CHAPTER VII
CONCLUSION

1. Human antibody phage display library which had 2.6 x 10^8 antibody diversity was constructed from peripheral blood mononuclear cells of 60 Thai blood donors. After phage rescuing, the titer of the phage particles was 6.5 x 10^{12} cfu/ml. This is the largest human antibody phage display library ever constructed to date.

2. Eighty-five percent of the phage particles in the library contained genes encoding human single chain variable antibody fragments (*huscFv*). Random sampling of 17 phage clones revealed that there were 15 DNA banding patterns of restriction fragment length polymorphism of the *Mva*I-cut *huscFv*.

3. Protein components of the Thai cobra, *N. kaouthia*, venom were studied by proteomics by two dimensional gel electrophoresis (2DE), peptide generation liquid tandem mass spectrometry (LC/MS-MS) and protein orthologs identification. Twenty-four spots of proteins in the 2DE-gel were analyzed and they could br grouped into six groups, *i.e.*, cobra venom factors, nerve growth factor β-chain, phospholipases, venom protein-II, cytotoxin-I, and neurotoxins.

4. The venom components of the *N. kaouthia* were also studied by using 2D-LC/MS-MS and protein orthologs identification. There were 61 orthologous proteins of the database that matched with the peptide sequences generated from the venom by using the 2D-LC/MS-MS. They were classified into 12 different groups according to their putative biological functions/activities: 1) cardiotoxins; 2) cobra venom factors; 3) a cysteine-rich venom toxin; 4) cytotoxins; 5) kaouthiagin; 6) mocarhagin; 7) muscarinic toxin-like proteins; 8) neurotoxins; 9) an oxoglutarate dehydrogenase complex;10) phospholipases; 11) serum albumin; and 12) weak toxin.

5. *N. kaouthia* venom was subjected to an ion exchange column chromatography and 11 protein profiles, *i.e.*, P1-P11 were obtained. The proteins in each profile were identified by either gel-based MALDI-TOF or 2D-LC/MS-MS. The P1 contained cobra venom factor, while the P3-P5 and P8 contained predominantly phospholipases, natrin (a novel component of *N. kaouthia* venom), phospholipases, and long α-neurotoxin, respectively.

6. The P1, P3, P4, P5 and P8 were used in a phage bio-panning to select phage clones displaying HuScFv specific to the proteins in the fractions from the constructed human antibody phage display library. The selected phage clones were used to infect *E. coli* and the soluble HuScFv were produced from the *E. coli*, purified, and tested for their specific binding with target antigens in indirect ELISA, dot-ELISA, and WB.

7. Mimotopes of HuScFv that specifically bound to long α-neurotoxin in P8, *i.e.*, clones P8/0/1, P8/9/1, P8/19/1, P8/7/2, P8/10/2, P8/22/3, and P8/31/3) were identified by using the 12-mer peptide displaying M13 phage library. The mimotope sequence of all phage clones was "TVNT" and this peptide is a homolog of "TVKT" peptide located in the loop-III of long α-neurotoxin of *N. kaouthia* which is an acetylcholine receptor binding domain of the Thai cobra venom.

8. The HuScFv specific to acetylcholine receptor binding domain of the long α-neurotoxin of the Thai cobra venom could rescue the mice from lethal envenomation at the efficacy equal to or better than the conventional horse anti-Thai cobra venom at an equal protein weight basis.

9. The HuScFv specific to the other part of the long α-neurotoxin and other venom proteins can be similarly produced. The combination of the HuScFv specific to several epitopes on long α-neurotoxin and other venom component (polyclonal antibodies to venom components) has potential as anti-venom therapeutic for the envenomed victims.

10. The so-constructed human antibody phage display library is a useful biological tool for production of fully human antibodies in the form of single chain antibody fragments to other epitopes, especially those of low immunogenic, toxic or self-molecules without immunization.