DNA-launched sindbis virus based replicon encoding the yellow fever virus ED-III protein

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Background: Yellow fever virus (YFV) is a positive sense single-stranded RNA virus that is transmitted by mosquitoes and is endemic in the tropical regions of Africa and South America. The World Health Organization has estimated the annual prevalence of yellow fever to be 200,000 cases, of which more than 90% is attributed to Africa. Although highly efficacious live attenuated vaccines are available, use of the vaccines in immunocompromised individuals are contra-indicated and, although rare, vaccine-associated viscerotropic adverse events have been reported. Hence development of a safer alternative that can complement the use of the live vaccines would have application. The aim of this study was to prepare a DNA-launched Sindbis based replicon encoding the ED-III protein of YFV and to characterize the expression of the ED-III protein in transfected cells prior to evaluation as a candidate vaccine in an animal model.

Methods & Materials: The gene encoding wild-type Asibi strain YFV ED-III protein was codon optimized and synthesized by GenScript. The gene was cloned into a DNA based expression system carrying the Sindbis genome and designated pSinED-III. The pSinED-III replicon was purified and utilized for the transfection of BHK21 cells. A similarly constructed DNA launched Sindbis replicon expressing a reporter gene, green fluorescent protein, was used as a transfection control. Transfected BHK cells were assayed 24 hours post-transfection for the expression of the ED-III protein using an indirect immunofluorescence assay (IFA). Anti-his 6 mouse monoclonal antibodies were used to detect the C-terminal histidine tag. In addition, sera obtained from mice immunized with bacterially expressed ED-III, and shown to have an IgG antibody response to ED-III, were used to detect ED-III protein in transfected cells.

Results: The presence of the gene encoding the ED-III protein in the construct was confirmed by PCR and sequencing using primers flanking the cloning site. The detection of the C-terminal histidine tag and the ED-III protein in an indirect IFA confirmed the expression of YFV ED-III protein in mammalian cells.

Conclusion: IFA confirmed the expression of ED-III protein from pSinED-III. Therefore the immunogenicity of the DNA-launched Sindbis virus based replicon will be evaluated using mice.

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