CHAPTER I

INTRODUCTION

The common vaccines currently in use consist of inactivated (killed) or live but attenuated (avirulent) bacterial cells or viral particles. The attenuated vaccines, whereby microorganisms are attenuated so that they lose their pathogenicity but still retain their capacity for transient growth within an inoculated host, provide prolonged immune-system exposure to individual epitopes on the attenuated organisms, resulting in increased immunogenicity and production of memory cells. As a consequence, these vaccines often require only a single immunization. In addition, the ability of the vaccines to replicate within host cells makes them particularly suitable for inducing a cell-mediated response. A major disadvantage of attenuated vaccines is the possibility of their reversion to a virulent form. Inactivation of the pathogen by heat or by chemical means make it no longer capable of replication in the host. However, repeated boosters of the killed vaccines are often needed to maintain the immune status of the host. In addition, the vaccines induce a predominantly humoral antibody response and are still associated to certain risks such as failure to kill all the microorganisms. Some of the risks associated with attenuated or killed vaccines can be avoided with vaccines that comprise specific, purified macromolecules derived from pathogens or synthetic peptide vaccines. Although these vaccines are safer, they are poorly immunogenic and tend to induce only a humoral antibody response. To raise the protective immunity, the use of conjugates or adjuvants is indispensable (Goldsby, Kindt and Osborne, 2000; Storni *et al.*, 2005).

A number of studies have reported so far on the application of biodegradable micro-/nanoparticles as vaccine adjuvants or vaccine delivery systems (O'Hagan, Singh and Gupta, 1998; Singh and O'Hagan, 2002), in particular, those made of poly(lactic acid) and poly(lactic-co-glycolic acid) (Hampl *et al.*, 1997; Singh *et al.*, 1997; Johansen *et al.*, 2000). They were utilized for delivering a variety of antigens, including tetanus (Alonso *et al.*, 1994; Johansen *et al.*, 2001; Thomasin *et al.*, 1996), human immunodeficiency virus (Moore *et al.*, 1995), malaria (Men *et al.*, 1996; Thomasin *et al.*, 1996), influenza (Lemoine *et al.*, 1998) antigens, etc. To meet the

desired properties, the microparticles could be controlled or modified in terms of their biodegradation and/or antigen release rate, making them very promising as efficient vaccine delivery (Thomasin *et al.*, 1996). However, these particles have a limitation in their preparation procedure, which requires the use of organic solvents and surfactants as well as sonication or homogenization. Such components and preparation process have been reported to impose deleterious effects on the activity of encapsulated proteins (Cegnar, Kos and Kristl, 2004; van de Weert *et al.*, 2000).

Chitosan has been recognized as a promising material for delivery of drugs and labile macromolecular compounds, attributed to its excellent physicochemical properties. It is considered as a biocompatible and biodegradable polymer of low toxicity (Illum, 1998; Mi *et al.*, 2002). It is also important to note that chitosan is soluble in aqueous mild acidic solution, which is an obvious advantage over other biodegradable polymers. Therefore, preparation of chitosan microparticles generally requires none of those components or preparation procedures, which impose detrimental effects on the activity of proteins and are required when most biodegradable polymers are used. In addition, there have been several reports of applying chitosan as a vaccine adjuvant for mucosal immunization (van der Lubben *et al.*, 2001c; 2001d), both intranasally (Illum *et al.*, 2001; Westerink *et al.*, 2001; Mills *et al.*, 2003) and orally (Calvo *et al.*, 1997; van der Lubben *et al.*, 2001a; 2001b; 2002).

In order to initiate the protective immunity, antigens or microparticulate vaccines, entering into the body, need to be taken up, processed and presented to T-helper cells by a group of cells called antigen presenting cells, including dendritic cells, macrophages, and B-lymphocytes (Singh and O'Hagan, 2003; Zinkernagel *et al.*, 1997). It was found that physicochemical properties, particularly particle size and surface characteristics, of the vaccine delivery systems or microparticles themselves play an important role on their interactions with the antigen presenting cells (Ahsan *et al.*, 2002; Tabata and Ikada, 1988; Thiele *et al.*, 2001).

This study was thus aimed at investigating the feasibility of developing chitosan microparticles of various physicochemical properties for delivery of protein

and vaccine to the antigen presenting cells. For this purpose, bovine serum albumin and Japanese Encephalitis antigen were used as a model protein and model antigen, respectively. The interaction between chitosan microparticles and two types of antigen presenting cells, *i.e.* dendritic cells and macrophages, were then explored. After all, the ability of the prospective microparticulate vaccines to initiate immune response was studied in mice. The poly(lactic-co-glycolic acid) microparticles were also prepared and studied for comparison. If possible, the relationship between the interactions of microparticles with cells and the *in vivo* immune response were evaluated.

The objectives of this study are:

1. To develop chitosan microparticles for delivery of protein and Japanese Encephalitis antigen by spray drying technique
2. To evaluate the effect of type and concentration of excipients, included in the microparticles, and modification of particle surface on physicochemical properties of microparticles
3. To study the interaction between microparticles of various physicochemical characteristics and two antigen presenting cells, *i.e.* dendritic cells and macrophages
4. To investigate the ability of antigen-loaded microparticles to initiate the *in vivo* immune response, compared with the commercial Japanese Encephalitis vaccine
5. To assess the relationship between *in vitro* interactions of microparticles with antigen presenting cells and the *in vivo* immune response, if possible