Development of Monovalent and Tetravalent Dengue Virus Vaccine Candidates Using Silkworm Expression System

学 位 論 文 要 約

Summary of Doctoral Thesis

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論文題目：

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Summary：Dengue is an arboviral disease, which threatens almost half the global population, and has emerged as the most significant of current global public health challenges. In this study, we prepared dengue virus-like particles (DENV-LPs) consisting of Capsid-Premembrane-Envelope (CprME) and Premembrane-Envelope (prME) polypeptides and envelope region from serotype 1, 2, 3, and 4, which were expressed in the silkworms using *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV) bacmid. CprMEs, prMEs, 2E, E1, E2, E3, and E4 were expressed in hemolymph, respectively, and the molecular weight of the purified proteins was 55 kDa. For the tetravalent DENV-LP formation, the mixed bacmids of CprME and mixed bacmids of PrME were co-expressed in hemolymph and have molecular weight of the purified proteins of 55 kDa of E protein (Fig. 1). 3CprME and 3prME expressed in the fat body due to removing capsid-anchor and had molecular weights of 85 and 75 kDa, respectively. The purified polypeptides formed spherical Dengue virus-like particles serotype 1 to 4 (DENV-LPs/CprMEs and /prMEs) with approximately 30–55 nm diameter (Fig. 2). TEM and IEM images revealed icosahedral shapes and antigens to the surface of a lipid bilayer of DENV-LPs (Fig. 3). The heparin-binding ELISA assay shows a positive relationship between absorbance and the quantity of EDIII (Fig. 4), which was supported by the ITC assay, showing a moderate binding affinity between heparin and DENV-LP. It also shows that the CPrME construct has a higher affinity to heparin, suggesting more spontaneous binding than the prME construct (Tabel 1) (Fig. 5). The high correlation between patient sera and DENV-LP reactivities revealed that these DENV-LPs shared similar epitopes with the natural dengue virus (Fig. 6). IgG elicitation studies in mice have demonstrated that DENV-LPs/CPrME1–4 elicit a stronger immune response than DENV-LPs/prME1–4 and DENV-LP/2E, which lend credence to this claim (Fig. 7). In terms of neutralization assay, prME constructs allow better protection to the vero cells against the DENV-2 NGC infection compared to CprME. Anti-2PrME adjuvanted and Anti-prME1–4 unadjuvanted

provide a similar 40% protection level compared to the other observed immunized groups (Figs. 8 and 9).

Table 1. The binding affinity of tetravalent DENV-LPs towards heparin

Construct	Sero-type	K_D (M)	K_a (M^{-1})	N	ΔG (kcal mol^{-1})	Ligand	Host	Method	Ref.
CprME	1–4	168.4×10^{-9}	5.94×10^{6}	0.926	–9.68	Heparin	*B. mori*	ITC	This study
	1–4	751.9×10^{-9}	1.33×10^{6}	0.786	–8.36	Heparin	*B. mori*	ITC	This study
E subunit protein	2	5.0×10^{-9}	-	-	-	*O*-sulfated heparin	*E. coli*	SPR	(Marks et al. 2001)
	2	5.0×10^{-9}	-	-	-	*N*- and *O*-sulfated heparin	*E. coli*	SPR	(Marks et al. 2001)
	2	15.0×10^{-9}	-	-	-	Heparin	COS-7	ITC	(Chen et al. 1997)
ZIKV-E		433.0×10^{-9}	-	-	-	Heparin	*E. coli*	SPR	(Kim et al. 2017)

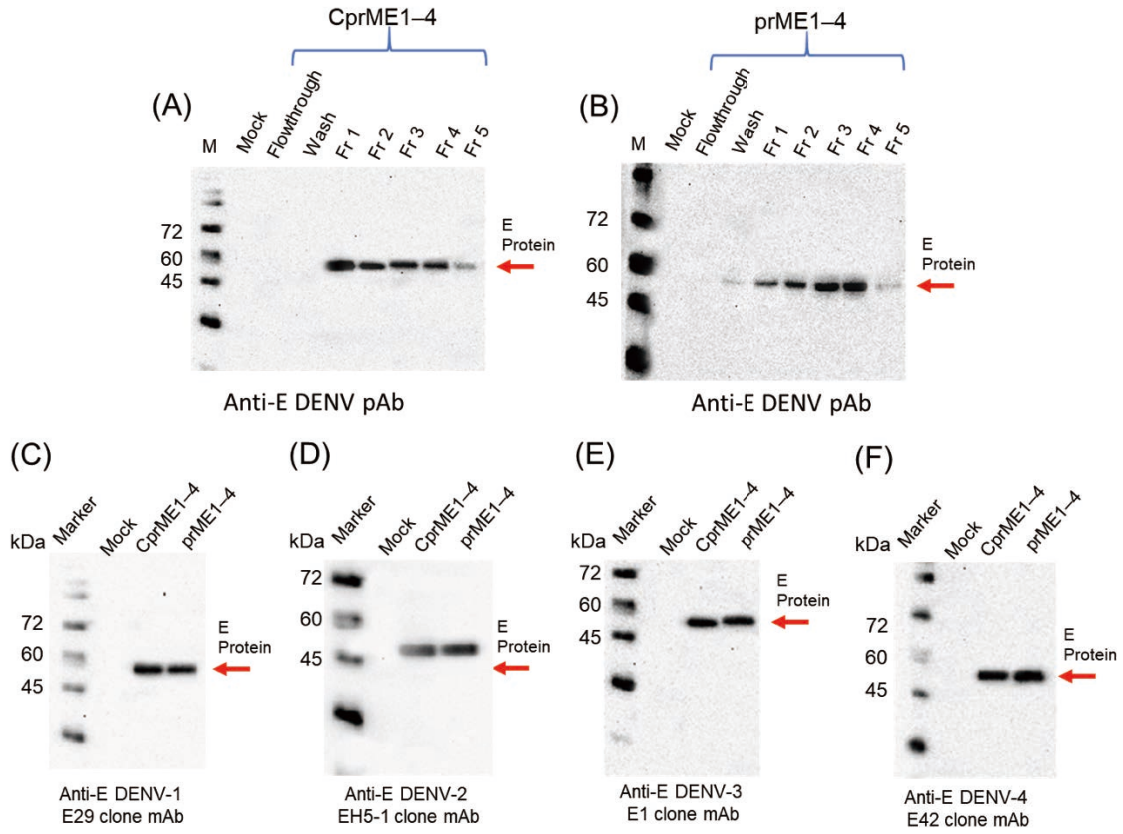

Fig. 1. Western blot of purified (A) CprME1–4 and (B) prME1–4 polypeptides. Each protein was purified from silkworm hemolymph using DDDDK-tagged protein magnetic purification. E protein was verified using polyclonal antibody anti-E-DENV. The E protein was verified using specific monoclonal antibody anti-E-DENV-1 E29 clone (C), anti-E DENV-2 3H5-1 clone (D), anti-E DENV-3 E1 clone (E), and anti-E-DENV-4 E42 clone (F).

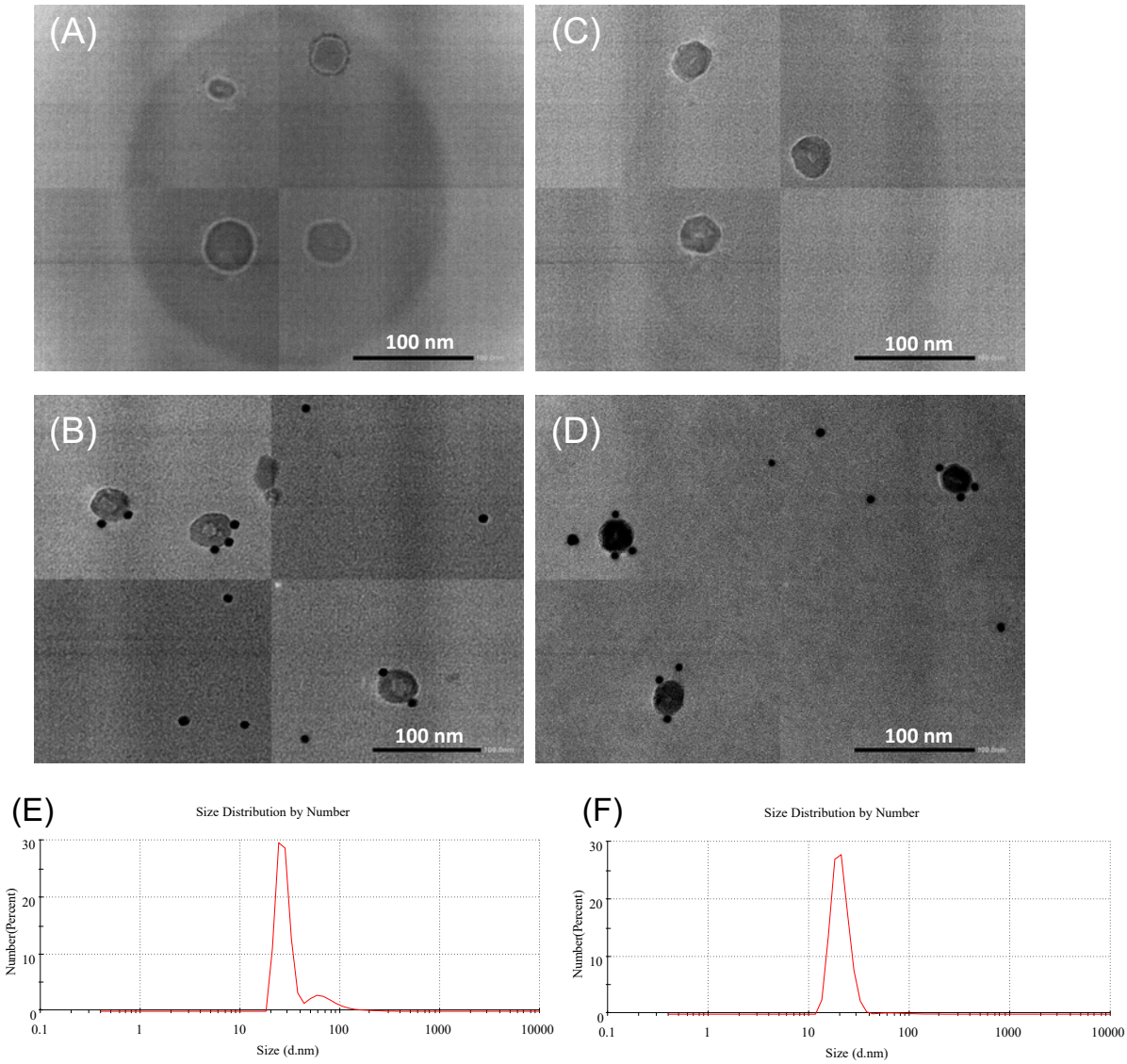

Fig. 2. TEM analysis of purified CprME1–4 (A) and prME1–4 (C) polypeptides. The purified CprME1–4 (B) and prME1–4 (D) polypeptides were immunogold-labeled using an anti-E polyclonal antibody and analyzed via IEM. Black spots in b and d indicate immunogold particles. Dynamic light scattering (DLS) was used to analyze the size distributions of CprME1–4 (E) and prME1–4 (F) polypeptides.

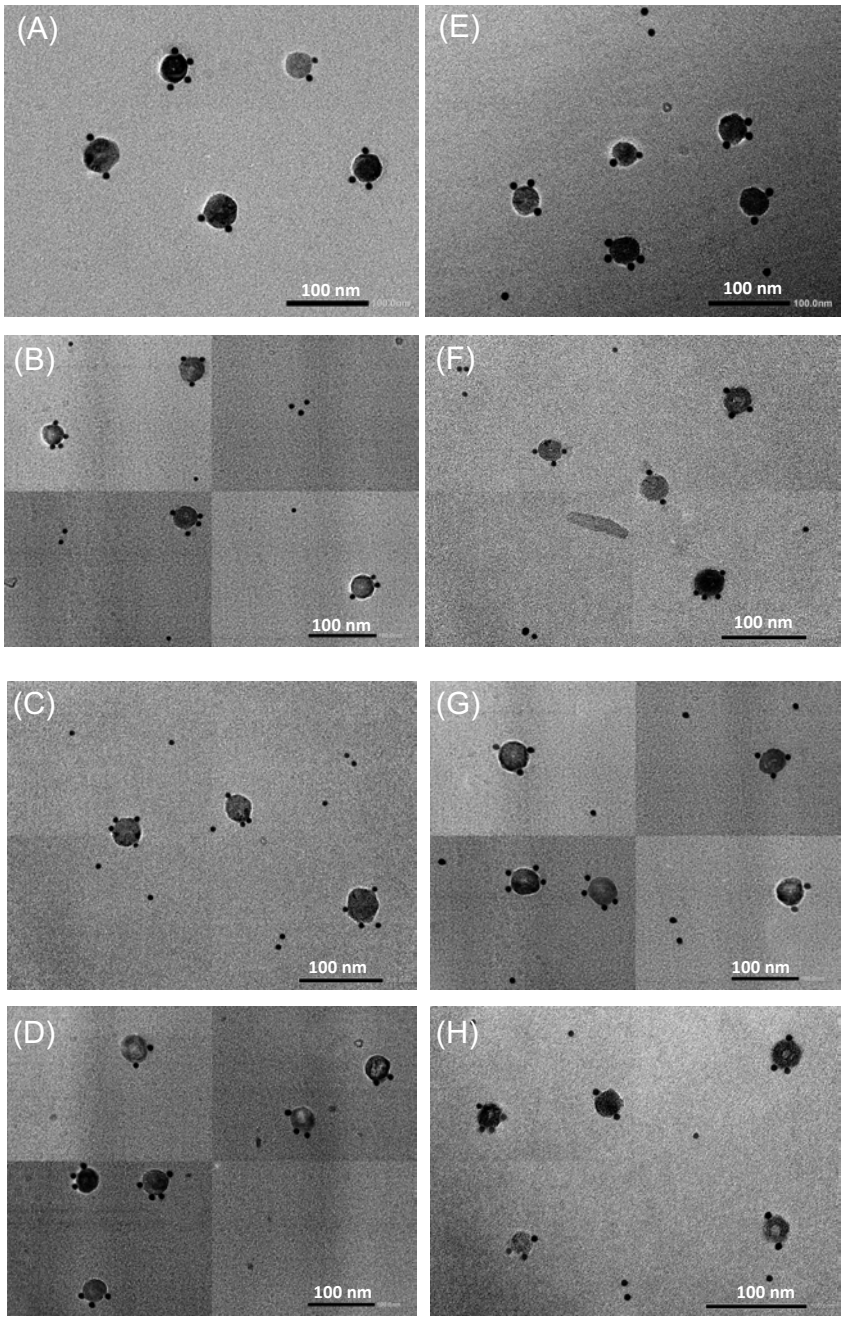

Fig. 3. DENV-LP/CprME1–4 and /prME1–4 was immunogold-labeled using anti-E-DENV-1 E29 clone (A, B), anti-E DENV-2 3H5-1 clone (C, D), anti-E DENV-3 E1 clone (E, F), and anti-E-DENV-4 E42 clone (G, H) and analyzed via IEM. Black spots in b and d indicate immunogold particles.

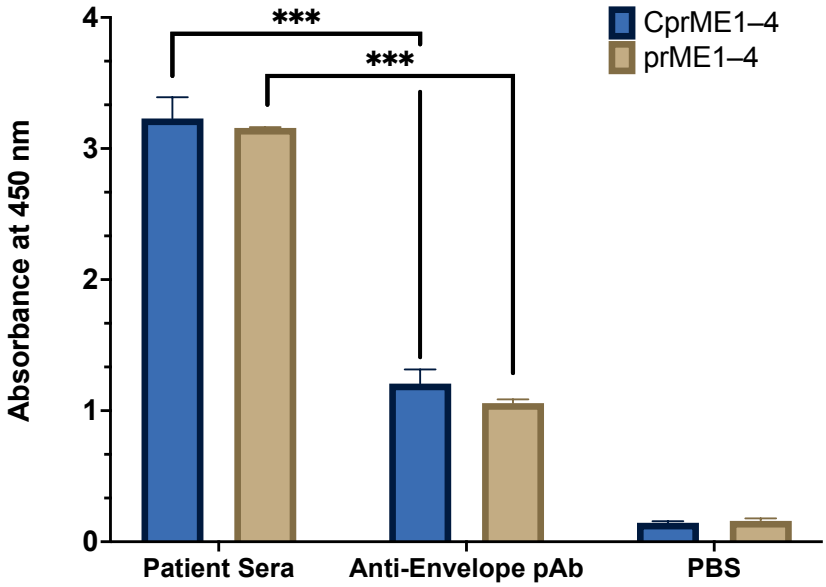

Fig. 4. The interactions between DENV-LP/CprME1–4, and /prME1–4 with mixed sera from dengue patients [rapid diagnostic test NS1(+)] were investigated (Welch t-test, *p<0.05, **p<0.01, ***p<0.001).

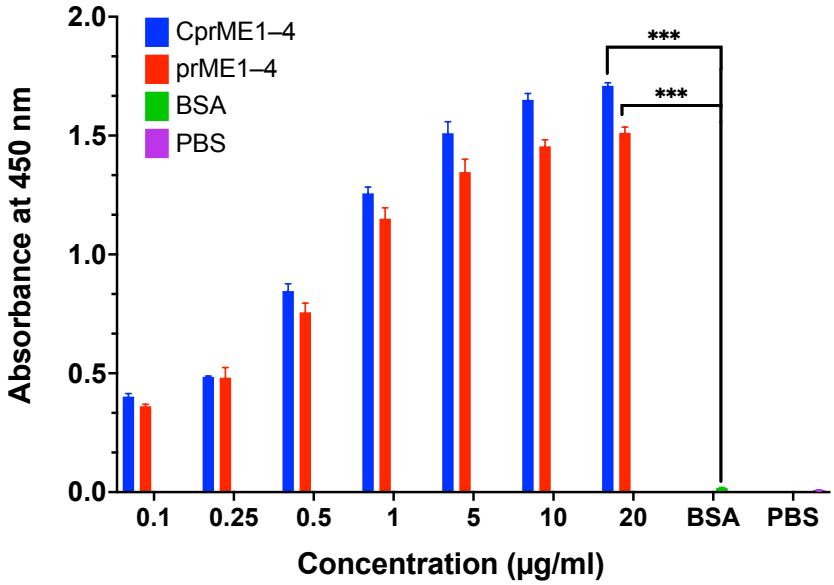

Fig. 5. Binding assay of DENV-LP/CprME1–4, and /prME1–4 to heparin using ELISA. Heparin (1.8 ng) was coated onto each well of an ELISA plate, each amount of purified protein was used for this ELISA, and 2 μg/ml BSA was used as a control which was carried out according to the protocol described in the Materials and methods (Welch t-test, *p<0.05, **p<0.01, ***p<0.001).

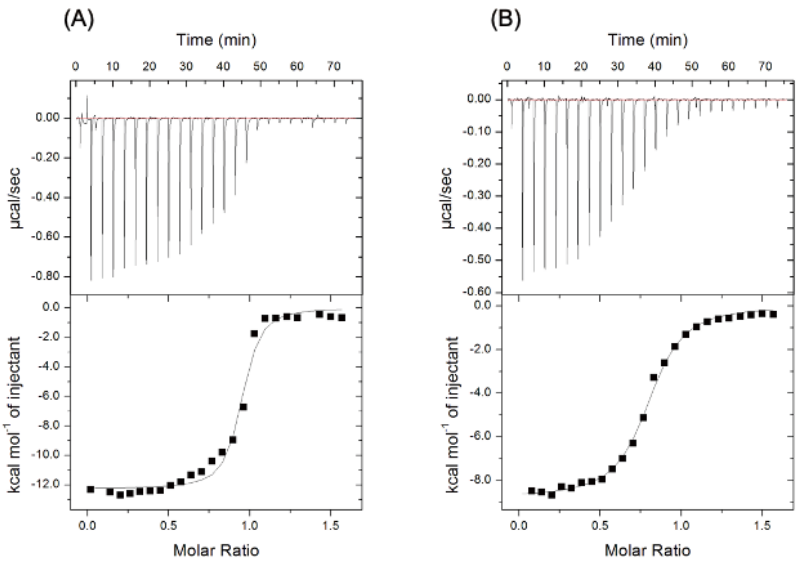

Fig. 6. The binding activities between DENV-LP/CprME1–4 (A), and /prME1–4 (B) toward heparin were examined by ITC.

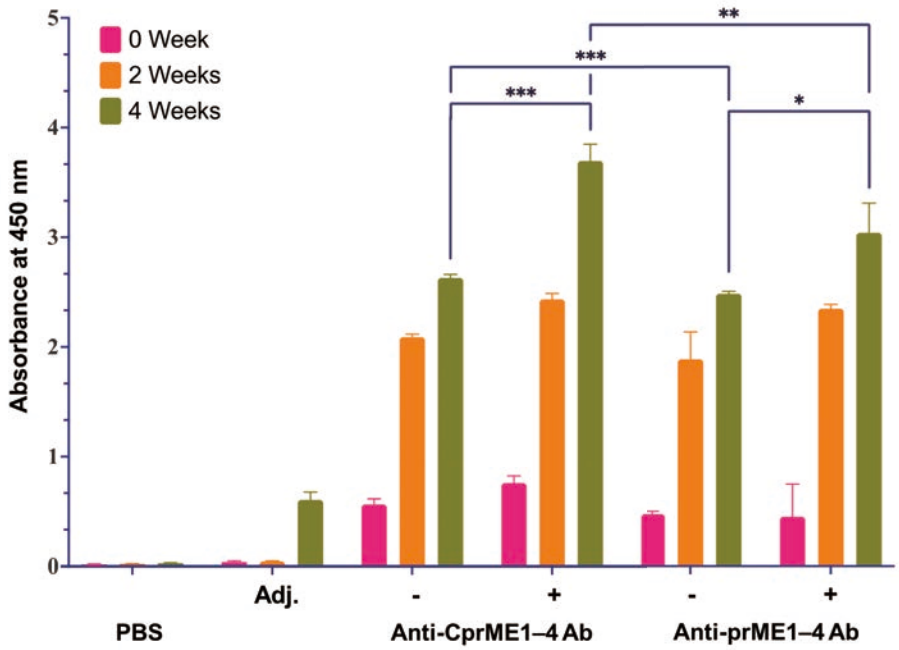

Fig. 7. Binding reactions were analyzed using direct ELISA as described. Specific IgG generation by DENV-LP/CprME1–4, and /prME1–4. BALB/c mice were intraperitoneally immunized with 50 µg of monovalent DENV-LP/CprME1–4 and /prME1–4. At 0, 2, and 4 weeks, sera were collected and used to test for binding of the specific IgG (Welch t-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$).

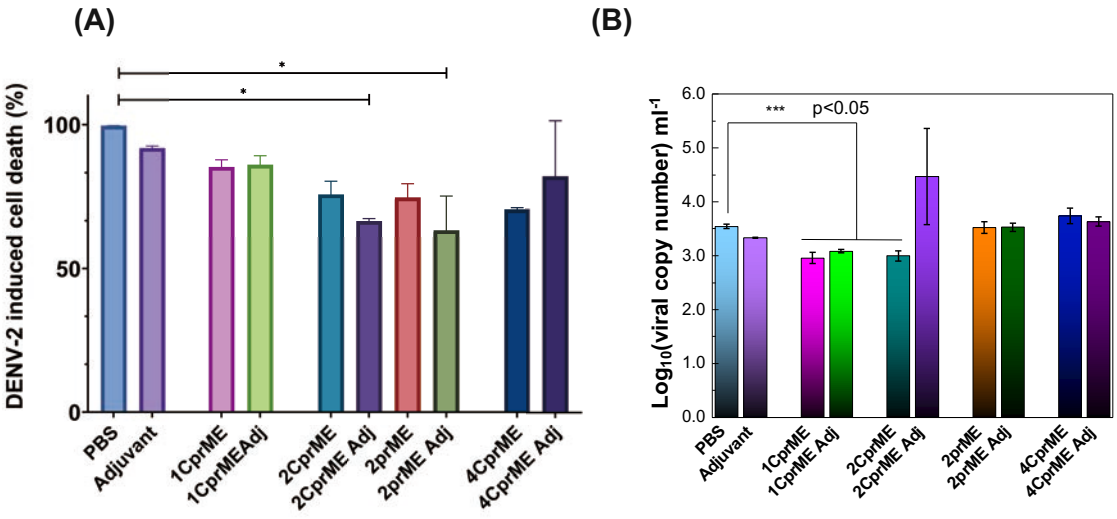

Fig. 8. MTT (A) and qPCR (B) assay in virus neutralization. Four-fold mice sera were used against 300 pfu/ml DENV-2 NGC (Welch t-test, *p < 0.05, ***p < 0.01).

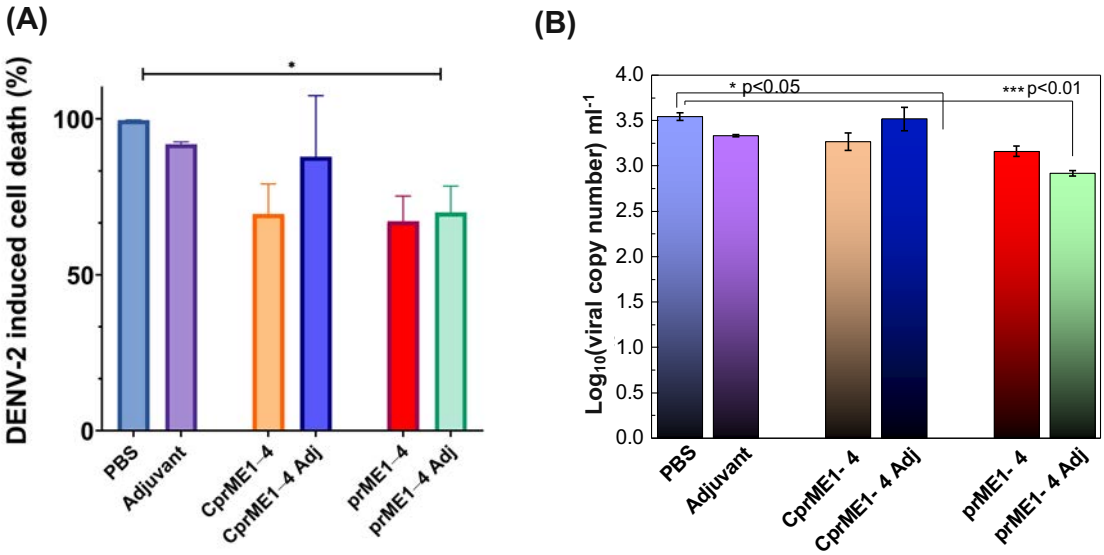

Fig. 9. MTT (A) and qPCR (B) assays in the virus neutralization of DENV-LPs tetravalent. Four-fold diluted mice sera induced by DENV-LPs tetravalent were used for neuralization against 300 pfu/ml DENV-2 NGC (Welch t-test, *p < 0.05, ***p < 0.01).