CHAPTER 7

SALIENT FEATURES OF THE WORK AND FUTURE PROSPECTS

SALIENT FEATURES

(1) Induction of the E.coli pATH NS1-2A expression system with indole- acrylic acid for 8 hrs was found to be optimum for maximum expression of the recombinant protein.

(2) The NS1 protein was found to be associated with the membrane surface of infected cells as determined by surface immunofluorescence and Triton X-114 phase partitioning.

(3) Dimeric NS1 protein appeared to be the predominant species found in JEV *infected cells.*

(4) Dimers were found to be resistant to SDS, 2-mercaptoethanol, Gu- HCl, urea, temperature upto 55°C, neutral and alkaline pH. This suggested an involvement of bonds other than hydrogen-hydrogen and disulphide bridges.

(5) In addition to dimers, NS1 was also found to exist in an oligomeric form. This may probably be important for transport of the NS1 protein from the endoplasmic reticulum to the cell surface.

(6) Glycosylation pattern suggested the presence of mannose-rich oligosaccharides but not complex-fucose type of glycans on the intracellular NS1 and NS1' protein.

(7) Anti-NS1 polyclonal and monoclonal antibodies did not exhibit haemagglutination inhibition, complement fixing and virus neutralizing activities.

(8) Although the recombinant NS1 protein lacked the N-terminus 59 residues it was able to confer partial protection in mice.

(9) Epitope mapping of the rNS1-2A protein with MAbs by the additivity index method revealed two distinct domains on the recombinant protein.

FUTURE PROSPECTS

The current study has given us an insight into some of the properties of the NS1 protein of JE virus. The significance of glycosylation of a non-structural protein and its dimerization are important queries yet to be resolved. The following questions addressed for future studies will help in a better understanding of the importance of cell-mediated immunity (CMI) in NS1 mediated protection and to also identify and ascribe biological function(s) to NS1protein.

(i) To compare the relative protective potentials of native and full-length NS1/NS1-2A recombinants expressed in vaccinia or baculovirus expression systems.

(ii) Traditional efforts at development of attenuated live JE virus vaccines have not been entirely successful to date. Subunit vaccines might provide an acceptable alternative. Inclusion of biologically active immunodominant T helper cell reactive epitopes on the NS1 protein may help in improving the efficacy of the subunit vaccines.

(iii) Virus specific cytotoxic T lymphocytes (CTLS) have been reported to play an important role in recovery from certain virus infections. Efforts should therefore be directed to investigate whether primary (*in vivo*) and secondary (*in vitro*) CTLS are generated against the NS1 protein in natural human infections or in infected mice.

(iv) In order to understand the role of NS1 protein in infection, nucleotide substitution(s) can be introduced in the NS1 encoded region of an infectious clone using site-directed mutagenesis and its effect on viral pathogenesis can be studied.