Long-term protection against dengue viruses in mice conferred by a tetravalent DNA vaccine candidate

DEAR EDITOR,

The development of an effective tetravalent vaccine against dengue viruses (DENVs) has become a world priority. We previously showed that four monovalent dengue DNA vaccines expressing premembrane (prM) and envelope (E) proteins displayed effective protection against corresponding challenges in mice. Thus, to elucidate the overall immunity and persistence of the tetravalent formulation (TetraME), we evaluated the humoral and cellular immune responses as well as the long-term protection in the current study. TetraME-immunized mice displayed increased production of Th1/Th2-typed cytokines upon stimulation with heterologous DENV antigens. Moreover, high levels of tetravalent DENV antibodies and sterilized immunity were detected long-term (30 weeks after immunization). These findings provide feasible validation for the potential utility of this vaccine formulation.

DENVs are mosquito-borne flaviviruses. The DENV genome contains positive single-stranded RNA encoding three structure proteins and seven non-structure proteins. Among them, prM and envelope E proteins contain epitopes of both cellular immunity and neutralizing antibodies and are therefore often used as molecular targets for vaccine development (Wang et al., 2018). DENVs have four distinct serotypes (DENV1–4) and infection by any serotype can cause dengue fever and/or life-threatening dengue diseases. Recently, due to mosquito-favorable factors such as global warming and increased human population movement, the incidence of dengue is on the rise worldwide (Wilder-Smith et al., 2019) and has become a global public health concern.

Vaccination is the most effective approach against dengue and has been the focus of virologists for many years. Common dengue candidate vaccines are predominantly composed of either live-attenuated or recombinant chimeric vaccines (Shrivastava et al., 2017), with many still under development. At present, Dengvaxia is the only licensed vaccine against all four serotypes of DENV. However, the World Health Organization (WHO) has recommended it only be used in populations previously exposed to DENVs, indicating its limitation in application (Lee et al., 2018). An ideal tetravalent dengue vaccine avoids interference among components and provides long-term and balanced protection against all four serotypes (Fatima & Syed, 2018; Prompetchara et al., 2019). DNA vaccines offer a series of advantages, such as mobilizing the cellular and humoral arms of the immune response and providing prolonged protection against a range of pathogens (Fynan et al., 2018). We previously manufactured four constructs expressing each DENV prM and E proteins, named pV-D1ME–pV-D4ME, which were individually evaluated in regard to immunogenicity and protection in BALB/c mice (Chen et al., 2016; Sheng et al., 2019; Zheng et al., 2017). In the current study, immunocompetent BALB/c mice were vaccinated with four monovalent prM/E-based DNA vaccine candidates (TetraME), after which we investigated the balanced and long-term tetravalent protection. All animal experiments were performed under approval of the Animal Experiments and Experimental Animal Welfare Committee of Chinese Capital Medical University (AEEI-2015-066). All animal experiments were performed under diethyl ether anesthesia, and all efforts were made to minimize suffering.

As shown in Figure 1A, mice were thrice immunized with 50 μg of each monovalent vaccine or pV vector into the quadricep muscles of all four limbs via electroporation (EP) at three-week intervals. To characterize the production of Th2 (IL-4)/Th1 (IFN-γ)-type cytokines in response to DENV1–4, splenocytes harvested from mice one week after final immunization with either TetraME or pV were plated at 3×10^5 cells per well in pre-coated enzyme-linked immunospot plates to quantitatively measure IL-4 or IFN-γ expression. When individually stimulated with DENV1–4 antigens, significantly secreted and comparable levels of the two cytokines were observed in the TetraME vaccination groups compared with

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the control groups (Figure 1B, P<0.05 or P<0.01). The higher levels of IL-4 and IFN-γ indicated functional cytotoxic T cell activity, which contributed to the clearance of virus-infected cells. These cytokine results suggest that vaccination with TetraME elicited Th1/Th2 mixed immune responses to DENV1–4.

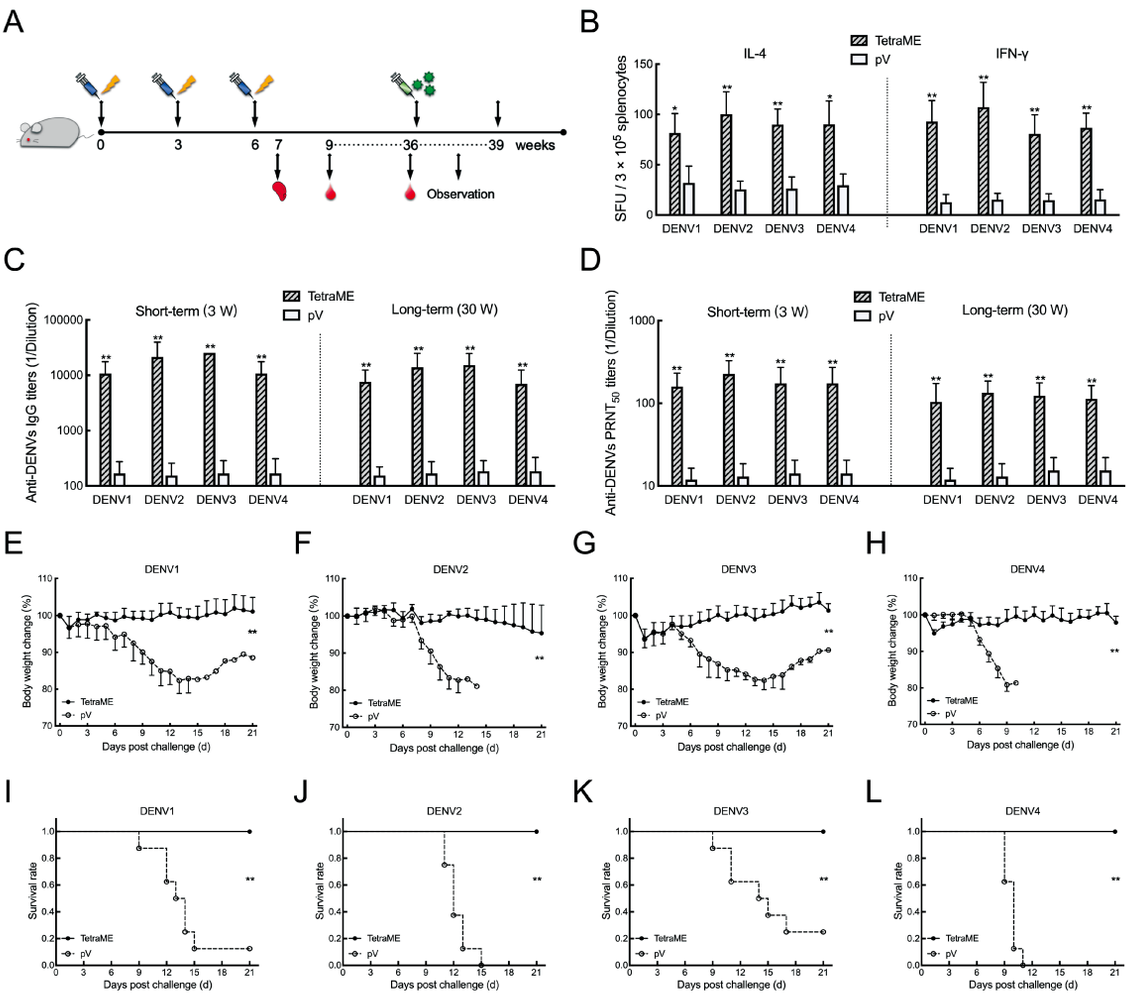

Figure 1 Tetravalent dengue DNA vaccine candidate (TetraME) induces cytokine and short- and long-term humoral immune responses and provides protection against four serotypes of DENV in BALB/c mice

A: Mouse experimental workflow. Groups of mice were immunized by intramuscular electroporation with 50 μg of either monovalent dengue DNA vaccine candidate or vector (pV) in each limb individually and were boosted twice at three-week intervals. Splenocytes were obtained one week after final immunization, and sera were collected three and 30 weeks after final immunization, respectively. Subsequently, vaccinated mice were challenged with 1×10^6 PFU of DENV1, 200 PFU of DENV2, 1×10^6 PFU of DENV3, or 1×10^5 PFU of DENV4. Body weight changes and survival rates were observed for 21 consecutive days after challenge. B: Splenocyte-secreted IL-4 and IFN-γ upon DENV1-4 antigen stimulation. SFU: Spot forming unit. Short- and long-term IgG antibodies (C) and nAb titers (D) against DENV1-4 in sera of immunized mice. Body weight changes (E–H) and survival rates (I–L) were monitored daily for 21 consecutive days. Mice exhibiting more than 20% loss in weight were humanely euthanized for ethical reasons. n=8, results are expressed as means±SD. *: P<0.05; **: P<0.01.

To detect short- and long-term DENV-specific IgG antibodies, enzyme-linked immunosorbent assays was used three- and 30-weeks post-vaccination, as described previously (Wang et al., 2018). The TetraME vaccination generated high levels of tetravalent IgG antibodies three weeks after the last immunization. The IgG antibody titers towards DENV1, DENV2, DENV3, and DENV4 were 1:10 763, 1: 21 527, 1: 25 600, and 1: 10 763, respectively, and were significantly different from their corresponding controls (Figure 1C, P<0.01). Notably, the IgG antibodies in the sera of immunized mice remained elevated with titers of 1: 7 610, 1: 13 959, 1:15 222, and 1:6 979, respectively, at 30-weeks post-vaccination, indicating a decreasing trend with time but relatively long-term immunogenicity.

Generally, the level of DENV-specific neutralizing antibodies (nAbs) can be used as a predictor of protective immunity. Thus, short- and long-term anti-DENV nAb titers in the sera of immunized mice were measured using a plaque reduction neutralizing test, as reported previously (Wang et al., 2019). The viral strains were DENV1 strain Hawaii, DENV2 strain Tr1751, DENV3 strain H87, and DENV4 strain H241, respectively. Over the short-term, sera from TetraME-vaccinated mice displayed broad neutralizing potency against heterologous DENVs. The nAb titers against DENV1–4 were 1: 160, 1: 226, 1: 174, and 1: 174, respectively, which were greater than their corresponding controls (Figure 1D, P<0.01). Similar to the dynamic changes in IgG titers, at 30 weeks after the last immunization, the nAb titers in the sera of the TetraME groups were 1: 104, 1: 135, 1: 123, and 1: 113, respectively, which were lower than their short-term titers. However, an anti-DENV nAb titer of 1: 10 implies protection against challenge (Zhang et al., 2015). Thus, these data indicate that the three EP doses of TetraME induced potent and long-term antibody responses with strong neutralizing activity.

Notwithstanding, the inevitable waning of nAb with time following TetraME vaccination raises concerns about whether the protective efficacy of the vaccine can persist long enough. Therefore, we performed DENV challenge experiments at week 30 after the last vaccination. Mice were challenged intracerebrally with either DENV1 (strain Hawaii) at a dose of 1×10^6 plaque-forming units (PFU), DENV2 (strain Tr 1751) at a dose of 200 PFU, DENV3 (strain H87) at a dose of 1×10^6 PFU, or DENV4 (strain H241) at a dose of 1×10^5 PFU. Each experiment was independently repeated three times. All TetraME-vaccinated mice showed only slight body weight losses, ranging from 3.4%–6.3% (Figure 1E–H, P<0.01) and survived the challenge with different DENV serotypes. In contrast, mice in the pV groups showed obvious body weight losses, ranging from 17.4%–19.2% after DENV challenge and all died, except for DENV3 group mice, which showed a 25% survival (2/8). This indicated that vaccination of mice with TetraME was sufficient to induce prolonged protective immunity against lethal challenge.

Over the last decade, progress with DNA vaccines has lagged behind that of others due to their limited immunogenicity (Kudlacek & Metz, 2019). To address these concerns, EP combined with multiple immunization strategies has been applied, resulting in dramatically improved immunogenicity and sustained expression of antigen-encoding DNA vaccines (Li & Petrovsky, 2016; Sheng et al., 2016). Here, our dengue DNA vaccine candidate (TetraME) generated robust DENV-specific cellular and humoral immune responses as well as long-term protective efficacy against four DENV serotypes in mice, which is beneficial in several aspects: firstly, DNA vaccination by intramuscular EP can generate significant antibody responses that persist for at least half a year (Babiuk et al., 2007) as well as antigen-specific T cell responses that persist for 40–60 weeks (Davis et al., 1995; Gurunathan et al., 1998); secondly, cross-protection among serotypes can be induced by monovalent vaccines. For example, infection or immunization with one DENV serotype can confer substantial cross-protection against heterologous serotypes for an average duration of two years (Reich et al., 2013), consistent with our results. However, the characteristics of cross-immunity for each monovalent DNA component to other serotypes of DENV warrant further in-depth investigation. In the current study, we used immunocompetent BALB/c mice vaccinated with four monovalent prM/E-based DNA vaccine candidates (TetraME) and verified their long-term tetravalent protection up to 30-weeks post-vaccination. These data should provide a basis for further development and testing of this vaccine formulation in larger animals in combination with EP delivery.

In conclusion, we determined the duration and protection of resulting tetravalent antibodies by vaccinating mice with a tetravalent dengue DNA vaccine. For the first time, we demonstrated that DENV-specific nAb titers remained relatively constant and conferred full protection for up to 30 weeks after immunization. Thus, this study provides promising data for the further development of tetravalent DNA vaccines against dengue.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

R. W. designed and performed the experiments, analysed the data, and wrote the manuscript; F. J. Y. analyzed the data and reviewed the manuscript; X. Y. Z. and X. Z. L. helped with the experiments; D. Y. F. contributed reagents and materials; H. C. designed the research; J. A. principally designed the experiments, revised the manuscript, and directed the project. All authors read and approved the final version of the manuscript.

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