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Smallpox vaccines for biodefense

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ABSTRACT

Few diseases can match the enormous impact that smallpox has had on mankind. Its influence can be seen in the earliest recorded histories of ancient civilizations in Egypt and Mesopotamia. With fatality rates up to 30%, smallpox left its survivors with extensive scarring and other serious sequelae. It is estimated that smallpox killed 500 million people in the 19th and 20th centuries. Given the ongoing concerns regarding the use of variola as a biological weapon, this review will focus on the licensed vaccines as well as current research into next-generation vaccines to protect against smallpox and other poxviruses.

1. History

Smallpox is thought to have originated near Egypt and the Near East between 5000 and 10,000 years ago [1]. From here the disease spread into Asia, Europe and northern Africa. Smallpox was brought to the western hemisphere by European explorers and military expeditions with devastating results. It is estimated that it killed millions and contributed to the downfall of the Incan and Aztec empires [2]. By the 17th century, smallpox was endemic throughout most of the world. By the early 1900s a far milder version of smallpox (alastrim—caused by variola minor) began to circulate throughout North and Central America and in the UK.

In 1796 Edward Jenner published his landmark findings that vaccination with cowpox would prevent infection with smallpox [3,4]. While the concept of vaccination spread rapidly throughout the world, it was not until the Intensified Smallpox Eradication Program of 1967 that much progress was made in controlling the disease on a large scale [2]. Massive vaccination campaigns occurred worldwide and were combined with sophisticated surveillance systems to detect outbreaks, which were then contained using the ring vaccination approach [5]. The last naturally occurring case of smallpox was in 1977, and one year later smallpox claimed its last human life after a laboratory exposure in England. In May 1980 the World Health Organization declared that smallpox had been eradicated [6].

2. Pathology

Infection typically occurred primarily through the respiratory route or contact with contaminated clothing and bedding, although airborne exposure cannot be ruled out [2,7]. This was followed by a prolonged, asymptomatic incubation period of 12–14 days which culminated in a 2–4 day prodromal phase marked by high fever, headache, backache, nausea and prostration [8,9]. The characteristic rash began on the face and extremities and eventually covered the entire body. The lesions begin as small reddish macules which, over the next several weeks, enlarge and become pustules several millimeters in size. The lesions eventually scab over leaving behind extensive scarring. Other long-term sequelae can include blindness, encephalitis, secondary infections and arthritis. Clinical symptoms varied widely and are likely attributable to infectious dose, host health, pre-existing immunity and genetic factors of both host and virus. Fatality rates can be as high as 30%, with death usually attributed to toxemia, hypotension and multi-organ failure; however the exact causes of death are not fully understood and it is likely that both viral cytopathic effects and inflammatory immune mediators contribute to mortality [9].

3. Prevention and treatment

The earliest effective preventive measure for smallpox was variolation, the intentional introduction of smallpox material into a healthy host [1,2]. There were two main techniques: (1) dried scab material inhalation, and (2) pus from active lesions scratched into the skin. Both approaches resulted in full-fledged cases of smallpox, the vast majority of which had far milder symptoms with case fatality rates of 1–2%. One of the first large-scale uses of this treat-

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Table 1
The use of smallpox as a bioweapon.

Year	Incident
1500s	Spanish explorers bring smallpox-contaminated items to American Indians. Resulting outbreaks likely kill millions.
1760s	French and Indian War—British army gives contaminated blankets to Indian tribes allied with the French, with mortality rates reaching 50%.
1776s	Revolutionary war—British forces in Boston send infected civilians out to Continental Army, causing outbreaks among American soldiers.
1800s	Westward spread of American settlers—contaminated items given to Indian tribes causing wide-spread outbreaks.
1860s	Civil War—contaminated clothing sold to Union troops
1980s–?	Numerous countries, including Russia, Iraq, Syria, North Korea, and China have robust biological weapons programs.

ment in North America was when General Washington ordered the variolation of US troops during the Revolutionary War [10].

The eradication efforts were made possible, in part, by the availability of an effective vaccine. Although Jenner's initial work focused on cowpox, by the mid-20th century most vaccines were based on vaccinia, an immunologically cross-protective poxvirus. Although the origins of vaccinia virus are unknown, the vaccine was remarkably effective, with take rates over 97%. Protection was believed to last for 3–10 years and revaccination was required every 10 years [2]. Recent research has shown that immune responses to vaccinia can be detected over 70 years after vaccination, however, the level of protection afforded by these immune markers is not known [11–18].

There are few treatments for smallpox, underscoring the need for safe and effective vaccines. Hyperimmune serum obtained from recent vaccine recipients has been used to produce vaccinia immune globulin (VIG) which is used to treat both smallpox outbreaks and vaccine complications [19]. Similarly, cidofovir and related anti-viral agents have shown inhibitory activity against poxviruses in vitro and in animal challenge models [20–22]. Poxvirus anti-virals are an area of active research and are likely to play key roles for supplementing vaccines in biodefense preparations against smallpox.

4. Smallpox as a bioweapon

With the recent rise in terrorism activity (including bioterrorism), and concerns regarding state-sponsored biological weapons programs have led to significant efforts in the areas of biodefense and biopreparedness [23]. Both the Soviet Union and Iraq are known to have had large biological weapons programs which included smallpox [24,25]. Variola major, the causative agent of smallpox is considered a top bioterrorism agent [26]. (1) It is a disfiguring illness with a high mortality rate, (2) it is a highly contagious pathogen with an extremely low infectious dose, (3) the disease has a long, asymptomatic incubation period, (4) there are no effective treatments and limited vaccine stocks, (5) an outbreak would cause wide-spread panic and societal disruption and, (6) since vaccination ceased over 30 years ago, and the present population is largely susceptible and vaccine recipients are likely to have waning immunity. Notably a contemporary release of smallpox would not be its first use as a weapon (Table 1).

5. Current vaccines

First generation vaccines developed during the eradication effort consist of live vaccinia virus (see Table 2). Dryvax®, the vaccine used in the US is based on the New York City Board of Health (NYCBOH) strain of vaccinia and together with the Lister strain used in Europe, Africa and Asia was deemed safer than other vaccine strains [2]. First generation vaccines were produced by infecting the abdomen and flanks of calves or other large animals. The infected areas were then scraped to collect the lymph exudate and virus was purified, stabilized with glycerol and phenol, and in many cases lyophilized. The resulting vaccine was introduced into the

Table 2
Smallpox vaccines and vaccine candidates.

Vaccine name	VACV strain	Details
Conventional vaccines		
Dryvax	NYCBOH	Lyophilized vaccine used in US during eradication [27]
APSV	NYCBOH	Frozen liquid vaccine used in US during eradication [34]
Lancy-Vaxina	Lister	Lyophilized vaccine used world wide during eradication [27]
Tissue culture based, live virus vaccines		
ACAM1000	NYCBOH	Grown in MRC-5 cells. Improved safety profile in animals, immunogenicity similar to Dryvax [45]
ACAM2000	NYCBOH	Grown in Vero cells Improved safety profile in animals, immunogenicity similar to Dryvax [45]
CCSV	NYCBOH	Tissue culture vaccine grown in MRC-5 cells. Similar immunogenicity to Dryvax [57]
Elstree-BN	Lister	Immunogenicity is equivalent to conventional Lister vaccines and Dryvax [51]
Replication competent, attenuated vaccines		
MVA	Ankara	Passaged through chick embryo fibroblasts. Lost 15% of genome and cannot replicate in human cells. Used at end of eradication with few adverse events [62]
LC16m8	Lister	Attenuated vaccine used in Japan at end of eradication era with an improved safety profile [59]
NYVAC	Copenhagen	Attenuated strain missing 18 ORFs. Improved safety profile but has not been widely used [67]
dVV-L	Lister	UDG gene has been deleted to improve safety but has no not been widely used [72]
IMVAMUNE	MVA	Bavarian Nordic reformulation of MVA vaccine [65]
TBC-MVA	MVA	Therion Biologics reformulation of MVA vaccine [66]
Subunit vaccines		
Protein	VACV	H3L based [98]; A33R, B5R, L1R, based [103]; Polyvalent w/D8 protein [127]
DNA	VACV	A27L, A33R, L1R, B5R based [105]; A27L, A33R and B5R based [128]
DNA	VARV	A30, B7, F8 proteins [109]