CHAPTER II

LITERATURE REVIEW

Polymeric microparticles for vaccine delivery

Traditional vaccines consist of live attenuated pathogens, whole inactivated organisms or inactivated toxins. In many cases, these approaches have been very successful at inducing protective immune response. Additionally, most live vaccines are able to induce cell-mediated immune response against pathogens, which often establish chronic infections, including human immunodeficiency virus, hepatitis C virus, tuberculosis, and malaria. However, some live attenuated vaccines can cause disease in immunosuppressed individuals and some pathogens are difficult or impossible to grow in culture (*e.g.* hepatitis C virus). Meanwhile, non-living vaccines have generally proven ineffective at inducing such type of immune response. Many traditional inactivated vaccines (*e.g. Bordetella pertussis*) also contain components that can cause undesirable effects and safety problems. As a result, several new approaches to vaccine development have emerged, which may have significant advantages over more traditional approaches. These approaches include: (1) recombinant protein subunit, (2) synthetic peptides, (3) protein-polysaccharide conjugates, and (4) plasmid DNA. While these new approaches may offer important safety advantages, a general problem is that the vaccines alone are often poorly immunogenic. Traditional vaccines contain many components, some of which can elicit additional T cell help or function as adjuvants, for example, bacterial DNA in whole cell vaccines. Unfortunately, these components have been eliminated from many new generation vaccines. Therefore, there is an urgent need for the development of potent and safe adjuvants and/or delivery systems, that can be used with newer generation vaccines, including DNA vaccines (Goldsby, Kindt and Osborne, 2000; O'Hagan, MacKichan and Singh, 2001; 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 vehicles (Thomasin *et al.*, 1996).

Polymeric microparticles as efficient adjuvant

The adjuvant effect achieved as a consequence of the entrapment of antigens within microparticles has been known for many years. The enhanced immunogenicity of particulate antigen is unsurprising, since pathogens are particulates of similar dimensions and the immune system has evolved to deal with these (O'Hagan, Singh and Gupta, 1998; Singh and O'Hagan, 2002). Microparticulate delivery systems are taken up by macrophages and dendritic cells, present multiple copies of antigens to the immune system and promote trapping and retention of antigens in local lymph nodes, leading to enhanced antigen presentation, the release of cytokines and the induction of an immune response (O'Hagan, Singh and Gupta, 1998).

Uchida, Goto and Foster (1994) reported that the single subcutaneous administration of ovalbumin-loaded poly(lactic-co-glycolic acid) microspheres with different diameters into mice induced good antibody response above ovalbumin saline negative control at 3, 6, 9, and 12 weeks after inoculation. Especially, 0.16% ovalbumin-loaded microspheres having mean volume diameter of 3.5 μm exhibited the best immune responses with values greater than those obtained after inoculation with adjuvants such as complete Freund's adjuvant or alum as positive control. Similar results were obtained from hepatitis B core antigen-loaded microspheres (Uchida *et al.*, 1998) and ovalbumin-loaded microparticles, made of poly(oxyethylene)-poly(oxypropylene) block copolymer (Newman, Todd and Balusubramanian, 1998). In addition, the ability of microparticles to initiate immune response could be further enhanced by co-administration with other adjuvants (O'Hagan *et al.*, 1991; Newman *et al.*, 1998; Jung *et al.*, 2001)

There have been several other polymeric microparticles developed for vaccine delivery, including chitosan, chitosan/ethylene oxide-propylene oxide block copolymer (Calvo *et al.*, 1997), sodium alginate (Kidane *et al.*, 2001), poly(methylmethacrylate) (Kreuter and Liehe, 1981), sulfobutylated poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (Jung *et al.*, 2001), poloxamer/poly(lactide-co-glycolide) composite (Tobio *et al.*, 1999) microparticles, etc.

Polymeric microparticles for single immunization

One major problem of most vaccines is that multiple immunizations are needed in order to obtain protective immune response. Many vaccinees often fail to complete the vaccination program, especially those in the developing countries or in the country. Development of vaccines, which are able to induce effective immunity after a single immunization, would be the best solution. Poly(lactic acid) and poly(lactic-co-glycolic acid) of different molecular weights or different ratios between lactic acid and glycolic acid yield different biodegradation rate and hence different antigen release rate. For example, poly(lactic-co-glycolic acid) of ratio between lactic acid and glycolic acid at 75:25 owns slower biodegradation rate and subsequently slower antigen release rate than the polymer of 50:50 ratio. As a consequence, it is able to present the antigen to immune cells longer and initiate higher immune response than the other polymer. Based on this principle, single-shot vaccines could be developed (Coombes *et al.*, 1996).

Another approach is a mixture between one dose of a common vaccine and multiple doses of microparticulate vaccines as booster doses (Cleland *et al.*, 1998; Shi *et al.*, 2002). Furthermore, O'Hagan, Singh and Gupta (1998) reported several potential microparticulate vaccines, which could induce prolonged immunity after only single immunization, for a variety of antigens, including tetanus toxoid, diphtheria toxoid, hepatitis B surface antigen, etc.

Induction of cell-mediated immunity with microparticulate vaccines

There have been numerous studies mentioning the induction of cell-mediated immunity after immunization with microparticulate vaccines (O'Hagan *et al.*, 1993;

Maloy *et al.*, 1994; Moore *et al.*, 1995; Vordermeier *et al.*, 1995; Nixon *et al.*, 1996; Ma *et al.*, 1998; Newman *et al.*, 1998; Newman, Todd and Balusubramanian, 1998). This type of immunity is very useful in eradication of infected or cancerous cells.

Microparticles for mucosal immunization

Most currently in use vaccines are administered by injection. It would be more convenient in many ways such as easy to be administered, reduction of undesirable effect of injection, convenient for booster immunization, etc., if the vaccines could be immunized mucosally. The most convenient mucosal administration is per oral. It was found that oral vaccination in microparticulate form could initiate both mucosal and systemic immune response (Eldirdge *et al.*, 1990; Challacombe *et al.*, 1992; Challacombe, Rahman, and O'Hagan, 1997; Jung *et al.*, 2001; Kidane *et al.*, 2001).

However, oral vaccines essentially need special protection from gastric acidity and intestinal enzymes, even though the vaccines are already encapsulated into microparticles (Singh and O'Hagan, 2002). An alternative and promising route of administration is intranasal vaccination, which is as convenient as the oral vaccination and could induce comparable immune response to the oral vaccination at a smaller dose (Jung *et al.*, 2001).

Microparticles for optimal immune response

Microparticulate vaccines could be prepared in different particle sizes, in order to optimize the immune response. It was revealed that size of microparticles is one of the important factors in determining the ability of vaccines to initiate the immune response (Uchida, Goto and Foster, 1994). Microparticles of size smaller than 10 μm are able to initiate the immune response better than the larger microparticles, due to the increased uptake of the vaccines into lymphatic system and subsequently better approach to antigen presenting cells (Tabata, Inoue and Ikada, 1996). Nevertheless, the large particles with size greater than 10 μm could be sequestered but not translocated and act as a long-term depot instead (Eldridge *et al.*, 1991).

In addition, surface modification of microparticles for special purposes is also possible, for examples, preparation of cationic microparticles for adsorption of DNA vaccines (Briones *et al.*, 2001; Walter and Merkle, 2002), preparation of anionic microparticles for adsorption of antigenic protein (Kazzaz *et al.*, 2000), surface binding to IgG ligand for targeting the antigen to antigen presenting cells by Fc receptor-dependent phagocytosis (Bot *et al.*, 2001), or preparation of microencapsulated liposome systems in order to improve vaccine efficacy (Machluf *et al.*, 2000), etc.

Microparticles for delivery of DNA vaccines

In principles, DNA vaccines is able to initiate both humoral and cell-mediated immune response (Davis and McCluskie, 1999 cited in Howard and Alpar, 2002), if DNA could be delivered to cytosol and subsequently to the nucleus of antigen presenting cells and the transcription eventually occurs, resulting in intracellular production of antigen.

Direct administration of naked DNA into the body would result in degradation of DNA by nuclease enzymes, which exist throughout the body. In addition, this method of immunization yields low transfection efficiency and could not reproduce the effect. Application of microparticles as carriers for DNA delivery is one promising approach to solve such problems. DNA or DNA-cationic polymer complex of poly(L-lysine) and poly(L-ornithine) could be encapsulated into microparticles (Benoit *et al.*, 2001; Howard and Alpar, 2002). On the other hand, DNA could be adsorbed on the cationic surface of microparticles, prepared by incorporation of cationic components such as cetylpyridinium bromide (Briones *et al.*, 2001), polyethylenimine or poly(butyl methacrylate-[2-dimethyl aminoethyl] methacrylate-methyl methacryalte) (Walter and Merkle, 2002). It was revealed that the delivery systems were well taken up by macrophages and could initiate the production of high serum IgG in mice, compared with administration of naked DNA (Benoit *et al.*, 2001; Walter and Merkle, 2002).

Conclusions

It is obvious that polymeric microparticles are very versatile in delivery of many types of antigens, including peptides, proteins and DNA. In addition, microparticles also offer the possibility for modification in order to achieve specific needs such as the development of single-shot vaccines. It is also essential to note that the microparticulate vaccines could efficiently elicit both humoral and cell-mediated immunity. These evidences apparently support the potential of microparticles as an efficient delivery system for vaccines.