Chapter 2

Exposition

To the problem:
2.0 Exposition to the problem

Japanese encephalitis virus (JEV) (Genus: Flavivirus, Family: Flaviviridae) is the etiological agent of Japanese encephalitis, responsible for acute illness and death. It is transmitted to humans by mosquitoes leading to infection of the central nervous system and encephalitis. Besides humans, it causes encephalitis in horses and stillbirth or abortion in pigs. JEV has covered a vast geographic area of Asia and parts of Oceania (Hurk et al., 2009). It has become a worldwide public health problem because of spreading into new areas and potential for further expansion. Nearly half of the human population falls in countries where JEV occurs, globally 50,000 cases are reported with 15,000 mortality rate per year mainly in countries where no mass vaccination has been carried out (Solomon et al., 2008).

Vaccination appears to be a promising way of controlling such outbreaks. The available, formalin inactivated or live-attenuated vaccines (SA 14-14-2) have certain limitations, such as high cost of production, allergic reaction or reversion to virulence. Hence, there is a need for the development of safer, potent and cost-effective vaccine which can elicit both the arms of immune response, such as DNA vaccines. DNA vaccine is a circular plasmid including a gene encoding the target antigen under the control of a promoter. It is less costly to produce than peptide or protein vaccines, and is chemically stable under a variety of conditions. Recombinant genetic vaccine for JEV using different gene products have been tried, but the response was suboptimal. Therefore the present attention has been shifted towards the improvement of DNA vaccine modulated through several immunological adjuvants.

Successful immune response requires engagement of T cell receptor with MHC-peptide on professional Antigen presenting cell (APC) as first signal. Simultaneously, second signal in the form of various co-stimulatory molecule engagements is necessary for sustained immune response. Failure to have this second signal may lead to reduced immune response or even anergy.

In the conventional DNA vaccines the expression is controlled by a nonspecific (CMV) promoter making it possible to express the protein in all the cells that get transfected including that of nonprofessional APCs. Moreover, it can be argued that widespread expression of a gene through viral cytomegalovirus (CMV) promoter may lead to some adverse effect.

DC specific promoter has shown promising results, it also has some inconsistencies. A study has reported that targeting of DC was insufficient to optimally induce T cell immunity and the role of non-DC should be explored for sustained effector functions.
In another study, DC as one of the APCs, was targeted with the gene of interest, (Brocker et al., 1997). However, it was seen that exclusive targeting of DC during DNA vaccination to express the immunogen was insufficient to induce optimum T cell immunity. Hence the role of other professional APC (Macrophage and B-cells) as a target cell for DNA vaccine could not be ignored.

To further improve this approach and to develop strategies to improve DNA vaccines, antigen presentation in the form of targeted expression of desired antigen in professional APCs (Macrophages, Dendritic cell and B-cells) was explored. Therefore the approach used in this work was to target the antigen expression in professional APC (majorly macrophage) by using the promoters dominant in APC. Using JEV envelope protein as immunogen comparison in the form of protection from lethal challenge with JEV was attempted.

**Objective**

To study the cloning and expression of JE virus structural gene under the control of immune cell dominant promoters and study immune response and protection in-vivo when those recombinants are used as a plasmid vaccine.

The strategy adapted for the study was:

1) To select appropriate promoters from literature that would express dominantly in macrophage lineage cells.

2) To construct vectors with the selected promoters and study the expression profile using GFP reporter to evaluate the strongest promoter.

3) To modify the vector with JEV ‘E’ gene with the selected promoter and ubiquitous promoter.

4) To immunize mice with these vectors and undertake immunological studies including lethal challenge.