CHAPTER VII
CONCLUSION

The PT-neutralizing capability of murine MAb specific to S1 subunit (MAbPT6-2G6) was studied *in vitro*: hemagglutination activity (HA), *ex vivo*: CHO cell clustering (CC) activity and *in vivo*: leukocytosis-promoting (LP) activity. It was found that 1) MAbPT6-2G6 at 6.25 μg neutralized the hemagglutination caused by the HA100 of PT (125 ng), 2) MAb PT6-2G6 at 32.5 μg could mediate partial (~50%) inhibition of CC caused by a CCD50 of PT (5.76 pg), and 3) MAbPT6-2G6 could cause significant decrease ($p<0.05$) in LP mediated by 100 ng of PT in a dose dependent manner.

The specific-epitope of MAbPT6-2G6 was determined using peptide phage display technique. Six mimotope sequences of MAbPT6-2G6 were obtained and grouped into 6 different types: M1; LTPCRN (1 phage clone), M2; LTPCRTK (2 phage clones), M3; LSRIFIAN (5 phage clones), M4; LLTVRRA (2 phage clones), M5; VISIVGV (1 phage clone) and M6; SCVNSLL (1 phage clone). All of them matched to the amino acids 213-225 (TSRRSVASIVGTL) of S1 subunit that located on the surface of the S1 and at juxtaposition of the S2-S4 heterodimer of PT. The finding implied that the amino acids 213-225 (TSRRSVASIVGTL) is the mimotpe of MAbPT6-2G6. The MAbPT6-2G6 neutralized the PT-mediated hemagglutination by specific binding to S1 subunit at amino acids 213-225 (TSRRSVASIVGTL) juxtaposed to S2-S4 heterodimer of the B subunit lead to stereologically hindering the binding region of the dimer, resulting in abolishing the hemagglutination of PT. While, the inhibition of the CHO cell clustering and leukocyte-promotion activities of PT by previously incubating the PT with the MAbPT6-2G6 specific to S1 subunit should not be mediated by interfering the enzymatic function of the S1. But, MAbPT6-2G6 prevents 1) the binding of PT to target cell by stereologically hindering the B subunit, and 2) the intracellular entry of the toxin by forming the large PT-antibody complexes and by the hydrophilicity of MAbPT6-2G6 that are unable to traverse the hydrophobic plasma membrane.

For MAbPT6-2G6 humanization, *PT6-2G6-huscFv* was successfully constructed by grafting the murine CDRs of PT6-2G6 hybridoma onto the selected human immunoglobulin frameworks using SOE-PCR technique. The *PT6-2G6-huscFv* DNA sequences was cloned into phagemid. Recombinant phages carrying PT6-2G6-HuScFv were rescued by M13KO7 helper phages and subjected to select the phage carrying specific PT6-2G6-HuScFv to PT by bio-panning method. Five phage clones that had negligible binding signal to the negative control antigen (BSA) were selected and used to transfect the non-suppressor HB2151 *E. coli* for soluble HuScFv expression. It was found that only HB2151 *E. coli* harboring PT6-2G6-HuScF no. 1 can produce soluble PT6-2G6-HuScF. By Western blot analysis with anti-E Tag antibody, PT6-2G6-HuScF was located at approximatedly at 35 kDa and showed binding affinity against PT by soluble HuScFv-ELISA. Under IPTG-induction at 30^{o}C, PT6-2G6-HuScFv was secreted into periplasm of HB2151 *E. coli*. PT6-2G6-HuScFv contained in periplasmic fraction was purified and subjected to determine its antigenic specificity and neutralization activity to PT. It was found that PT6-2G6-HuScFv possessed the same antigenic specificity of MAbPT-2G6 to PT by recognizing S1 subunit of PT and the inhibition activity to PT of original MAb by neutralizing the PT-mediated hemaglutination. Therefore, humanized-murine PT6-2G6-ScFv has a high potential for use as a non-antimicrobial therapeutic alternative in order to effectively resuscitate the seriously affected patients from pertussis.