### TABLE OF CONTENT

			Page
ACK	NOWL	EDGEMENTS	iii
ENG	LISH A	BSTRACT	iv
тна	I ABST	'RACT	vii
LIST	OF TA	ABLES	xv
LIST	OF FI	GURES	xvi
ABB	REVIA	TIONS	xviii
СНА	PTER	I: INTRODUCTION	
1.1	Stater	nent of problems	1
1.2	Litera	ture reviews	4
	1.2.1	Immune system	4
		1.2.1.1 Innate immunity	6
		1.2.1.2 Adaptive immunity	7
		1.2.1.2.1 Humoral mediated immunity	10
		1.2.1.2.2 Cell-mediated immunity	12
	1.2.2	CD4 molecule	/ <sup>17</sup> rsitv
	1.2.3	Hybridoma technology and monoclonal	24
		antibody production	
	1.2.4	Production of anti-CD4 monoclonal	30
		antibodies used in this study	
1.3	Object	tives	31

# Page

#### **CHAPTER II: MATERIALS AND METHODS**

2.1	Chemicals, antibodies and instruments used in this study 32		
2.2	Hybridoma cultivation and culture supernatant production 32		
2.3	Determination of specific reactivity of anti-CD4 mAbs		
	in cult	ture supernatants by COS cell transfection technique	
	2.3.1	COS cell transfection	32
	2.3.2	Indirect immunofluorescent assay of transfected	33
		COS cells with anti-CD4 mAbs	
2.4	Large	scale production and purification of anti-CD4 mAbs	34
	2.4.1	Large scale production of anti-CD4 mAbs	34
	2.4.2	Purification of anti-CD4 mAbs by	34
		affinity chromatography	
	2.4.3	Determination of the purity of purified	35
		anti-CD4 mAbs	
	2.4.4	Determination of the reactivity of purified anti-CD4	35
		mAbs by COS cell transfection technique	
2.5	Immu	noprecipitation of CD4 protein by anti-CD4 mAbs	35
	2.5.1	Biotinylation of cell surface molecules	35
	2.5.2	Preparation of cell lysates and immunoprecipitation	36
	2.5.3	Chemiluminescence detection system	37 <b>e</b>
2.6	Epitop	pe mapping of anti-CD4 mAbs by cross blocking analysis	37

		Page
	2.6.1 Preparation of FITC-conjugated purified anti-CD4	37
	mAbs	
	2.6.2 Peripheral blood mononuclear cells (PBMCs)	38
	preparation	
	2.6.3 Cross blocking experiment	38
2.7	Reactivity of anti-CD4 mAbs with CD4 molecule	39
	expressed on lymphocytes and monocytes	
	2.7.1 Determination of saturated concentration of	39
	anti-CD4 mAbs	
	2.7.2 Determination of anti-CD4 mAbs reactivity	39
	with lymphocytes and monocytes	
2.8	Study the effect of anti-CD4 mAbs on the regulation of	40
	anti-CD3 induced PBMCs proliferation	
	2.8.1 Immobilization of anti-CD3 mAb	40
	2.8.2 Carboxyfluorescein diacetate succinimidyl ester	40
	(CFSE) labeling of PBMCs	
	2.8.3 Proliferation assay	41
2.9	Study the effect of anti-CD4 mAbs on the regulation of	41
	anti-CD3/CD28 induced monocyte-depleted lymphocte	
	proliferation <b>B h t S h e s e</b>	
	2.9.1 Immobilization of anti-CD3 mAb	41
	2.9.2 CFSE labeling of monocyte-depleted lymphocytes	41

		Page
	2.9.3 Proliferation assay	42
2.10	Study the effect of anti-CD4 mAbs on monocyte oxidative	42
	burst induction	
	2.10.1 Phorbol myristate acetate (PMA) activation	42
	(Control group)	
	2.10.2 Effect of anti-CD4 mAbs on monocyte oxidative burst	43
	(Experimental group)	
СНАР	TER III: RESULTS	
31	Specificity of anti-CD4 monoclonal antibodies used in this study	44
3.2	Large scale production and purification of anti-CD4 mAbs	45
3.3	Immunoprecipitation of CD4 molecule using purified	50
	anti-CD4 mAbs	
3.4	Epitope recognition by anti-CD4 mAbs	52
3.5	Reactivity of anti-CD4 mAbs with CD4 molecules expressed	54
	on lymphocytes and monocytes	
	3.5.1 Determination of the saturated concentration of anti-CD4	54
	mAbs for immunefluorescence staining	
	ight by Chang Mar Only	
	3.5.2 Reactivity of anti-CD4 mAbs on CD4 molecules expressed	59 <del>-</del>
	on lymphocyte and monocyte surfaces	
3.6	Functional study of CD4 molecule	62

	Pa	age
3.6.1 Effect of the generated anti-CI	D4 mAbs on anti-CD3 62	2
induced PBMC proliferation		
3.6.2 Effect of the generated anti-CI	D4 mAbs on 6'	7
anti-CD3/CD28 induced mono	ocyte-depleted	
lymphocyte proliferation		
3.6.3 Effect of the generated anti-CI	D4 mAbs in 70	0
oxidative burst induction		
CHARTER IV. DISCUSSION & CONCL	USION 7	5
CHAFTER IV. DISCUSSION & CONCLU		2
REFERENCES	8	0
APPENDICES	8	9
APPENDIX A	90	0
APPENDIX B	9.	3
APPENDIX C	VER 94	4
APPENDIX D	93	5
CURRICULUM VITAE	10	)8

xiv

#### LIST OF TABLES



**ลิขสิทธิ์มหาวิทยาลัยเชียงใหม** Copyright<sup>©</sup> by Chiang Mai University All rights reserved

# LIST OF FIGURES

Figur	e งามยหตุ	Page
1.1	Innate and adaptive immunity	5
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 CD4+ lymphocytes	14
1.6	Mechanisms of cytotoxic T lymphocytes	16
1.7	Structure of CD4 molecule	21
1.8	Function of CD4 molecule	22
1.9	CD4 is a receptor for HIV	23
1.10	Metabolic pathways of DNA synthesis	25
1.11	Pathway of nucleotide synthesis in the present of	28
	antifolate drug	
1.12	Monoclonal antibody production	29
3.1	Immunofluorescence analysis of specific	47
	reactivity of anti-CD4 mAbs	
3.2	SDS-PAGE analysis of the purified anti-CD4 mAbs	48
3.3	Immunofluorescence analysis of specific reactivity of purified	49
	anti-CD4 mAbs	
3.4	Immunoprecipitation of CD4 molecule by purified	51
	anti-CD4 mAbs	

Figure		Page
3.5	Flow cytometric analysis of lymphocytes and monocytes	55
	stained with various concentrations of anti-CD4 mAbs	
3.6	The saturated concentration of anti-CD4 mAbs for	57
	determination of CD4 molecules expressed on	
	lymphocytes and monocytes	
3.7	Flow cytometric analysis of lymphocytes and monocytes	60
	by stained with anti-CD4 mAbs MT4, MT4/2, and MT4/3	
3.8	The reactivity of anti-CD4 mAbs MT4, MT4/2 , and MT4/3	61
	on lymphocytes (A) and monocytes (B)	
3.9	Effect of anti-CD4 mAbs on anti-CD3 induced	63
	lymphocyte proliferation	
3.10	Effect of anti-CD4 mAbs on lymphocyte proliferation	65
3.11	Effect of anti-CD4 mAbs on anti-CD3/CD28	68
	induced monocyte-depleted lymphocyte proliferation	
3.12	Effect of anti-CD4 mAbs MT4, MT4/2, and MT4/3 in	71
	the induction of monocyte oxidative burst	

Copyright<sup>©</sup> by Chiang Mai University All rights reserved

### ABBREVIATIONS

°C of the second	Degree Celsius
(v/v)	volume: volume ratio
%	Percentage
μg	Microgram
μί	Microliter
APC	Antigen presenting cell
BSA	Bovine serum albumin
CD	Cluster of differentiation
CFSE	Carboxyfluorescein
	diacetate succinimidyl ester
СМІ	Cell-mediated immunity
CTL 41 IIN	Cytotoxic T lymphocyte
DHE	Dihydroehidium
DMEM	Dulbecco's Modified Eagle Medium
E. coli	Escherichia coli
EDTA by Chia	Ethylenediamine tetraacetic acid
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
gp	Glycorotein
HGPRT	Hypoxanthine guanine phosphoribosyl
	transferase

xviii

HIV	Human immunodeficiency virus
HMI	Humoral mediated immunity
HRP	Horseradish peroxidase
IFN ANEL?	Interferon
Ig	Immunoglobulin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
	Interleukin
IMDM	Iscove's Modified Dulbecco's Medium
kDa	Kilodalton
L	Liter
LPS	Lipopolysaccharide
Μ	Molarity
mAb	Monoclonal antibody
mg	Milligram
МНС	Major histocompatibility complex
ml	Milliliter
MW	Molecular weight
NaCl	Sodium chloride
NaHCO <sub>3</sub>	Sodium bicarbonate
ng rignts	Nanogram S e la V e O
OD	Optical density
PAMPs	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell

PBS	Phosphate buffer saline
PE	Phycoerythrin
PGN	Proteins and peptidoglycans
pH	Power of hydrogen
РКС	Protein kinase C
РМА	Phorbol myristate acetate
PMSF	Phenylmethyl-sulphonylfluoride
RBC	Red blood cell
SD	Standard deviation
SDS-PAGE	sodium dodecyl sulfate-
	polyacrylamide gel electrophoresis
TCR	T cell receptor
T <sub>H</sub>	Helper T lymphocytes
TLR	Toll-like receptor
TNF	Tumor necrosis factor
ТуК	tyrosine kinase

**ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่** Copyright<sup>©</sup> by Chiang Mai University All rights reserved