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Poster presentation

Expression and characterization of recombinant Japanese encephalitis virus NS1 protein in Drosophila S2 cell

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Background

Japanese encephalitis virus (JEV) is a member of the genus Flavivirus, family *Flaviviridae*. Flavivirus NS1 glycoproteins are essential proteins, which exhibit a high degree of sequence homology. The NS1 protein is secreted from infected cells as a soluble hexamer and can induce protective immune response in mice against lethal encephalitis. However, its role in neuroinvasion of JEV remains unknown. Our aim is to identify the role of NS1 protein in JEV neuropathogenesis. In this study, we have produced recombinant JEV NS1 protein in Drosophila S2 cells and have characterized its biochemical, biological and antigenic properties.

Results

The full-length and N- and C-terminal fragments of NS1 protein of JEV Nakayama strain were amplified from cDNA and inserted into a pMT vector. These plasmids were transfected into S2 cell. Limit dilution and cell cloning were carried out to subclone NS1 S2 cells to generate stable cell lines expressing NS1. Ni-NTA and size exclusion FPLC column were used to purify the secreted proteins. S2 cell expression system provided a high amount of purified full-length protein (1L supernatant yielded 2~5 mg of protein with purity over 90%). We observed one form of ~300 kD of NS1 by size exclusion column, corresponding to the hexameric form of full-length NS1. Digestion by Endo H and PNGase F generated a 2~4 kD decrease of NS1 molecular weight. Study of NS1 binding to different lectines demonstrated the highly mannose and hybrid glycosylation forms of NS1 N-glycans. Purified proteins were used to vaccinate C3H mice and showed high degree of protection against a challenge of 100 LD50 of JEV. MAbs against NS1 were produced and their specificity in Flavivirus genus was characterized.

Conclusion

These studies contribute to better characterize NS1 protein produced in S2 cells and to understand its role in neuropathogenesis.

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