

CONTENTS

	Page
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
CONTENTS	vii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xix
CHAPTER	
I INTRODUCTION	1
II OBJECTIVES	4
III LITERATURE REVIEW	5
1. <i>Bordetella pertussis</i> and pertussis	5
1.1 General description of <i>B. pertussis</i>	5
1.2 Epidemiology of pertussis	7
1.3 Pathogenesis and clinical manifestations	7
1.4 Pertussis therapy	11
2. Pertussis toxin	11
2.1 Structure of pertussis toxin	11
2.2 Biological activities of pertussis toxin	13
2.3 Binding of pertussis toxin to host cell	15
2.4 Entry of pertussis toxin into host cell	18
2.5 Activation of pertussis toxin	18
3. Antibody therapy	20
4. Recombinant antibody	23
4.1 Antibody molecule	23
4.1.1 Antibody structure	23
4.1.2 Mechanisms responsible for diversity of antigen binding sites	24
4.2 Antibody engineering technology	24

CONTENTS (cont.)

	Page
4.3 Humanization of murine antibodies	28
4.4 Production of recombinant antibodies	31
5. Phage display technology	34
5.1 Phage display vehicles	38
5.2 Screening phage display libraries: Bio-panning	41
5.2.1 Library amplification	42
5.2.2 Bringing phage and target together	42
5.2.3 Washing and elution	43
5.2.4 Re-infection into host cells	44
6. Peptides and proteins displaying T7 phage	44
6.1 Biology of T7 phage	44
6.2 T7 phage vectors	46
6.3 Construction of random heptapeptide peptide T7 phages	48
7. Recombinant antibodies displaying filamentous phages	49
7.1 Biology of filamentous phages	49
7.2 Phagemid cloning vectors	51
7.3 Construction and screening of antibody displaying phages	52
7.3.1 Construction of ScFv displaying phage	52
7.3.2 Types of antibody repertoires	53
7.3.3 Selection of antibody libraries: “bio-panning”	55
7.4 Production of soluble ScFv molecules	58
IV MATERIALS AND METHODS	59
1. Purified pertussis toxin (PT)	59
2. <i>Escherichia coli</i> strains	59
3. Murine hybridoma clone PT6-2G6	59
4. Preparation of MAbPT6-2G6	60
5. Purification of MAbPT6-2G6 by protein G affinity column chromatography	60

CONTENTS (cont.)

	Page
6. PT neutralizing activities of MAbPT6-2G6	60
6.1 <i>In vitro</i> assay: hemagglutination assay (HA) and hemagglutination-inhibition (HI) test	60
6.2 <i>Ex vivo</i> test: CHO clustering inhibition (CCI) assay	61
6.3 <i>In vivo</i> test: leukocytosis-promotion (LP) inhibition test	61
7. Determination of the mimotope of MAbPT6-2G6 by using a peptide phage display library	62
7.1 Random heptapeptide peptide T7 phage library	62
7.2 Bio-panning	63
7.3 PCR and DNA sequencing of selected T7 phages	63
7.4 Local alignment of selected phage sequences with S1 sequence	64
7.5 Locating the epitope recognized by MAbPT6-2G6 on the S1 model	64
8. DNA amplification and CDR identification of VH and VL genes of murine hybridoma clone PT6-2G6	64
8.1 Preparation of RNA of PT6-2G6 hybridoma	64
8.2 cDNA synthesis	65
8.3 PCR amplification of hybridoma <i>PT6-2G6-VH</i> and <i>VL</i> genes	65
8.4 Determination of hybridoma <i>PT6-2G6-VH</i> and <i>VL</i> coding DNAs by agarose gel electrophoresis	66
8.5 Cloning of <i>PT6-2G6-VH</i> and <i>VL</i> genes	66
8.6 CDR identification of the murine <i>PT6-2G6-VH</i> and <i>VL</i> coding DNA sequences	67
8.7 Selection of the most matched-human VH and VL sequences to the murine VH and VL sequences	67
9. Production of humanized-murine <i>scFv</i> sequence (<i>huscFv</i>)	67

CONTENTS (cont.)

	Page
9.1 Construction of humanized-murine <i>scFv</i> sequence	67
9.2 Cloning of PT6-2G6- <i>huscFv</i> DNA to phagemid vector	68
10. Production of ScFv displaying phages	70
10.1 Growth of <i>E. coli</i> containing the recombinant phagemid	70
10.2 Rescue of ScFv displaying phages by M13KO7 helper phages	70
10.3 Phage precipitation by PEG/NaCl	70
11. Selection of pertussis toxin specific phages by bio-panning	72
12. Screening of specific phages from the enriched phage-sub-library	72
12.1 Transfection of <i>E. coli</i> with the enriched phage clones	72
12.2 Phage ELISA	73
13. Production of soluble humanized-murine ScFv (HuScFv)	74
13.1 Infection of HB2151 <i>E. coli</i> cells with phage displaying specific HuScFv to PT	74
13.2 Small scale expression of soluble HuScFv	74
13.3 Verification of soluble PT6-2G6-HuScFV	75
13.4 Determinating the reactivity of PT6-2G6-HuScFv against PT by soluble HuScFv-ELISA	75
14. Large scale expression and purification of soluble PT6-2G6-HuScFv	76
15. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	77
15.1 Preparation of samples for loading into a slab gel	77
15.2 Running the gel	77
15.3 Staining and destaining of protein bands	78
16. Western blot analysis	78

CONTENTS (cont.)

	Page
V RESULTS	79
1. Determination of antigenic specificity of MAbPT6-2G6 to PT subunit	79
2. Neutralization test of MAbs	79
2.1 Inhibition of PT mediated- hemagglutination by MAbPT6-2G6	79
2.2 Neutralizing activity of MAbPT6-2G6 on PT mediated- CHO cell clustering	82
2.3 Neutralization of PT mediated- leukocytosis-promotion by the MAbPT6-2G6	82
3. Mimotopes of MAbPT6-2G6 identified by using T7 heptapeptide phage display library	86
3.1 Selection of phage mimotopes by bio-panning	86
3.2 PCR and DNA sequencing of the selected T7 phages	86
3.3 Comparison of MAb bound phage sequences (mimotope sequences) with peptide sequence of S1 protein	89
3.4 Localization of the S1 epitope recognized by MAbPT6-2G6 on the molecular model of PT	100
4. DNA amplification and CDR identification of VH and VL genes of murine hybridoma	102
4.1 Total RNA extraction from PT6-2G6 hybridoma cells	102
4.2 Amplification of VL and VH genes of PT6-2G6 hybridoma cells	102
4.3 Cloning of PT6-2G6 VL and VH genes into a cloning vector	105
4.4 DNA and deduced amino sequence analysis	105
4.5 Amino acid sequence analysis and alignment	105

CONTENTS (cont.)

	Page
4.6 Identification of CDRs of MAbPT6-2G6-VL and VH fragments	111
4.7 Selection of high homology human immunoglobulin frameworks to those of the murine MAbPT6-2G6	113
5. Construction of <i>PT6-2G6-huscFv</i> sequence	117
5.1 Amplification of humanized <i>PT6-2G6-VL</i> and <i>VH</i> fragments	117
5.2 Amplification of <i>PT6-2G6-huscFv</i> by splice overlapped extension PCR	122
6. Construction of phagmid carrying <i>PT6-2G6-huscFv</i>	124
7. Selection of phage displaying specific HuScFv to PT	128
8. The production of soluble PT6-2G6-HuScFv in HB2151 <i>E. coli</i>	131
8.1 The small scale expression of soluble PT6-2G6-HuScFv	131
8.2 Determinating the bacterial fraction containing soluble PT6-2G6-HuScFv	134
8.3 Soluble HuScFv-ELISA of PT6-2G6-HuScFv 1.1 and 1.3	136
8.4 DNA and deduced amino acid sequence of PT6-2G6-HuScFv clone 1.1	139
9. Large scale expression and purification of soluble HuScFv	143
10. Determination of antigenic specificity of PT6-2G6-HuScFv	145
11. Hemagglutination inhibition (HI) assay of PT6-2G6-HuScFv	147
VI DISCUSSION	149
VII CONCLUSION	160
REFERENCES	162
APPENDIX A	189
APPENDIX B	191
APPENDIX C	193

CONTENTS (cont.)

	Page
APPENDIX D	198
APPENDIX E	201
APPENDIX F	203
APPENDIX G	206
APPENDIX H	209
APPENDIX I	213
BIOGRAPHY	215

