Safe and Effective Smallpox Vaccine Development Using DNA Vaccines and In vivo Electroporation

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Abstract

Few viruses have elicited more of its threat potential as a tool of bioterrorism than smallpox. In the post-9/11 “Amerithrax” environment, the threat of an intentional release of smallpox has led to renewed efforts to develop a safer vaccine, with fewer side effects, that could be administered to the general public. DNA vaccines administered through the use of enhanced delivery using electroporation could provide a platform for delivering a smallpox vaccine. Previously published data have shown that an 8 plasmid combination vaccine consisting of VACV antigens (specifically, A4L, A27L, A33R, A56R, B5R, F9L, H3L, and L1R) delivered to rabbits and nonhuman primates followed by electroporation elicited robust humoral and cell-mediated immune responses. Furthermore, non-human primates were protected from lethal challenge with monkeypox, showing that this vaccine platform is effective. This review summarizes recent data supporting vaccine development using DNA and electroporation to protect the general public in the event of a bioterror incident using smallpox.

Keywords: Smallpox; DNA Vaccine; Electroporation; Intradermal; Monkeypox; Challenge.

Introduction

Smallpox is an infectious disease that has killed more people worldwide than almost any other single pathogen [1]. Smallpox infections had been reported as far back as thousands of years ago [2] and organized smallpox prevention began with Edward Jenner’s cowpox-origin vaccination in the 1800’s. Due to the diligence of various health organizations including WHO in the 1950’s and 1960’s, smallpox was declared to be eradicated [3] and systematic prophylactic vaccination for the U.S. public was ceased. However, public attention has remained on the potential for this agent to be used in bioterrorism. Furthermore, in the event of an actual outbreak, the inadequate stocks of smallpox vaccine made using an outdated vaccine production method would likely tax the public health system. Large portions of the US population are unvaccinated and thus susceptible, while there is a significant number of both immunosuppressed individuals and other members of the US population with contraindications to the current licensed vaccine.

Additionally, it has been shown that there was a loss of immunity in vaccines that received Vaccinia virus (VACV) during the period of smallpox pandemic and until eradication in 1979 [4]. The recent outbreaks and epidemics of poxviruses in both the U.S. [5] and Africa [6,7] of the closely-related monkeypox virus have heightened fears of a new pandemic. This potential threat has caused a re-examination of the vaccine status of the public [8]. Thus, there is renewed interest in a more deployable smallpox vaccine [1]. Recent research efforts have been focused on increasing our understanding of poxvirus immunity in order to develop safe and effective next-generation vaccines.

Previous Smallpox Vaccine Development and Experience in Humans

The previously approved vaccine, Dryvax* (Wyeth Laboratories), is a lyophilized preparation of live VACV derived from calf lymph [9] and is administered by scarification with a bifurcated needle. However, use of this vaccine is limited because of adverse reactions that range from non-serious flu-like symptoms and rash to life threatening complications (i.e. eczema vaccinatum, encephalitis, and progressive Vaccinia). In addition, this vaccine is contraindicated in important groups of the population - those individuals that are immunocompromised (i.e. organ or bone marrow transplant recipients, HIV-positive individuals, cancer patients receiving chemotherapy or radiotherapy), pregnant and breastfeeding women, and children under the age of 12 months. ACAM2000 (manufactured by Acambis, now Sanofi Pasteur), a second-generation Vaccinia virus-infected cell culture-based smallpox vaccine, is delivered similarly to Jenner's vaccine by scarification. ACAM2000 also closely matches the safety of Dryvax in both non-clinical and clinical trials, which may include risks of inoculating close contacts, eczema vaccinatum [10] and in particular myopericarditis [11]. Therefore, it is imperative that a vaccine be developed that is both safer and as effective as live virus-based vaccines.

DNA Vaccines

DNA vaccines have been shown to be safe and with shorter preparation times compared to the production of live viruses. Traditionally in animals, DNA vaccines have been used as a prime for viral vectors or proteins as the prevailing theory was that DNA-based vaccines cannot be boosted without the use of adjuvants. This theory was further exacerbated by the inability of researchers to replicate promising results from rodent studies to larger mammals including human clinical trials. Thus, while DNA vaccines offered a level of safety compared to other vaccination methods, they were not shown to be efficacious.

Several methods have been suggested to improve the

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immunogenicity of DNA vaccines and electroporation (EP) in particular has consistently improved the expression of naked DNA transfer. Recent in vivo EP has been shown to increase the level of expression by an order of 3 magnitudes over plasmid injection alone [12] and has been shown efficacy in multiple species of animals for a variety of infectious disease targets [13-16]. Studies have concluded that intramuscular (IM) injection followed by EP may be a feasible method for DNA vaccine delivery for clinical use [17-19] resulting in activation of both cellular and humoral immune responses [16,18-20].

While EP remained a method that gained relative acceptance for enhancing DNA transfection and expression in animal studies, transition of this technology from the bench to the bedside has been rather slow. In response to this need, the CELLECTRA® 2000 constant current EP device was developed. This device generates constant electrical current EP throughout the tissue, with mild tissue damage and increased vaccine or therapeutic plasmid expression [21-25]. EP has been previously shown to improve the immune potency of DNA vaccines by enhancing the uptake and expression of the transgene [17,26] and increasing the immune responses in a tumor animal model system [27]. EP has been utilized to enhance the immune potency against HIV [20,28], Chikungunya virus [29], HPV [16], and influenza virus [30,31].

Skin vaccination using DNA and EP also has the advantage of direct transfection of antigen presenting cells that are present in the skin, such as Langerhans cells and dermal dendritic cells. These cells in turn can express and present the antigens recruiting an immune response through both T-cell and B-cell activation. Furthermore, the EP itself may aid this by causing inflammation and thus recruiting the immune response to the site of vaccination in the skin. Essentially, this platform technology can use the body’s natural and very immunopotent barrier organ as an antigen-expression system to elicit immune responses against these antigens. DNA injection to the skin delivered with EP has been used in previous studies [32-36] and has recently been highlighted for its potential as delivery tool for eliciting protective titers against H1N1 influenza antigens and protection against H5N1 challenge [37].

Smallpox DNA vaccines – previous animal experience

A DNA vaccine approach has previously been used to protect mice and nonhuman primates from lethal poxviral challenges [38-43]. The 2 to 4 VACV antigens selected have been shown to yield both a humoral and cell-mediated immune response [38-47]. In a mouse model, IM delivery of plasmid DNA encoding the VACV antigens A4L, A27L, and H5R followed by a protein boost elicited a humoral and cell-mediated immune response [38-47]. The 2 to 4 VACV antigens selected have been shown to yield both a humoral and cell-mediated immune response [38-47]. The 2 to 4 VACV antigens selected have been shown to yield both a humoral and cell-mediated immune response [38-47].

Nevertheless, antigenic interference has also been described using these methodologies [40]: mice vaccinated with LIR alone developed neutralizing antibodies and were partially protected, however, mice vaccinated with a combination of A33R and LIR were not protected [39]. In a follow up study, Golden et al. [48] were able to enhance the neutralizing antibody response to LIR by adding a tissue plasmominogen activator signal sequence to the LIR gene.

Using the gene gun method of delivery, Hooper et al. [40] showed that vaccination of mice with a combination of LIR and A33R conferred greater protection than either gene alone. Similar results were observed in mice administered A27L and B5R in that greater protection, but not complete protection, was observed with the combination of the antigens compared to either gene alone [40]. The combination of the four VACV genes (A27L, A33R, B5R, and LIR) elicited the greatest defense against challenge, conferring complete protection in mice. Protection from challenge was also observed when rhesus macaques were vaccinated with the four gene VACV combination [41]. Vaccination with the same combination of antigens followed by a novel skin EP in mice mounted a robust immune response consisting of strong neutralizing antibody titers that exceeded that of the live virus [42]. Together, these results suggest that polyvalent smallpox vaccines could be most effective against smallpox challenges.

Developing a Multivalent, Highly Concentrated Vaccine in Rabbit Model

To the best of our knowledge, the DNA vaccine candidates to date for smallpox have comprised of a maximum of 4 or 5 VACV antigens [38,40-42] and have never comprised of an 8 plasmid combination possibly due to the lack of feasibility of this approach. We have used highly concentrated DNA, which allows for the of 8 antigens within the same vaccination.

Nevertheless, a multivalent vaccine that can be administered as a single preparation may be needed to offer protection in humans, for this and other disease models. Thus, a recent study evaluated the efficacy of a multi-plasmid combination vaccine (consisting of 8 VACV antigens: A4L, A27L, A33R, A56R, B5R, F9L, H3L, and L1R) in eliciting a potent immune response in several different animal models using IM delivery using EP [49]. These antigens (details in Table 1) were chosen because of their ability to elicit strong humoral and cellular immunity, and have provided protection in animal models [38-47]. Antigens were synthesized, codon-optimized, an IgE leader sequence was added, and cloned into the pVAX1 plasmid backbone. Each plasmid was scaled up at high concentration (between 7.4 and 13.6 mg/mL) by VGXI (The Woodlands, Texas, USA). The issue of antigenic interference was also explored, as specific responses to each of the antigens included in the DNA vaccine preparation were measured.

Table 1: Biological properties of the cloned VACV genes.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Size (kDa)</th>
<th>Functional properties</th>
<th>Infectious form association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4L</td>
<td>39</td>
<td>Viral core protein synthesized late post-infection and involved in viral core assembly.</td>
<td>IMV</td>
<td>[50]</td>
</tr>
<tr>
<td>A27L</td>
<td>14.0</td>
<td>Required for formation and viral assembly.</td>
<td>IMV</td>
<td>[51]</td>
</tr>
<tr>
<td>H3L</td>
<td>37.5</td>
<td>C-terminal transmembrane protein involved in morphogenesis.</td>
<td>IMV</td>
<td>[52,53]</td>
</tr>
<tr>
<td>F9L</td>
<td>24</td>
<td>Related to L1R. Essential for virus entry and cell fusion.</td>
<td>IMV</td>
<td>[54]</td>
</tr>
<tr>
<td>L1R</td>
<td>27.3</td>
<td>Myristoylated type I membrane protein involved in viral assembly.</td>
<td>IMV</td>
<td>[55]</td>
</tr>
<tr>
<td>A33R</td>
<td>20.5</td>
<td>Type II membrane protein involved in actin tail formation.</td>
<td>EEV</td>
<td>[56]</td>
</tr>
<tr>
<td>A56R</td>
<td>85</td>
<td>Related to hemagglutinin</td>
<td>EEV</td>
<td>[57]</td>
</tr>
<tr>
<td>B5R</td>
<td>35.1</td>
<td>Type I membrane protein involved in viral egress.</td>
<td>EEV</td>
<td>[58]</td>
</tr>
</tbody>
</table>

Table 1: Biological properties of the cloned VACV genes.

*All antigens described have been shown to be essential for viral replication.

The intracellular mature virion (IMV) is retained within the infected cell, while the extracellular enveloped virion (EEV) form of the virus is actively secreted from cells contributing to the efficient dissemination of the virus in vitro [59] and in vivo [60]. Although not clearly understood, there has been suggested that the IMV form is responsible for host-to-host spread and the EEV form is thought to be primarily involved in long-range spread within the host [61].
Antigenic interference has been previously described [40] and it is quite possible that a multivalent vaccine of 8 plasmids would lack efficacy or a few of the antigens would not be immunogenic and thus possibly be detrimental to the response to challenge. Rabbits were used to compare the efficacy of an 8 vs. a 4 plasmid combination following EP. The results from this study suggested that statistically the level of binding antibody response between the 4 and 8 plasmid combinations were identical other than on Day 42 for BSR (P < 0.05). Binding antibody titers to the antigens reached from 1:15,000 to 1:50,000 for antigens delivered via IM or ID injection, respectively, followed by electroporation. A similar level of response was observed in between the 4 and 8 plasmid combination, which indicates that the immune response was not diminished by increasing the number of antigens (data not shown). This conclusion is based on the detection of binding antibodies for A27L, BSR, and H3L although it is quite possible a different response would have been observed with other antigens.

**Monkeypox Challenge and Demonstration of Protection Using Eight Antigens**

The multivalent smallpox vaccine was tested in non-human primates using three vaccinations, either via intramuscular or intradermal route [49]. Since the 8 distinct plasmid constructs were manufactured at higher concentrations, the vaccines were administered into three total IM and ID vaccinations at 4 weeks intervals (weeks 0, 4 and 8). Control animals were sham vaccinated. All animals were challenged at week 13 with monkeypox virus (2 x 10^7 PFU). Morbidity was measured through changes in body temperature, body weight and using a tabulated number of visible monkeypox lesions. IM and ID vaccinated animals were well protected against morbidity and appearance of lesions as compared to control animals. Furthermore, viral loads were several logs lower in vaccinated animals. Neutralizing antibody titers were higher in ID vaccinated animals and correlated with fewer visible lesions and faster recovery from peak viral load. Animals were protected from lethal challenge using a regimen using DNA vaccination delivered by electroporation. Thus, the possibility of co-delivering 8 plasmids could be a significant advantage, both in a challenge against a single disease, or in a combination vaccine in an effort to elicit broad cross-protection.

**Conclusions**

Importantly, the scientific field continues to make progress in developing a safer and more effective smallpox vaccine, by showing immune responses in multiple large animal species. Overall, the combination of highly concentrated DNA with EP has resulted in a feasible approach for the administration of multivalent vaccine using 8 different antigens that conferred protection against challenge. Besides safety, further development of a DNA-based smallpox vaccine will have to demonstrate efficacy in the form of a measureable correlate of protection and future clinical trials will need to address this important question. These findings will aid the development and translation of this technology to humans for a more safe and effective smallpox vaccine to protect against the threat of a bioterror attack using the smallpox virus.

**References**


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47. Demkoovciz W, Maa JS, Esteban M (1992) Identification and characterization of vaccinia virus genes encoding proteins that are highly antigenic in animals and are immunodominant in vaccinated humans. Vaccine 29: 3507-3515.


