CHAPTER I
INTRODUCTION

Pertussis or whooping cough, a fatal human respiratory diseases especially among infants and young children, is caused by a gram-negative bacterium named *Bordetella pertussis*. Human gets infection directly by inhalation of the aerosol droplets containing the causative bacteria coughed out from the infected individual (Hewlett *et al*., 1997). *B. pertussis* bacteria initially adhere to the respiratory ciliated epithelium using many kinds of adhesins expressed on the bacterial surface, *e.g.* filamentous hemagglutinin, fimbrial agglutinogens, pertactin (an outer membrane protein) which bind to several host components, *e.g.* various sulfated sugars (chondroitin, dextran, heparan), integrin (CR3), fibronectin (Relman *et al*., 1990; Relman 1995; Babu *et al*., 2001). The attached bacteria then multiply rapidly (colonization) and produce toxic substances for host immune evasion and bacterial survival and pathogenesis. Virulence factors of *B. pertussis* have been extensively reviewed elsewhere (Relman, 1995; Babu *et al*., 2001). Among them are the above mentioned colonization factors and adenylate cyclase (Weiss *et al*., 1984), demonecrotic toxin (Livey and Wardlaw, 1984), tracheal cytotoxin (Goldman *et al*., 1982; Rosenthal *et al*., 1987), type-III secretion system (Kerr *et al*., 1999), and pertussis toxin (PT) (Babu *et al*., 2001). Pertussis toxin (also called leucocytosis-promoting factor, islet-activating protein, or pertussigen) is the principal exotoxin of *B. pertussis*. It is an A-B_5 toxin consisting of A or S1 subunit which is an ADP-ribosyl transferase and a B pentamer made up of two dimers, *i.e*, S2S4 and S3S4, and one S5, which is responsible for binding of the holotoxin to the host cell surface. PT exists in two forms, either cell-bound or secreted. The cell-bound PT functions as one of the bacterial colonizing factors (Carbonetti *et al*., 2003, 2005). Once the PT bound to the target host cell *via* its B subunits, the A (S1) subunit is cleaved-off and traverses the plasma membrane into the cytoplasm where it transfers ADP-ribose moiety from NAD to a cysteine residue at position 352 of the α-subunit of the membrane-bound inhibitory guanosine-binding protein (Gi-protein). The normal function of this Gi protein [adenylate cyclase inhibition by means of guanosine triphosphate (GTP)

hydrolysis to guanosine diphosphate (GDP)], is disrupted. The result is a sustained adenylate cyclase activation and, in the effect, the massive increment of intracellular cyclic adenosine mono-phosphate (cAMP) (Bruni *et al*., 1985). The increase of intracellular cAMP causes cellular function disruption including defective in all steps of phagocytosis and cytotoxicity (Meade *et al*.,1984). PT *via* the S1 subunit causes also systemic leucocytosis, termed leucocytosis-promotion (LP), histamine sensitization and islet cell activation (IA) with massive release of insulin (Katada and Ui, 1979). The PT B subunit not only binds to the target cell for S1 intracellular delivery but was found also to have immuno-(pathology) potentiating effects, *e.g*. T-lymphocyte mitogenicity (Strnad *et al*., 1987).

Fortunately, pertussis is vaccine preventable and vaccination against the disease is a routine practice of the Extended Program of Immunization (EPI) of most, if not all, countries. Currently, two vaccines are used in pertussis prophylaxis. The first inactivated whole cell vaccine (WCV) is prepared from *B. pertussis* strain which expresses agglutinogens (proteinaceous fimbrial appendages protrude from the bacterial surface). This vaccine is highly protective but frequently causes local adversed reactions in the vaccinees and occasionally induces the more serious systemic reaction including acute neurological illness and death (Brody and Sorley, 1947; Byers and Moll, 1948). This is because the vaccine still contains several active toxic components of the bacteria, such as PT, adenylate cyclase, and endotoxin (Geier *et al*., 1978). As such, acellular pertussis vaccine (ACV) consisting principally of chemically/genetically inactivated PT and other purified components of *B. pertussis*, *i.e.* filamentous hemagglutinin, pertactin, and fimbrial agglutinogens formulated with aluminium hydroxide adjuvant was developed and used in Japan since 1981 (Sato *et al*., 1984; Storsaetor *et al*., 1990). Potency of the ACV is as high as the WCV with only one-tenth toxicity as judged by leucocytosis promotion, histamine sensitization, and endotoxin activities (Sato *et al*., 1984). Thus the ACV is currently in used also in the EPI of several other countries.

Although effector mechanisms of protective immunity against pertussis is not completely understood and may involve both humoral and cell mediated immune mechanisms (Dolby *et al*., 1975), evidences in the literature have pointed out that antibodies play important part in protection against *B. pertussis* infection and in

resuscitation from pertussis severe morbidity especially those directed against PT (Sato and Sato, 1990; Halparin *et al*., 1991). Several murine monoclonal antibodies specific to various PT subunits were tested for their protective activities in infant mice against the aerosol and intracerebral challenge with *B. pertussis* (Sato and Sato, 1990; Sato *et al*., 1991). It was found that while many of the monoclonal antibodies especially those directed against S1 were highly protective when passively administered to the mice, several other preparations directed against the same protein were less effective or did not confer any immunity at all (Sato and Sato, 1990; Sato *et al*., 1991). The finding indicates that only certain epitopes in the S1 protein are important in neutralizing PT activities. However, no attempts have been made to identify the protective epitopes of the PT protein.

In spite of the high vaccination coverage, however, there has been resurgence of pertussis during the past few decades in both developing and developed countries (Bass and Wittler, 1994; De Serrese *et al*., 1995; Andrew *et al*., 1997). The upsurge might be due to several attributes: *e.g.* waning of the vaccine induced immunity in adolescence and adults, an emergence of a vaccine escape *B. pertussis* mutant, vaccines did not contain appropriate and/or adequate amount of protective immunogen, improper regimens of vaccination. While further investigations are needed to pinpoint the causes of the pertussis resurgence, a non-antimicrobial therapeutic regimen such as an immunotherapy using neutralizing antibodies is required as an adjunct to the current supportive treatment measures in order to effectively resuscitate the seriously affected patients.

In this study, the PT neutralizing activity of a murine monoclonal antibody specific S1 subunit was determined. An epitope important in the PT activities of the S1 specific murine monoclonal antibody was determined using phage display peptide library. A humanized-murine single chain variable fragment of anti-S1 antibody was produced and evaluated for PT neutralizing activity.