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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.

Keywords

smallpox vaccine; variola virus; vaccinia virus; biodefense

History

Smallpox is thought to have originated near Egypt and the Near East between 5,000 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 1900's 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

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human life after a laboratory exposure in England. In May of 1980 the World Health Organization declared that smallpox had been eradicated. [6]

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]

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 treatment 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 twentieth 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 antivirals are an area of active research and are likely to play key roles supplementing vaccines in biodefense preparations against smallpox.

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 widespread 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).

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 epidermal layer of the skin using a bifurcated needle with multiple punctures. Production of these vaccines ceased in the 1970's and 1980's.

Three to four days following successful vaccination a pock forms at the vaccine site as a result of local viral replication and inflammatory response. Over the first week, the pock grows to 10-15 mm in size, fills with pus and eventually drains and scabs over. Approximately 3-4 weeks after vaccination the scab will fall off leaving a small scar.

Humoral responses begin to develop within 2 weeks of vaccination, with neutralizing antibody titers peaking around day 28. [16,27] Antibody titers decline in the first several years following vaccination and then stabilize and remain constant for decades. [11] Robust CD4 and CD8 T cell responses occur after immunization and reach their peak between 2-5 weeks post vaccination. Similar to humoral immunity, virus-specific T cells can be detected for decades following vaccination, with CD4+ T cells showing greater longevity. [11] Unfortunately, the magnitude of the protective immune response has not been clearly defined. One study showed that subjects with neutralizing antibody titers > 1:32 were less susceptible to infection[28], while a similar study found that titers >1:20 were protective. [29] Unfortunately, no similar data are available for cell-mediated responses.

The live virus vaccines used during the eradication efforts were not without side-effects and complications, in fact they are among the most reactogenic of all FDA-approved vaccines. Common side effects include: fever, headache, pruritis, lymphadenopathy, fatigue, muscle aches and nausea. Although rare, life-threatening conditions such as postvaccinal encephalitis, progressive vaccinia and eczema vaccinatum, myopericarditis or generalized vaccinia can occur. [30-32] Historically, for every million vaccine recipients there were 1-2 deaths and hundreds of cases with complications severe enough to require hospitalization. [33] If the current population of the United States were vaccinated, it might result in several hundred deaths and tens of thousands of hospitalizations. In the absence of endemic smallpox this risk was not acceptable, and large-scale vaccination halted in 1972 in the United States and by 1980 in most other countries as well. From a biodefense standpoint these vaccine safety concerns would be of secondary importance to the need to quickly and efficiently break the chain of transmission and contain the outbreak.

As concerns regarding bioterrorism increased in the earliest years of the 21st century, national stockpiles of smallpox vaccines were found to be extremely limited. In 2005 the stockpile was estimated to have 15 million doses of lyophilized Dryvax vaccine and 85 million doses of frozen Aventis Pasteur Smallpox Vaccine (APSV). [34] Other countries were found to have similarly limited stockpiles of smallpox vaccine, enough for an estimated 10% of the world

population. [35] In the early 2000's the US government re-instituted large scale vaccination programs among the military and among civilian healthcare workers. [32,36,37] Consequently considerable effort was put into extending the current stocks and development of replacement vaccines. Given the lack of endemic smallpox all new smallpox vaccines are being compared to Dryvax® in terms of both safety and immunogenicity. [38] Controlled studies indicated that Dryvax and other first-generation vaccines (APSV, Lister) could be diluted at least 5-fold without a significant drop in either take rates or immunogenicity[39-43], although other groups have argued that dilution will increase the risk of ineffective inoculations and may hamper ring vaccine strategies. [44]

Acambis and Baxter laboratories plaque purified a NYCBOH isolate from Dryvax® with an improved safety profile in animal models and used this strain to create a cell-culture based vaccine; ACAM2000. [45,46] This vaccine has been through multiple clinical trials and was found to have the same take rate, lesion size and a similar rate of adverse events as Dryvax. [47] Neutralizing antibody titers induced by ACAM2000 were slightly lower than Dryvax in one study[47] but equivalent in others[45,48], while T cell responses showed no significant differences. In a non-human primate study, ACAM2000 was found to be as effective as Dryvax in preventing disease following a lethal monkeypox challenge. [49] Interestingly, ACAM2000 takes rates decreased significantly upon dilution. [48] ACAM2000 received FDA approval in 2007[50]. By 2004 Acambis had produced 200 million doses of the vaccine for the national stockpile[45] and is contracted to produce more.

Development of New Vaccines

In addition to licensed vaccines, a number of other vaccine candidates are in various stages of development. [51-54] These include other live vaccinia strains, live attenuated strains deficient in one or more viral genes (believed to be safer alternatives to conventional vaccinia strains), and inactivated or subunit vaccines that potentially offer even greater safety profiles.

Live smallpox vaccines produced in tissue culture

Initial work on tissue culture smallpox vaccines was performed in the 1970's, and again emphasized in the early 2000's as replacements for the aging calf-lymph products. These vaccines sought to retain the immunogenicity of the live vaccines while controlling the production conditions in order to curtail non-sterile handling of the vaccine components, and eliminate bacterial, viral, and fungal adventitious agents. [55] These vaccine candidates include: ACAM1000 and ACAM2000 produced by Acambis from plaque purified NYCBOH isolates grown in MRC-5 and Vero cells respectively[45,56], Cell-culture smallpox vaccine (CCSV), another NYCBOH-based vaccine grown in MRC-5 cells and developed by DynPort, [57], and Elstree-BN, a Bavarian-nordic product based on the Lister strain.[51] Most of these strains have undergone extensive side-by-side testing with Dryvax® or Lister vaccines and have similar safety and immunogenicity profiles. [58]

Replication competent, attenuated smallpox vaccines

During the eradication effort a number of attempts were made to create less pathogenic virus strains with a corresponding increase in vaccine safety. [34] While most of these newer strains were not sufficiently immunogenic for use in vaccines, several promising candidates remain.

LC16m8 was developed through serial passage of VACV Lister through rabbit kidney cells. [59,60] The resulting strain was less virulent and was used in Japan in the 1970's. [60] LC16m8 was found to have similar take rates and levels of neutralizing antibodies than Lister, with fewer adverse events and febrile reactions. [61] LC16m8 is one of the candidates for the creation of a US stockpile of attenuated smallpox vaccine.

Modified Virus Ankara (MVA) was developed through the serial passage of VACV Ankara through chick embryo fibroblasts. [62] After 572 passages, the virus lost ~15% of its genome and the ability to replicate in most mammalian cells. [63] It was used in Germany in the late 1960's to vaccinate over 100,000 individuals with very few adverse events. As smallpox did not circulate in Germany during or after this time period, there are no efficacy data on MVA. Newer versions of MVA are being produced by a number of manufacturers including: ACAM3000 by Acambis (www.clinicaltrials.gov # NCT00079820 last accessed 7/14/2009), IMVAMUNE by Bavarian Nordic[64,65], and TBC-MVA by Therion Biologics Corporation [66]. MVA is also a top candidate for inclusion in the US stockpile as an attenuated vaccine.

NYVAC, another highly attenuated vaccine strain was derived from the parental Copenhagen vaccinia strain by deletion of 19 ORFs including the thymidine kinase, ribonucleotide reductase, complement binding, hemagglutinin and other genes.[67] While NYVAC is deemed to be a "safer" vaccine candidate, direct comparisons with Dryvax or Lister-based vaccines have shown that NYVAC possesses inferior immunogenicity. [18,68,69] From the standpoint of biodefense preparations against poxviruses, NYVAC is not an optimal vaccine candidate. On the other hand, foreign antigens can be readily incorporated in NYVAC based vectors and induce robust immunity. NYVAC is therefore a promising vehicle for the development of vaccines against other diseases. [69-71]

A replication defective vaccine based on the Lister strain (dVV-L) was created through the deletion of the uracil-DNA-glycosylase (UDG) gene. [72] The resulting vaccine, while replication incompetent outside of certain cell lines which provide exogenous UDG is still capable of initial infection and early gene expression. Early work found that this vaccine candidate had an enhanced safety profile compared to wild-type Lister vaccine. [73] Mouse vaccination and lethal challenge studies found that dVV-L induced protective T and B cell responses against cowpox or vaccinia virus but required higher vaccine doses to prevent death in response to more virulent natural mouse pathogen, ectromelia virus. [74]

The Dairen strain was used primarily in Japan during the eradication effort and more recently was used to create the attenuated Dairen-I strain.[75,76] This strain lacks several host range and immunomodulatory genes [77] which restrict its replicative potential and may enhance its safety profile. Similar to NYVAC, Dairen-I has served as a useful vector in the development of vaccines against mycobacterium, HIV, SARS and other pathogens. [78-80]

A variety of other attenuated vaccinia virus strains have been created through the selected deletion or alteration of host range genes, virulence factors and immunomodulatory genes. [81-87] These recombinant vaccinia strains have been shown to grow to lower titers in various organs, and/or exhibit less toxicity and morbidity in immunocompromised animal models. Other groups have used genetic engineering to incorporate growth factors, cytokines, chemokines and innate immune activators into vaccinia virus in an attempt to boost immune responses after infection. [88]

Overall, attenuated vaccinia strains are far safer in animal models, especially those with defective immune responses, and in a number of cases (MVA, LC16m8) induce fewer side effects in human populations. Unfortunately a growing body of literature indicates that they are less immunogenic than their conventional vaccine counterparts, requiring high doses of antigen and/or repeat immunization to induce similar levels of antibody titers or cellular immunity.[18] Another interesting observation is that subclones with markedly different behavioral characteristics exist within live viral stocks, and outgrowth of these lines may have unforeseen effects on safety, immunogenicity or both.[89] In the absence of endemic smallpox it is not known if these vaccines will provide full protection or merely attenuate disease symptoms. One potential option would be to use these safer vaccines in individuals with

compromised immunity, while another approach would be to provide these vaccines to everyone in the population as a priming dose. In this case, a partially protected population may serve as a deterrent to bioterrorism and the conventional vaccine would always be available in the case of an outbreak or biological attack. In any case, these attenuated vaccine formulations are a valuable component of biodefense preparations.

Inactivated smallpox vaccines

In the mid-20th century a great deal of effort went into the development of inactivated smallpox vaccines, with limited success. [90,91] Although these vaccines exhibited far greater safety profiles, and induced strong humoral immunity; the resulting responses were unable to control infection and prevent illness in animal models. [92,93] This may be attributed to the lack of key immunogenic epitopes within the killed vaccines. These formulations lack the extracellular enveloped virions which contain an extra lipid membrane with several additional viral proteins. [94-96] These proteins are targets of neutralizing antibody responses and immunity to both the intracellular and extracellular virions is likely needed for complete protection.

Subunit-based smallpox vaccines

The development of protein, peptide or nucleic acid based subunit vaccines for preventing smallpox is an active area of investigation. Most groups have focused on the inclusion of viral proteins targeted by humoral immunity, although with the recent identification of CD4 and CD8 T cell epitopes, cellular responses to poxviruses are beginning to be taken into account as well. [97,98] [99] Several groups have found that combinations of membrane and virion proteins (A27L, A33R, B5R, H3L, L1R) or DNA are far more effective than single protein vaccines and provide excellent protectin in both mice and non-human primate models. [99-106] One consideration that must be taken into account is the genetic diversity of poxviruses. The vaccines are all based on vaccinia virus whereas the most important pathogenic poxviruses are variola and monkeypox. Cross-protective immunity is a direct result of antigenic similarity between the viruses. Using whole viruses provides a large mixture of vaccinia antigens, many of which are highly conserved between poxvirus strains. With protein or DNA based subunit vaccines the selection of antigens is severely limited. Heterogeneity within individual proteins may have negative consequences on vaccine efficacy. [107,108] One alternative to this is to base vaccine components on variola homologs rather than the vaccinia proteins. [109] The identification of HLA class I and class II restricted epitopes from poxviruses has opened the door to the development of safe and immunogenic peptide-based vaccines. [110-117] [118]

Another area of investigation is the use of different delivery routes, including mucosal vaccines [119,120] and plant-based formulations. [121]

Conclusions and looking to the future

Given the tremendous effect a biological attack with smallpox would cause, it is imperative that we are properly prepared. Vaccines are among the most cost-effective of healthcare measures and play key roles in biodefense preparations both through deterrent action and as preventive measures. Although several elements of our bio-preparedness efforts are now being accomplished (sufficient vaccine doses for the US population, new anti-virals), much work remains as many countries have insufficient doses for their population and live virus vaccines remain problematic in today's environment of extreme sensitivity to adverse vaccine reactions. Numerous potentially safer vaccine products are in various stages of research and development but will require rigorous testing to ensure that they induce immunity equivalent to conventional smallpox vaccines. To this end, further work needs to be done to refine animal models for testing new smallpox vaccines as small animal studies using different poxviruses can produce

conflicting results, and non-human primate models do not fully recapitulate human exposure to smallpox. [122]

Another avenue of research that should be further developed is that of immunogenetics and understanding why some individuals are predisposed to ineffective vaccine responses or even adverse events. [123-125] The development of simple screening tests would allow us to steer individual patients toward vaccines best suited to their individual requirements. [126]

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Table 1

The use of Smallpox as a Bioweapon

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

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]