefit profile of smallpox vaccination is complex (Table 3). The risks associated with currently licensed vaccines probably do not justify their pre-event use in groups with a very low perceived risk of smallpox exposure. However, the latter type of risk is dependent on the unpredictable nature of terrorists and may be stratified among different groups; for example, deployed military forces may be at higher levels of exposure risk. Additionally, the general level of perceived risk may increase abruptly should a terrorist event occur. Such an unpredictable situation argues for continued research on safer smallpox vaccines. New-generation vaccines that are demonstrated to have significantly improved safety profiles after adequate human studies may alter the risk-versus-benefit assessment.

Five-year view

Current stockpiles of first- and second-generation smallpox vaccines serve as an important contingency position for emergent circumstances. Newer-generation smallpox vaccines that employ highly attenuated and/or nonreplicative forms of vaccinia or subunit vaccine approaches, some with promising preclinical data, may provide significantly safer, effective alternatives over the next 5 years that will enhance biodefense strategies. Viral subunit strategies, in particular, may provide a flexible platform in the future upon which to build capabilities for protection against genetically altered forms of smallpox.

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Key issues

- First-generation smallpox vaccines, comprising live vaccinia virus grown largely in the skin of calves, have a well-documented track record of effectiveness in preventing smallpox but are associated with uncommon, serious adverse events that may limit their use.
- Newer-generation smallpox vaccines that employ either vaccinia grown in tissue culture (second generation) or highly attenuated vaccinia viruses (third generation) may retain efficacy; although both first- and second-generation vaccines are associated with a significant risk of myopericarditis that limits their acceptability in pre-event settings. Third-generation vaccines may improve upon the safety profile of other smallpox vaccines, although there are insufficient data to determine this conclusively.
- The feasibility of deploying smallpox vaccines is dependent on a risk-versus-benefit assessment, in which the probability of exposure to smallpox through bioterrorism must be weighed against the risks and potential benefits of smallpox vaccines. The unpredictable nature of terrorism may compel this evaluation to be more qualitative than quantitative.
- Because there remains a potential risk of smallpox exposure that depends on specific scenarios, newer-generation smallpox vaccines that retain clinical efficacy and improve upon safety are needed.

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