

## **Immunological analysis of Nodavirus capsid displaying the domain III of Japanese Encephalitis Virus envelope protein**

### **ABSTRACT**

Japanese encephalitis virus (JEV) is the pathogen that causes Japanese encephalitis (JE) in humans and horses. Lethality of the virus was reported to be between 20–30%, of which, 30–50% of the JE survivors develop neurological and psychiatric sequelae. Attributed to the low effectiveness of current therapeutic approaches against JEV, vaccination remains the only effective approach to prevent the viral infection. Currently, live-attenuated and chimeric-live vaccines are widely used worldwide but these vaccines pose a risk of virulence restoration. Therefore, continuing development of JE vaccines with higher safety profiles and better protective efficacies is urgently needed. In this study, the *Macrobrachium rosenbergii* nodavirus (MrNV) capsid protein (CP) fused with the domain III of JEV envelope protein (JEV-DIII) was produced in *Escherichia coli*. The fusion protein (MrNV-CPJEV-DIII) assembled into virus-like particles (VLPs) with a diameter of approximately 18 nm. The BALB/c mice injected with the VLPs alone or in the presence of alum successfully elicited the production of anti-JEV-DIII antibody, with titers significantly higher than that in mice immunized with IMOJEV, a commercially available vaccine. Immunophenotyping showed that the MrNV-CPJEV-DIII supplemented with alum triggered proliferation of cytotoxic T-lymphocytes, macrophages, and natural killer (NK) cells. Additionally, cytokine profiles of the immunized mice revealed activities of cytotoxic T-lymphocytes, macrophages, and NK cells, indicating the activation of adaptive cellular and innate immune responses mediated by MrNV-CPJEV-DIII VLPs. Induction of innate, humoral, and cellular immune responses by the MrNV-CPJEV-DIII VLPs suggest that the chimeric protein is a promising JEV vaccine candidate.

**Keyword:** Japanese encephalitis vaccine; *Macrobrachium rosenbergii* nodavirus; Virus-like particles (VLP); Domain III; Cytokines; Cytotoxic T-lymphocytes