CHAPTER IV

DISCUSSION AND CONCLUSION

The DNA-based vaccination is a new technique for immunizing against pathogens. By this technique, gene encoding protein is inserted into eukaryotic expression vector. After introduction of the plasmid DNA into host body, the encoded protein is produced and acts as antigen for induction of immune responses. Consequently, this alternative strategy of vaccine development is worthy of investigation, though, due to its inherent advantages, including long-lasting immune response, induction of both cell-mediated and humoral immunity. In addition, DNA vaccines are relatively inexpensive and easy to produce. It is a simple methodology and a broader spectrum of immune responses for achieving greater efficacy similar to natural infection (Shi *et al.*, 1999). Thus, DNA vaccines have been effectively applied in association with several diseases such as hepatitis B (Davis *et al.*, 1996), hepatitis C (Hu *et al.*, 1999), malaria (Weiss *et al.*, 2000), AIDS (Xu *et al.*, 2003) and cancer (Paglia *et al.*, 1998).

Recently, phage display is a high potential technology for producing functional recombinant proteins. This technique has been used as a very effective way for producing large numbers of diverse proteins, peptides and molecules that perform specific functions (Griffith, 1993; Barbas, 1993; Winter *et al.*, 1994; Burton, 1995; Ladner, 1995; Neri *et al.*, 1995; Hoogenboom, 1998; Ladner, 2000; Seigel, 2001; Tayapiwatana and Kasinrerk, 2002; Tayapiwatana *et al.*, 2003; Intasaia *et al.*, 2003).

This technique can also be used to study receptor and antibody binding site (Griffith, 1993; Winter *et al.*, 1994), protein-ligand interactions (Cesareni, 1992), and to improve or modify the affinity of proteins for their binding partners (Neri *et al.*, 1995; Ladner, 1995; Burton, 1995). The fusion proteins have also been made between peptides and a range of phage structural proteins at positions which preserve the biological properties of fusion partner protein (Perham *et al.*, 1995), glutathione S-fusion protein (Yip *et al.*, 2001), and filamentous hemagglutinin fusion protein (Kiel *et al.*, 2000). The strategy has been previously applied to induce a protective immune response *in vivo* against hepatitis B (Meola *et al.*, 1995) and malaria (Demangel *et al.*, 1996; Greenwood *et al.*, 1991). Moreover, phage display is applied to use for displaying the antibody fragment (ScFv) against GPIIIa integrin. The ScFv fragment has ability to inhibit the binding of fibrinogen to platelets and react with endothelial cells (An *et al.*, 2002).

More recently, Tayapiwatana *et al.* (2003) have applied this technique to generate phage expressing a leukocyte surface molecule, CD147. The CD147 cDNA from the mammalian expressing vector was amplified by PCR and subcloned into the phagemid expression vector, pCommb3HSS. The recombinant phagemid vector, pCom3H-CD147, was transformed into *E. coli* strain TG-1 and XL-1 blue and subsequently cultured for phage display. The sandwich ELISA was performed for detection of the CD147 which display on phage particle. It was found that the correct size of CD147-truncated gpIII fusion protein is 38 kDa and both the *E. coli* strain and growth condition were important for obtaining the correct conformation of CD147 displayed on phage particle. In additional, it was possible to deliver fusion protein for vaccine immunization with phage display carrier system. The phage display carrier

systems have substantiated to elicit strong immune responses, able to medicate prevention in animal models (D'Mello *et al.*, 1997; Wan *et al.*, 2001). The use of phage display has the additional advantage of increasing the half-life of the peptide in the circulation and providing T-cell help for the induction of a strong antibody response, even without added adjuvant (Yip *et al.*, 2001). Finally, the phage display carrier system can be produced inexpensively in large quantities and increased correctly folded peptides (Yip *et al.*, 2001; Forrer *et al.*, 1999).

CD147 molecule is a leukocyte surface molecule, which was designated as CD147 molecule at the Sixth International Workshop and Conference of Human Leukocyte Differentiation Antigens (HLDA workshop) (Stockinger *et al.*, 1997). This molecule was also known as basigin (Miyauchi *et al.*, 1991), M6 antigen (Kasinrerk *et al.*, 1992) and extracellular matrix metalloproteinase inducer (EMMPRIN) (Biswas *et al.*, 1995). It is a glycoprotein of type 1 transmembrane protein with a molecular mass of 50-60 kDa (Stockinger *et al.*, 1997). CD147 is strongly up-regulated on T cells upon activation, indicating a function in T cell biology (Kasinrerk *et al.*, 1992; Stockinger *et al.*, 1997) and widely expressed on hemopoietic and non-hemopoietic cells. CD147 is, therefore, speculated to be an essential molecule in the immune system. However, function of CD147 molecule is so far unclear and the study of the production of polyclonal antibody in mice using pCDM8-CD147 and phage-displayed CD147 has never been performed. Thus, the alternative method for production of polyclonal antibody, two immunization methods, DNA based and phage-display immunization were studied.

The immunogen preparation step is one of important factors in sensitizing the immune responses. To obtain the high quality of specific polyclonal antibodies, high

purity of immunogen is essential. However, the processes to obtain enough amount of immunogen are usually cumbersome. Regarding the well developed recombinant DNA technology, there are certain manipulations for triggering the immune cells in producing hyperimmune serum. The DNA-based immunization is a strategy which has been demonstrated as an efficient method for inducing the immune responses. This technique has been actively applied in the development of vaccine formulation (Hasan *et al.*, 1991; Hassett and Whitton, 1996; Whitton *et al.*, 1999). Besides, DNA-based immunization strategy is an alternative method for efficient production of polyclonal and monoclonal antibodies (Barry *et al.*, 1994; Kasinrerk *et al.*, 1996; Kasinrerk *et al.*, 1997; Kasinrerk and Tokrasinwit, 1999; Kasinrerk *et al.*, 2002; Puttikhunt *et al.*, 2003). In addition to DNA-based immunization, recently, phage display technology was demonstrated to be another application in provoking antibody production against polypeptide immunogens (Yip *et al.*, 2001). In this circumstance, protein of interest was displayed on the filamentous phage particle and used as an immunizing agent.

In this study, the efficiency of polyclonal antibodies against CD147 protein using both DNA-based and phage-displayed polypeptide immunization was compared *in vivo* studies. Plasmid DNA encoding CD147 and phage-displayed CD147 protein were generated by standard methods. Before employed as an immunogens, both preparations demonstrated there ability in producing the target polypeptide, CD147. In the sera of mice immunized with phage-displayed CD147 induced more potent antibody response than pCDM8-CD147 (approximately 10-20 times) by indirect ELISA. Apart from high antibody titer observed, the level of antibodies was maintained for a long period of time by phage immunization. In addition to indirect

ELISA, flow cytometric assay was employed for demonstrating the occurrence of anti-CD147 antibodies directed against the surface form of CD147 in immunized mice. Stable CD147 expressing BW5147 cells were generated (Chiampanichayakul *et al.*, 2002) and used as a specific target for the staining of produced antibodies. According to the FACS histogram, the mean fluorescent intensity obtained from mouse immunized with the phage-displayed CD147 was stronger in comparison to mouse immunized with pCDM8-CD147. These data correlated with the results from the indirect ELISA assay indicating a higher CD147 antibody titer in sera of mice immunized with phage-displayed CD147.

The contrast in antibody titers induced by DNA-based and phage-displayed polypeptide immunization may due to the different mechanisms of antibody induction. By DNA-based immunization, naked DNA was introduced and allowed *in vivo* expression of gene encoding exogenous protein (Donnelly *et al.*, 1997; Hasan *et al.*, 1991). The quantity of foreign protein expressed in mice depends upon the effectiveness in uptaking of the immunized plasmid DNA and the efficiency of *in vivo* transcription and translation pathway in certain cell types. In contrast, by phage display system, the protein of interest was expressed *in vitro* before immunization. Thus, immunization of protein expressed on phage particle is comparable to the conventional protein immunization. The phage display carrier systems have demonstrated to elicit strong immune responses as it could increase the half-life of the peptide in the circulation and providing T-cell help for the induction of a strong antibody response (Yip *et al* ., 2001). The T-cell epitopes situate in the coat protein of phage which carries the target B-cell epitopes on its gpIII molecule. In addition, the huge size of phage particle promotes the potential of immunosurviallance to

discover the displayed immunogen. These reasons may explain why the antibody responses observed in phage immunization was higher and sustained longer than DNA-based immunization. The strategy of phage-displayed polypeptide immunization has a high potential in producing the antibodies against small peptide fragments regardless of chemical conjugation with the carrier proteins.

In conclusion, in this study the effectiveness of DNA based and phage-displayed polypeptide immunizations in the induction of antibody response was investigated. It was found that the phage display carrier systems induced stronger immune responses. To the best of our knowledge the comparison of these two different molecular biology methods in preparing the immunogens was firstly described, herein. The established procedures will be further applied for the production of both polyclonal and monoclonal antibodies against the interesting molecules in the near future.