CHAPTER 7

SUMMARY & CONCLUSIONS
The present work entitled “Development of DNA vaccine for complete protection in pre- & post- exposure studies in murine model of rabies” involves cloning of glycoprotein gene of rabies virus in specialized DNA vaccine vectors. These vectors have been employed to achieve sub-cellular targeting of the encoded gene. The encoded glycoprotein is expected to be directed towards the MHC Class I and Class II pathway for improving its processing and presentation. The purpose of this targeting was to assess the generation of an ideal immune response for conferring protective immunity. The main findings of this work are as follows:

- The structural gene for glycoprotein of rabies virus was cloned in DNA vaccine plasmids bearing target sequences.
- The authenticity of DNA chimeras and ability of directing sequences to target the encoded antigen to desired sub-cellular locations was established by transfection followed by sub-cellular fractionation and immunoblotting.
- Vaccination of mice with all the DNA vaccine constructs led to the generation of anti-glycoprotein antibodies. All the modified (targeted) vaccines elicited higher antibody levels than the unmodified one.
- We found a strong IgG1 response in all the DNA constructs. Even though IgG2a antibodies were produced, the ratios of IgG1/IgG2a were consistently more than one, thus emphasizing on the Th2 bias.
- DNA chimeras induced potent rabies virus neutralizing antibody (RVNA) response and conferred protection upon intracerebral rabies virus challenge.
- pgp.LAMP-1 DNA vaccine comprising of glycoprotein with C-terminal lysosomal associated membrane protein (LAMP-1) signal sequence generated highest rabies virus neutralizing antibody titer in mice (16 IU/ml). However, it imparted partial protection (60 %) against challenge with 20 LD50 of rabies challenge virus standard (CVS).
- pgp.LAMP-1 DNA vaccine also generated high RVNA titers (8 IU/ml) in dogs.
- To improve outcome of vaccination, ultimately towards enhancement of the immune response; we investigated different routes for DNA vaccine delivery, varied doses of DNA and influence of adjuvant supplementation.
Three routes of delivery, namely, gene gun immunization, intramuscular injection and oral administration were assessed for conferring potent immune response against rabies. Intramuscular administration of rabies DNA vaccine was found to elicit strongest humoral immune response against rabies.

The optimum dosage via intramuscular injection was established as 100 μg.

Adjuvants - EMULSIGEN® and EMULSIGEN® D (ED) were tested for their immune augmentation potential. The adjuvant ED was found to enhance the magnitude of immune response elicited against the rabies DNA vaccine.

The highest immune response pertaining to IgG antibody titer, with a predominantly IgG1/IgG2a subclass distribution, effective cellular immunity and high level of RVNA titer was attained by the optimized DNA vaccine formulation comprising of intramuscular administration of 100 μg of DNA vaccine supplemented with EMULSIGEN®D.

In pre-exposure prophylaxis, three dose regimen of this formulation generated high RVNA titer (32 IU/ml) and conferred complete protection against challenge with 20 LD₅₀ of CVS.

For post-exposure efficacy analysis, rabies was experimentally induced with 50 LD₅₀ of CVS. Subsequent therapy with five doses of the formulation completely prevented rabies in BALB/c mice, which maintained protective RVNA titers of 4 IU/ml. WHO recommends rabies protective threshold titer as 0.5 IU/ml.

Enhancement of plasmid DNA production was attempted by fed batch culture, whereby 68.79 mg/l of pgp.LAMP-1 plasmid could be attained.

The prime endeavor of this work was to develop a highly potent, safe and cost effective DNA vaccine against rabies which would confer complete protection upon pre- and post-exposure prophylaxis. Thus, this optimized DNA vaccine formulation provides an avenue for preventing and controlling rabies.