CHAPTER 1

INTRODUCTION
Rabies, progressive fatal encephalitis [Rupprecht et al., 2002] is caused by rabies virus of genus Lyssavirus. It can infect all mammals. Majority of rabies cases reported from developed countries involve wild animals like raccoons, skunks, bats and foxes. However, it is a major health concern in developing countries, which account for more than 99% of all human deaths from rabies [WHO, 1998]. Exposure to rabid dogs is the cause of bulk (>99%) of human rabies deaths world-wide [Smith & Seidel, 1993]. The number of deaths from rabies worldwide is estimated to range from 40,000 to as high as 70,000 per year [WHO, 2001]. About 95% of them occur in Asia and Africa. India in particular, has severe problem, with as many as 30,000 human deaths and 2 million people requiring post-exposure vaccination yearly. Stray and community dogs cause vast majority of human cases. 30% to 60% of the victims of dog bites are children under the age of 15. Wound cleansing and immunizations, done as soon as possible after suspect contact with an infected animal and following WHO recommendations, can prevent the onset of rabies in virtually 100% of exposures. Once the signs and symptoms of rabies start to appear, there is no treatment and the disease is almost always fatal. Globally, the most cost-effective strategy for preventing rabies in humans is by eliminating rabies in dogs through animal vaccinations.

Though potent and safe cell culture derived inactivated vaccines are available, their efficacy may be compromised by disruption of cold chain storage, poor general health status of the subject, poor vaccination techniques, requisite of multiple boosters, high cost. As a consequence, several approaches are currently being investigated experimentally; out of which DNA vaccines appear to be particularly promising as they can induce persistent cell-mediated and humoral immune responses to antigens isolated from a variety of viral, bacterial and parasitic pathogens. Apart from their immunogenicity, DNA vaccines offer several practical advantages; these include: safety, cost effectiveness, ease of purification, stability, storage and transportation at room temperature.

In this regard, various DNA vaccination strategies have been shown to provide protection against lethal rabies virus challenge. A large body of evidence shows that glycoprotein antigen of rabies virus is the prime pathogenicity determinant. It is also the major antigen responsible for eliciting protective immune response being the primary target of the host humoral [Wiktor et al., 1973; Cox et al., 1977] as well as cellular immune responses [Macfarlan et al., 1986; Celis et al.,
Introduction

Thus, in the present study, we employed glycoprotein for development of DNA vaccination strategy by trafficking it to sub-cellular compartments. Efficient delivery of antigens to both MHC Class I and II processing and presentation pathways is required for generating an ideal immune response comprising of both cell mediated and antibody immune response. Accordingly, this study investigates strategies for targeting of glycoprotein to MHC Class I and II pathways for improving its antigenicity, immunogenicity and protective efficacy. We further undertook enhancement of immune response by optimization of immune parameters like delivery route, dose of DNA vaccine and adjuvant supplementation.

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. In view of this, following were the major aims and objectives of the study undertaken:

- Cloning of glycoprotein gene of rabies virus (ERA strain) in DNA plasmids bearing various signal sequences for targeting the expressed protein to sub-cellular locations accordingly.
- Evaluation of the authenticity of DNA vaccine constructs by transient transfection in BHK-21 cells followed by sub-cellular fractionation and immunoblot analysis.
- Characterization of the immune response generated by DNA vaccine chimeras in murine model.
- Evaluation of immune response in canine model.
- Optimization of immune parameters for maximal immunogenicity.
- Evaluation of the optimized DNA vaccine formulation to confer protection against lethal intracerebral rabies virus challenge, on pre- and post-exposure.
- Enhancement of DNA vaccine plasmid production.