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Immunization with electroporation enhances the protective effect of a DNA vaccine candidate expressing prME antigen against dengue virus serotype 2 infection

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ABSTRACT

Purpose: We aimed to use the dengue virus (DV) serotype 2 as a proof of principal for testing the efficacy of a DNA vaccine candidate via *in vivo* electroporation in mice and rabbits prior to the development of a tetravalent vaccine.
Methods: Different dosages of DNA pVAX1-D2ME encoding DV2 *prME* genes were vaccinated in mice via intramuscular injection or *in vivo* electroporation, immune responses and protection were determined. In DNA-vaccinated rabbits via electroporation, antibody titer and protein expression were tested.
Results: In mice, 50 μg of pVAX1-D2ME via electroporation elicited effective anti-DV2 responses and conferred significant protection against DV2 challenge. Moreover, anti-DV2 IgG and neutralizing antibodies were successfully induced in rabbits immunized with pVAX1-D2ME via electroporation and the expression of the interest protein was observed at local sites.
Conclusions: Enhanced immunogenicity and protective effect conferred by pVAX1-D2ME via electroporation show great promise for the development of a dengue tetravalent DNA vaccine.

1. Introduction

Dengue virus (DV), which co-circulates as four distinct serotypes (DV1–4), is the causative agent of dengue and severe dengue. The latter includes dengue hemorrhagic fever and deadly dengue shock syndrome. An estimated 390 million individuals are infected annually worldwide, particularly in tropical and subtropical regions, of which 96 million manifest clinically [1]. In 2014, southern China's Guangdong province witnessed a logarithmic increase in weekly dengue fever cases [2], with the total number of cases exceeding 45,000 – 15 times the number of dengue fever cases in 2013 [3,4]. The development of virus-specific prevention measures and treatments is an urgent public health priority.

Significant progress has been achieved and several vaccine candidates have been evaluated in I–III phases of clinical efficacy trials, and elicited varying levels of serotype specific immunity [5–8]. Theoretically, a secondary DV infection with a different serotype is believed to increase the risk of severe dengue *via* the effect of antibody (Ab) dependent enhancement. Hence, a desirable dengue vaccine should be tetravalent and elicit protective immune responses against all four serotypes. One vaccine candidate that has drawn researchers' interest is the CYD tetravalent dengue vaccine (TDV) comprising four recombinant, live, attenuated viruses (Sanofi-Pasteur, France). Large-scale phase III studies in various endemic countries [9–13] have shown that CYD-TDV induced both humoral and cellular immune responses and led to fewer hospitalizations for virologically confirmed dengue. More recently, in a CYD-TDV phase III trial in five Latin American countries where dengue is endemic, new results have demonstrated that the vaccine efficacy was raised to >60%, but the serotype-specific efficacy was 42.3%

Abbreviations: Ab, antibody; ANOVA, analysis of variance; CTL, cytotoxic T lymphocyte; DV, dengue virus; E, envelope; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; EP, electroporation; E:T, effector:target; FBS, fetal bovine serum; GMT, Geometric mean titer; HRP, horseradish peroxidase; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; IM, intramuscular; LD50, median lethal dose; MEM, minimum essential medium; MHC, major histocompatibility complex; NAb, neutralizing Ab; OD, optical density; prM, premembrane; PRNT, plaque reduction neutralization test; PFU, plaque-forming unit; SCID, severe combined immune deficient; SD, standard deviation; SFU, spot-forming unit; SI, stimulation index; TDV, tetravalent dengue vaccine; Th, helper T cell.

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for DV2 [13]. Because vaccination-related adverse events were observed [10], serious concerns must be raised regarding the safety of this live attenuated vaccine. Additionally, the three-dose schedule (administered at 0, 6, and 12 months) might limit administration of the full course in travelers. Although Sanofi Pasteur announced that the first DV vaccine was licensed in Mexico, the Philippines, and Brazil, it is still imperative that safer, more effective and less costly vaccines be developed.

As a simple and efficient technique, DNA vaccination represents an attractive alternative to other methods [14], as it confers many advantages, such as excellent stability and cost-effectiveness, non-spreading properties, high safety, and long-term persistence of immunogens [15]. More importantly, plasmid-encoded peptides can be not only presented to the major histocompatibility complex (MHC) class I molecules but also processed through the MHC class II pathway after the capture of secreted antigens. Both intracellular and extracellular presentations are capable of inducing antigen-specific cellular and humoral immune responses [16]. In recent years, electroporation (EP) has been shown to substantially increase the delivery of DNA to cells, resulting in elevated antigen expression and improved immune response magnitude [15]. More recently, Flingai and colleagues demonstrated that synthetic DNA plasmids engineered to express modified Ab against multiple DV serotypes can protect mice from dengue disease, indicating that DNA may play an important role in the fight against infectious diseases [17]. The licensed vaccines for horses, fish, and dogs and the first licensed EP-delivered product for swine [18,19] re-energized DNA vaccine research in larger animal models as well as in human clinical trials.

DV contains a positive single-stranded RNA that encodes three structural proteins, capsid, premembrane (prM) and envelope (E) proteins, followed by seven non-structural proteins. Exposed on the virion surface, the E protein contains the immunological epitopes recognized by neutralizing Abs (NAbs) [20,21]. The prM protein is processed during export and is essential for the correct conformation of the E protein [22]. Therefore, the *prM* and *E* genes were used as major target molecules for vaccine candidates and were capable of inducing DV-specific cellular and humoral immune responses in several studies [8,23–25]. Previously, we constructed a DNA vaccine candidate expressing the DV2 prME protein using the eukaryotic expression vector pCAGGSP7 [26,27]. When delivered by intramuscular (IM) injection, the DNA-immunized mice were partially protected with only a 30% survival rate against lethal DV2 challenge [27].

In this study, for the transition to a potential product that could be used in further clinical trials, the plasmid expressing DV2 prME was reconstructed using pVAX1, a unique US FDA-approved vector used in the development of DNA vaccines. The EP technique was used to facilitate DNA delivery and to enhance immune responses to DV2 in mice and rabbits. Our results suggest that immunization *via* EP with the DNA vaccine candidate pVAX1-D2ME could protect mice from lethal virus infection and could induce an effective DV2-specific Ab response in rabbits. This study using DV2 as a proof of principal should encourage further development of DV tetravalent DNA vaccines.

2. Materials and methods

2.1. Cells and viruses

Aedes albopictus C6/36 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) at 28 °C. Vero cells were grown in minimum essential medium (MEM) supplemented with 5% FBS at 37 °C. Mice fibroblast L929 cells were grown in RPMI 1640 supplemented with 10% FBS at 37 °C.

The DV2 (New Guinea C strain) was propagated in C6/36 cells. Viral titers were determined by plaque assay on Vero cells. Concentrated DV2 protein was prepared from the media of DV2-infected C6/36 cells containing 8% polyethylene glycol.

2.2. Ethics statement

BALB/c mice, severe combined immune deficient (SCID) mice and New Zealand white rabbits were purchased from Vital River Laboratories (Beijing, China). The animals were manipulated in strict accordance with the recommendations in the national guidelines for the care and use of animals in scientific research "Regulations for the Administration of Affairs Concerning Experimental Animals". The protocol was approved by the Institutional Animal Care and Use Committee of Chinese Capital Medical University (approval number AEEI-2015-066). All surgeries were performed under diethyl ether (in mice) or urethane (in rabbits) anesthesia, and all efforts were made to minimize suffering.

2.3. Construction of plasmids

The eukaryotic expression vector pVAX1 was constructed to be consistent with the FDA document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications". The DNA vaccine candidate was constructed for the expression of DV2 prME using the vector pVAX1 and designated as pVAX1-D2ME. Briefly, the DV2 prME fragment containing the signal sequence (GenBank accession number AF038403, nucleotides: 367 bp–2421 bp) was amplified by polymerase chain reaction from a full-length infectious clone of DV2 (New Guinea C strain). The Kozak sequence was inserted at the N-terminus. The gene of interest was digested with the *Nhe*I and *Xho*I restriction enzymes (MBI Fermentas, USA) and subsequently subcloned into the multiple cloning site of pVAX1 (Invitrogen, USA). Sequence analysis and enzyme digestion were performed to verify the recombinant plasmid, and the expression was confirmed by indirect immunofluorescence after transfection into Vero cells. The empty vector pVAX1 served as a negative control.

2.4. Vaccination

Animal grouping and immunization methods are shown in Table 1.

For IM injection, 50 μg of the pVAX1-D2ME was injected directly into the quadriceps muscle of the hind limb of six-week-old female BALB/c mice (hereafter referred to as the IM group) using a syringe with a 25-

Table 1
Vaccination groups and methods in mice and rabbits.

Group	DNA	DNA dosage (μg) in saline (μl)	Vaccination method	Vaccination times, interval (week)	EP parameters	
					Voltage (V)	Pulse length (ms)
BALB/c mice						
EP 50	pVAX1-D2ME	50, 50	IM + EP	3, 2	36	10
EP 5	pVAX1-D2ME	5, 50	IM + EP	3, 2	36	10
IM 50	pVAX1-D2ME	50, 50	IM	3, 2	–	–
pVAX1	pVAX1	50, 50	IM + EP	3, 2	36	10
New Zealand white rabbits						
EP 500	pVAX1-D2ME	500, 500	IM + EP	3, 3	60	50
pVAX1	pVAX1	500, 500	IM + EP	3, 3	60	50