

## TABLE OF CONTENTS

CONTENT	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
TABLE OF CONTENTS	ix
LIST OF TABLES	xiv
LIST OF FIGURES	xv
ABBREVIATIONS	xvii
CHAPTER I: INTRODUCTION	1
1.1 Statement and significance of the problem	1
1.2 Literature reviews	4
1.2.1 Immune system	4
1.2.1.1 Innate immunity	7
1.2.1.2 Adaptive immunity	8
1.2.1.2.1.1 Humoral mediated immunity (HMI)	10
1.2.1.2.1.2 Cell mediated immunity (CMI)	12
1.2.2 Monoclonal antibody	16
1.2.2.1 Hybridoma technique	16
1.2.2.2 Preparation of immunogen	22
1.2.2.3 Immunization protocol	25
1.2.2.4 Screening for antibody production	28
1.2.2.5 Fusion and screening of hybridomas	28
1.2.3 Application of monoclonal antibodies	30

1.2.3.1 Research reagent	30
1.2.3.2 Diagnosis	31
1.2.3.3 Therapeutic applications	32
1.2.4 Principle of immunoprecipitation technique	33
1.2.5 Principle of COS cell expression system	35
1.2.6 Principle of the production of BCCP fusion protein	37
1.2.7 CD4 protein	39
1.3 Objective	40
<b>CHAPTER II: MATERIALS AND METHODS</b>	41
2.2 Preparation of immunogen	41
2.2.1 Immunoprecipitated-bead technique	41
2.2.1.1 Biotinylation and preparation of cell lysates	41
2.2.1.2 Preclearing of cell lysates	42
2.2.1.3 Immuniprecipitation	42
2.2.1.4 Chemiluminescence detection system	42
2.2.1.5 Preparation of immunoprecipitate-beads for immunization	43
2.2.2 BCCP bead technique	43
2.2.2.1 Amplification of CD4 gene by PCR	43
2.2.2.2 Construction of plasmid encoding CD4-BCCP	44
2.2.2.3 Transformation	45
2.2.2.4 Plasmid preparation by alkaline lysis method	45
2.2.2.5 Expression of biotinylated CD4-BCCP protein	46
2.2.2.6 Analysis of biotinylated CD4-BCCP protein	47

2.2.2.6.1	Detection of biotinylated CD4-BCCP protein by Indirect ELISA	47
2.2.2.6.2	Detection of biotinylated CD4-BCCP protein by Western blot	48
2.2.2.7	Magnetic sorting	49
2.2.3	CD4-COS cell expression technique	50
2.2.3.1	Plasmid DNA preparation	50
2.2.3.2	COS cell transfection	51
2.2.3.3	Determination of COS cells expressing CD4 proteins	51
2.2.3.4	Immunomagnetic cell sorting	52
2.3	Immunization	52
2.4	Determination of antibody response in the immunized mice	52
2.4.1	Indirect immunofluorescence staining	52
2.4.2	ELISA	53
2.5	Hybridoma productions	53
2.5.1	Preparation of splenocytes	53
2.5.2	Cells fusion	54
2.5.3	Hybridoma screening	54
2.5.3.1	Screening of hybridomas by indirect immunofluorescence analysis	54
2.5.3.2	Screening of hybridomas by ELISA	55
2.5.4	Limiting dilution	55
2.6	Characterizations of monoclonal antibodies	56

2.6.1 Characterization of anti-CD4 mAbs by Western blotting	56
2.6.2 Determination of isotypes of monoclonal antibodies	56
2.6.3 Immunoprecipitation	57
2.6.4 Enumeration of the percentage of CD4 cells in peripheral blood lymphocytes	57
<b>CHAPTER III: RESULTS</b>	<b>59</b>
3.1 Immunogen preparation	59
3.1.1 Immunoprecipitated-beads technique	59
3.1.2 CD4-BCCP beads technique	62
3.1.2.1 Amplification of CD4 coding region	62
3.1.2.2 Expression of biotinylated CD4-BCCP protein	66
3.1.2.3 Determination of biotinylated CD4-BCCP	68
3.1.2.3.1 Determination of biotinylated CD4-BCCP by indirect ELISA	68
3.1.2.3.2 Determination of biotinylated CD4-BCCP by Western immunoblotting	70
3.1.2.4 Preparation of CD4-BCCP beads	71
3.1.3 CD4-COS cell expression technique	75
3.1.3.1 Preparation of CD4 DNA	75
3.1.3.2 Preparation of COS cell expressing CD4 protein	77
3.2 Mouse immunization	79
3.3 Hybridoma productions	87
3.4 Characterization of the generated monoclonal antibodies	92



3.4.1 Specificity of generated monoclonal antibodies characterization	92
3.4.2 Characterization of the generated anti-CD4 mAbs by Western immunoblotting and immunoprecipitation	95
3.4.2.1 Western immunoblotting	
3.4.2.2 Immunoprecipitation	94
3.4.3 Determination of isotype of the generated monoclonal antibodies	97
3.4.4 Enumeration of the percentage of CD4 <sup>+</sup> cells in peripheral blood lymphocytes by using the generated CD4 mAbs	99
<b>CHAPTER IV: DISCUSSIONS CONCLUSION</b>	101
<b>REFERENCES</b>	113
<b>APPENDIX</b>	121
<b>CURRICULUM VITAE</b>	141

**LIST OF TABLES**

<b>TABLE</b>	<b>PAGE</b>
1.1 Suggested dose of immunogens for mice	27
3.1 Summary of anti-CD4 monoclonal antibody productions using three different immunogens	89
3.2 The reactivity of generated mAbs with CD4 transfected COS cells and CD8 transfected COS cells	93
3.3 Determination of the percentage of CD4+ lymphocytes using the generated CD4 mAbs and standard reagent.	99

## LIST OF FIGURES

<b>FIGURE</b>	<b>PAGE</b>
1.1 Innate and adaptive immunity	6
1.2 Types of adaptive immunity	9
1.3 Effector functions of antibodies	11
1.4 The induction and effector phases of cell-mediated immunity	13
1.5 Effector functions of CMI	15
1.6 Metabolic pathways of DNA synthesis	17
1.7 Pathway of nucleotide synthesis in the present of antifolate drug	18
1.8 Monoclonal antibody production	21
1.9 The test sequence of an immunoprecipitation technique	34
3.1 Immunoprecipitation for preparation CD4 protein immunogen	61
3.2 Agarose gel electrophoresis of amplified PCR product	63
3.3 Agarose gel analysis of restriction of CD4 cDNA fragment and pAk400cb vector	63
3.4 Schematic illustration represents the pAK400cb-CD4 vector	64
3.5 Verification of pAk400cb-CD4	65
3.6 Characterization of pAk400cb-CD4	67
3.7 Determination of biotinylated CD4-BCCP by ELISA	69
3.8 Western blot analysis of bacterial extracts	71
3.9 Detection of biotinylated fusion protein	72
3.10 Flow cytometric analysis of captured CD4-BCCP	74
3.11 Immunofluorescence analysis of CD4-DNA transfected COS cells	76

3.12 Immunofluorescence analysis showing expression of CD4 protein in transfected COS cells	78
3.13 Analysis of antibody responses in mice sera immunized with CD4-BCCP beads	80
3.14 Determination of antibody responses in mice sera after immunization	81
3.15 Immunofluorescence analysis of CD4 antibodies in sera of CD4 immunoprecipitated beads immunization	83
3.16 Immunofluorescence analysis of CD4 antibodies in sera of CD4- BCCP immunization	84
3.17 Immunofluorescence analysis of CD4 antibodies in sera of CD4- COS cell immunization	85
3.18 Immunofluorescence of CD4 transfected COS cells with CD4 mAb MT4	86
3.19 Determination of CD4 antibody titer in sera of the immunized mice	87
3.20 ELISA analysis of the reactivity of mAb CD4-BCCP-1	91
3.21 Immunofluorescence analysis of specificity of generated monoclonal antibodies	93
3.22 Immunoprecipitation of CD4 protein from PBMCs using the generated CD4 mAbs	96
3.23 Determination of the isotype of the generated monoclonal antibodies	98



## ABBREVIATIONS

Ab	antibody
APS	ammonium persulphate
BSA	bovine serum albumin
BCCP	biotin carboxyl carrier protein
°C	degree Celcius
CD	cluster of differentiation
cDNA	complementary deoxyribonucleic acid
CMI	cell-mediated immunity
DEAE-Dextran	diethylaminoethyl-Dextran
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EtBr	ethidium bromide
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
g	gram or gravity
HAT	hypoxanthine aminopterin and thymidine
HGPRT	hypoxanthine guanine phosphoribosyltransferase
HMI	humoral-mediated immunity
Ig	immunoglobulin
IgG	immunoglobulin G

IgG1	immunoglobulin G1
IgG2a	immunoglobulin G2a
IgG2b	immunoglobulin G2b
IgG3	immunoglobulin G3
IgA	immunoglobulin A
IgM	immunoglobulin M
Igs	immunoglobulins
IMDM	iscove's modified Dulbecco's medium
kDa	kilodalton
M	molar
MEM	minimum essential medium
mg	milligram
ml	milliliter
mM	millimolar
mAb	monoclonal antibody
mA	milli ampere
NaN <sub>3</sub>	Sodium azide
O.D.	optical density
PBS	phosphate buffered saline
rpm	revolution per minute
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
μg	microgram
μl	microliter