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Nucleic acid (DNA) immunization as a platform for dengue vaccine development

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ABSTRACT

Since the early 1990s, DNA immunization has been used as a platform for developing a tetravalent dengue vaccine in response to the high priority need for protecting military personnel deployed to dengue endemic regions of the world. Several approaches have been explored ranging from naked DNA immunization to the use of live virus vectors to deliver the targeted genes for expression. Pre-clinical animal studies were largely successful in generating anti-dengue cellular and humoral immune responses that were protective either completely or partially against challenge with live dengue virus. However, Phase 1 clinical evaluation of a prototype monovalent dengue 1 DNA vaccine expressing prM and E genes revealed anti-dengue T cell IFNγ responses, but poor neutralizing antibody responses. These less than optimal results are thought to be due to poor uptake and expression of the DNA vaccine plasmids. Because DNA immunization as a vaccine platform has the advantages of ease of manufacture, flexible genetic manipulation and enhanced stability, efforts continue to improve the immunogenicity of these vaccines using a variety of methods.

1. Introduction

Q3 Dengue poses a tremendous global public health threat, occurring in approximately 100 countries [1]. Current strategies to prevent this disease include the use of mosquito repellants, vector control using insecticides, eliminating mosquito breeding sites and the use of bed nets. The effectiveness of the latter is questioned due to the day-biting nature of *Aedes* mosquitoes. As of the writing of this article, there are no FDA approved drugs or vaccines to treat or prevent dengue.

Given the global distribution of dengue with the circulation of multiple dengue serotypes in many different countries, the development of an effective tetravalent dengue vaccine is a top priority for U. S. military forces and public health agencies. For decades U. S. military scientists worked to develop a dengue vaccine. Investigators at the Walter Reed Army Institute of Research used traditional approaches to develop a live attenuated vaccine and currently are moving forward with a purified inactivated dengue vaccine.

Scientists at the Naval Medical Research Center (formally the Naval Medical Research Institute) pursued molecular platforms to complement the Army's dengue vaccine development programs. These efforts later were consolidated into a joint Army–Navy dengue vaccine program under the Military Infectious Diseases Research Program.

The various platforms utilized by Navy investigators as part of this joint effort primarily involved naked DNA immunization. To enhance the immunogenicity of experimental dengue DNA vaccines, several approaches were explored including DNA formulated in an adjuvant, virus vectored vaccines, use of immunostimulatory nucleic acid sequences and differing the routes of vaccine administration. Table 1 summarizes the different approaches. The preclinical and clinical successes and failures of each of these approaches is the subject of this review article.

2. Naked DNA immunization

2.1. DNA vaccine construct development

The dengue virus genome consists of three structural and seven nonstructural genes, in addition to 3′ and 5′ non-coding regions. The three structural genes code for capsid (C), pre-membrane (prM) and envelope (E) proteins. While immune responses are elicited primarily to the structural proteins as well as the nonstructural proteins NS1, NS3 and NS5, neutralizing antibody responses are directed primarily to epitopes on the envelope protein. Cellular immune responses are mostly generated against the nonstructural

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Table 1
U. S. navy DNA and virus vectored vaccine approaches.

Method	Pre-clinical	Clinical	Issues	References
Monovalent DNA. IM/ID needle injection.	Immunogenic in mice and NHP.	–	–	[1–5]
Monovalent DNA. Needle-free Biojector injection, IM.	Increased immunogenicity in NHP.	Weakly immunogenic. Weak antibody response but better T cell responses in a subset of individuals.	Antibody responses present only in high dose group (5 mg DNA).	[6,7]
Tetravalent DNA; single plasmid shuffled DNA approach.	Tetravalent immune responses in mice; weaker tetravalent responses in NHP.	–	–	[8,9]
LAMP chimera DNA approach.	Increased immunogenicity for dengue-2 DNA vaccine in mice.	Part of the tetravalent DNA vaccine cocktail.	Effect on dengue-2 could not be replicated for other serotypes.	[10,11]
Tetravalent DNA (mix of 4 monovalent DNAs) + Vaxfectin, IM, Biojector.	Increased antibody response to all 4 serotypes in NHP.	–	–	[12]
Tetravalent DNA (mix of 4 monovalent DNAs) + Vaxfectin, IM, needle.	Immunogenicity and safety demonstrated in white rabbits.	Clinical trial completed. Final report and manuscript in progress.	Maximum DNA that could be formulated and administered was 2 mg/dose.	[13]
VEE replicon particle (VRP) vaccines.	Monovalent dengue-1 & tetravalent mixtures immunogenic in mice and NHP. Better when used for boosting.	–	Difficult to produce large quantities. Generating replication competent virus is a concern.	[14]
Adenovirus vectored vaccines.	Tetravalent formulation immunogenic in mice and protective in NHP challenge studies.	–	Safety concerns about Ad-5 based vectors.	[15–17]

NHP = nonhuman primates; LAMP = lysosome associated membrane protein; Ad-5 = adenovirus serotype 5.

proteins. Given the epidemiological evidence in infants demonstrating that anti-dengue neutralizing antibodies appear to be sufficient to provide protection against dengue disease, early naked DNA vaccine development efforts centered on the use of the envelope gene.

The studies with dengue DNA vaccines outlined below proved the feasibility of this approach. They also informed us of the need to develop approaches and methods to improve the immunogenicity of the vaccine constructs. In the case of DNA vaccines, immunogenicity is a function of many variables such as the uptake of DNA by immunologically relevant cells, level and type of antigen expressed, and presentation of antigen to mount an immune response.

The first series of proof-of-principle experiments focused on the development of a monovalent dengue 2 (DEN-2) DNA vaccine. The construct consisted of the prM gene and 92% of the envelope gene of the New Guinea C DEN-2 strain, cloned into a plasmid vector provided by Vical Inc. The transmembrane portion of the E gene was omitted to promote secretion of the intracellularly expressed DEN-2 E protein. Murine studies with this vaccine demonstrated the production of anti-DEN-2 neutralizing antibodies that protected against intracerebral challenge with live DEN-2 virus [2].

Follow on work was performed to systematically determine the optimal configuration of a dengue DNA vaccine based on E protein antigen. We published murine studies showing that plasmid DNA vaccines containing dengue 1 Western Pacific 74 strain (DEN-1) prM and full length E (prM 100%E) were optimal in producing neutralizing antibody responses [3]. In that study, plasmids containing 80%E, prM 92%E and prM 100%E resulted in the secretion of dengue E proteins into culture media when transfected into 293 cells. Plasmids expressing prM 80%E did not adequately express DEN-1 E proteins. When the plasmids were administered to mice by intradermal (ID)/subcutaneous (SC) injection, only the 80% E and prM 100%E constructs generated high levels of neutralizing antibody, with the prM 100%E plasmid producing the highest and most durable response. Analysis of the culture supernatants of transfected cells revealed that the E protein secreted into the medium of cells transfected with prM 100%E, but not 80%E, was present in the form of virus-like-particles [3]. Based on these results, DNA vaccine constructs containing DEN-1 prM 100%E genes were chosen for evaluation of protective efficacy in non-human primate models. While there are a few other studies of dengue DNA vaccines based on E protein [4,5] and NS-1 protein [6–8], to our knowledge none have moved beyond evaluation in animal models.

2.2. Preclinical testing for immunogenicity and efficacy in non-human primates

The DEN-1 construct was tested in both rhesus macaques and *Aotus* monkeys for the ability to generate a protective immune response. Protective efficacy was assessed by challenge with live DEN-1 virus and subsequent measurement of duration/magnitude of the post-challenge dengue viremia.

Mode of vaccine delivery was also assessed in these experiments. Intramuscular (IM) and subcutaneous delivery of vaccines were the mainstay because of ease of administration and the ability to deliver relatively larger volumes. However, delivery via the ID route is believed to be more effective due to the presence of antigen presenting Langerhans cells that serve as a first line of defense against infectious pathogens. The vaccine in both non-human studies was therefore administered both by the ID and IM routes.

In the *Aotus* [9] study, there was a trend toward higher antibody responses when administered intradermally, but the differences were not statistically significant [9]. Small group sizes and the presence of keratinized tissue in *Aotus* skin may have contributed to not fully realizing the potential of intradermal delivery. Animals immunized both ID and IM showed some level of protection against live virus challenge.

In contrast to the results obtained using *Aotus* monkeys, ID immunized rhesus monkeys had poor neutralizing antibody responses [10]. The reason for the poor antibody responses in the ID immunized group was unclear, but it was thought that differences in the skin anatomy of the older rhesus macaques may have contributed. Consistent with the poor antibody response, the ID immunized animals were not protected against live virus challenge whereas the IM immunized rhesus monkeys showed partial protection.

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