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

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：カイコ発現系を用いた１価および４価デングウイルスワクチン候補の開発

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論文要旨：

Dengue fever is one of the fastest spreading vector-borne diseases at risk of dengue infection, with 3.9 billion people in 128 countries worldwide. Tropical and subtropical afflicted areas are solicitous for a vaccine against Dengue virus (DENV) to minimize damage from the infection of DENV. 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 (BmNPV) bacmid. After transformation to *Escherichia coli*, BmNPV/CPrMEs, /PrMEs, and 2E bacmids were obtained. For the tetravalent DENV-LP formation, the mixed bacmids of CprME and mixed bacmids of PrME were coexpressed in silkworm larvae. After the bacmids were injected into 5th instar silkworm, silkworm larval hemolymph and fat body were collected 5 dpi. CprMEs, prMEs, 2E, CprME1–4, prME1–4, subunit protein E1, E2, E3, and E4 expressed proteins in hemolymph, respectively, and the purified proteins' molecular weight was 55 kDa. 3CprME and 3prME expressed in fat body, due to the removing of capsid-anchor, and had molecular weights of 85 and 75 kDa, respectively. The purified polypeptides formed spherical DENV-LPs with approximately 30–55 nm in diameter. Transmission electronic microscopy (TEM) and immune electronic microscopy (IEM) images revealed icosahedral shapes, and antigens were displayed on the surface of a lipid bilayer of DENV-LPs. The heparin-binding ELISA assay shows a positive relationship between absorbance

and the quantity of EDIII, 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 and more spontaneous binding than the prME construct and suggests that the reaction can induce to immunize the host cell in the body. The high correlation between patient sera and DENV-LP reactivities revealed that these DENV-LPs shared similar epitopes with the natural dengue virus. IgG elicitation studies in mice have demonstrated that DENV-LP/CPrMEs elicit a stronger immune response than DENV-LP/prMEs and DENV-LP/2E. The mice sera immunized by monovalent and tetravalent DENV-LPs have protection capability against DENV-2 NGC.