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Construction and expression of DENV-2 NS2A protein

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DENV annually causes about 390 million human infections, leading to 96 million cases with manifest symptoms. The genome of flavivirus is a single-strand, positive-sense RNA of 11 kb in length. The ORF encodes a polyprotein which is co- or post-transnationally processed into three structural proteins (capsid [C], premembrane [prM], and envelope [E]) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) . Due to their high hydrophobicity, the three membrane proteins NS2A, NS4A, and NS4B remain poorly characterized. Dengue Virus-2 nonstructural protein 2A (NS2A) contains five transmembrane segments and is essential for viral replication and assembly and antagonizes the host immune response. Besides this, NS2A colocalizes with dsRNA, NS1, NS3, and NS5 within the replication complex.However several questions pertaining to the function of NS2A in modulating the host cell machinery is still elusive. Therefore to understand the function of this potential protein NS2A in host cell modulation, NS2A gene was PCR amplified, cloned in PET-151/D-TOPO vector. Further the cloned vector is transformed in BL21 star cells and subsequently protein is IPTG (1mM, 4hrs) induced and purified for the analysis. Afterwards, NS2A purified protein was used to immunize Wistar strain rats to obtain polyclonal antibody which would serve best to analyze several functions of NS2A including its localization within the host cell and its interaction with the host's proteins. In this context, rats were immunizedintraperitoneallywith 250ug of recombinant NS2A protein. Protein were mixed with fruend complete and incomplete adjuvant. Immunization scheme consisted in four doses at intervals of 15 days. The animal serum was obtained at each immunization and this was assessed by ELISA, immunofluorescence. Considerable degree of antibody protein NS2A was observed. Further these preliminary results will help unravel the hidden functions of NS2A, which promotes pathogenesis of dengue virus.

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