CHAPTER I
INTRODUCTION

Ophitoxemia or venomous snakebite remains a public health problem in many countries especially in the tropical areas like Asia and Africa (Theakston and Warrell, 2000). It is estimated that the incidence of snake envenomation could exceed 5 million per year; and about 100,000 of these develop severe sequelae (Chippaux, 1998). In Thailand, reported cases of snakebites are approximately 10,000 per annum (http://epid.moph.go.th). The monocellate Thai cobra, *Naja kaouthia,* causes the highest hospitalized incidence (Viravan *et al*., 1986). Treatment of snake envenomation requires proper medical management (Sutherland *et al*., 1979) and antivenom therapy. The latter is the therapeutic mainstay. Most of the conventional antivenoms are produced by immunizing horse. After the satisfactory level of antibodies in horse blood is reached, the gamma globulin is purified. The antivenoms are limitedly available in the form of either monospecies-specific (monovalent) or several species-specific (polyvalent). The former is usually prescribed for bitten cases which snake species can be identified while the latter are for those who are bitten by the unidentified species. It is generally accepted that there are several limiting factors in the production and use of the horse-derived antivenoms. Toxicity of the venoms limits the amount of an antigenic dose. As such, multiple-, spaced-, low dose, formulated in adjuvant and/or delivery vehicle, must be given intramuscularly at multi-sites to the animal over an extended period of time. Besides, certain components in snake venoms have been shown to down-regulated the immune response of the immunized host (Cardoso and Mota, 1997). For successful treatment, large amount of the heterologous antibodies must be infused into the snake bitten victim. Reactions including full range of anaphylactic manifestations, serum sickness, and other form of hypersensitivity often occur. These limitations emphasize the requirement of the immunotherapy using human derived antivenoms and new strategy for producing the therapeutic agents.

Phage display technology invented by Smith in 1985 is a powerful tool to display millions or even billions of diverse peptides or proteins in the forms of fusion

partners with the coat proteins of filamentous bacteriophages of *E. coli*, *e.g.*, M13 (Smith, 1985). One most successful application of the phage display technology is the production of human monoclonal antibodies using phage library that display more than 10^{10} human ScFv variants (Winter *et al.*, 1994). The high diversity of human antibody repertoire on B lymphocytes *in vivo* was emulated onto the surfaces of M13 *in vitro*. The phages antibody repertoire can be kept in a small scale which is easily to handle and use. The production of any antigen-specific human ScFv can be achieved rapidly without immunization process by bio-panning of the phage library with the antigen of interest. The technique overcomes the limiting factors found in the conventional immunization and murine hybridoma methods. The construction of non-immune antibody phage display library using variable region of heavy (VH) and kappa or lamda (Vκ or Vλ) chain-encoding DNA sequences, that can be obtained from naïve human peripheral blood B lymphocytes, has been used to produce antibody specific to toxic or low immunogenic molecules without immunization process.

In this research, human antibody phage display library was constructed from non-immune B-lymphocytes which were collected from peripheral blood of healthy Thai volunteers. DNA sequences-encoding VH and Vκ of human immunoglobulin (*VH* and *Vκ*, respectively) were amplified by using degenerate and non-degenerate primer sets that were designed from all immunoglobulin genes available in database with modifications. DNA sequences encoding human single-chain variable fragment (HuScFv) called *huscFv* were generated by spliced overlapped extension-PCR (SOE-PCR). The *huscFv* sequences were cloned into phagemid vector and the recombinant phagmids were introduced into competent *E. coli* host cells. Recombinant phages displaying HuScFv on their surface as fusion partner with pIII of phage coat were rescued from the transformed *E. coli* cells by infecting the host cells with helper phages (M13KO7). A library of HuScFv-displaying phages was obtained.

The constructed human antibody phage display was used in the production of human single chain monoclonal antibodies (HuScFv) specific to lethal components of the Thai cobra venom. The HuScFv were found to rescue the envenomed mice from venom lethality. Details of the experimental designs, actual experiments and the experimental results form the basis of this research.