PREFACE

Viruses form one of the major causative agents for diseases in humans, more so in children. Japanese encephalitis virus is one such virus that is predominant in many parts of Asia causing pediatric mortality and morbidity. Currently the widely used vaccine against this virus is mouse brain derived. There is a need to develop a more effective vaccine against this virus. In the present study, attempts were made to dissect out the epitopes from an immunodominant protein of the virus. Envelope glycoprotein is the protein that plays a central role at pathological and immunological level. Hence, most of the research in vaccine development against this virus has focused on this protein. The basic aim of this study was to analyze epitopes that would be immunogenic against the virus. These epitopes could be then incorporated in peptide based vaccine against the virus.

This thesis is divided into three basic studies in the form of chapter 2, 3 and 4. Generally, each chapter begins with an introduction and brief aim of the chapter. It is followed by results and the same are discussed immediately, though at certain places the comprehensive results are discussed. Every chapter carries its own references; hence, every chapter is a complete unit by itself. There are two appendices that mention the abbreviations and the protocols used.

The first chapter deals with general introduction to viral immunology and Japanese encephalitis virus with some mention about flaviviruses. The fields of viral immunology and flavivirolgy are too large and beyond the scope of this thesis. However, an attempt has been made to incorporate relevant findings from the literature in the introduction.

Chapter two describes the first part of the work that deals with prediction and analysis of B cell epitopes. B cell epitopes were identified from the sequence of the protein using more than one computer sustained algorithms. It was shown earlier, in our laboratory that potential epitopes could be predicted using computer algorithms, and their functional capability ascertained (**Kutubuddin *et al*, 1993, 1991**). Since they were theoretical outputs, an empirical proof of immunogenicity and antigenicity was required. This was done by synthesizing the peptides that represent the epitopes and analyzing them for immunogenicity and antigenicity. After the immune response was experimentally proven the region was further structural studies were carried out. These were carried out by reactivity of overlapping peptides representing the region. The peptide determinant in question was extended both at the N & C termini. Reactivity was assayed by binding studies between these overlapping peptides and monoclonal antibodies raised previously against the virus. The studies also incorporated variations in binding of the peptide to the surface and varying reacting conditions. The studies helped us in arriving at a state that was optimally reactive. Molecular modeling studies were also done in tandem in order to get insights about the structures.

This is followed by raising monoclonal antibodies against the antigenic determinant and subsequent characterization (Chapter three). It was essential to raise monoclonal antibodies to ascertain certain results that were obtained in the initial part of the study. Also, monoclonal antibodies are excellent immunological reagents. The answers sought by this study would help us in confirming whether the epitope was specific and virus neutralizing. The antibodies were assayed for their reactivity by routine immunological and virological methods. Since the epitope, as per the prediction and homology, was a neutralizing epitope, virus neutralizability of the monoclonal antibody both *in vitro* and *in vivo* was checked. In addition, since the epitope belonged to a virus specific region, neutralizability of this MAb with a closely related virus strain of Japanese encephalitis virus was checked the.

The last part of the work (Chapter four) was one step closer to a vaccine candidate by synthesizing chimeric peptides containing both the analyzed B and T cell epitopes. The immune response against these chimeric peptides was checked by routine assays. To check the haplotype dependence on immunogenicity different strains of mice were inoculated. The immune response was characterized by immunological & virological assays. Finally, the immunized mice were challenged with lethal dose of virus in order to check the potential of chimeric peptide as a plausible vaccine candidate.

The final part of the thesis briefly looks at the achievements of the present study and the future of JEV vaccine as a summation of the thesis.

In short, this thesis has tried to address the certain basic question(s):

- How effective are predictions and does the predicted epitope generate immune response ?
- Do peptide immunogens representing the epitopes elicit immune response?
- Does the anti-peptide immune response cross react with the intact virion ?
- Do the chimeric peptides containing both T and B cell epitopes provide an immune response that is better than only B cell peptide ?
- If yes, then whether this response is comparable to the vaccine in terms of antibody response and protection?

REFERENCES

Kutubuddin M, Gore MM, Banerjee K, Gosh SN, Kolaskar (1993) Analysis of computer-predicted antibody inducing epitope on Japanese encephalitis virus. ***Acta Virol***; 37: 417

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