

## TABLE OF CONTENTS

	<b>PAGE</b>
ACKNOWLEDGMENT	I
ABSTRACT	II
LIST OF TABLES	X
LIST OF FIGURES	XI
ABBREVIATIONS	XII
 I. INTRODUCTION	 1
 II. LITERATURE REVIEWS	 3
A. Leukocyte surface molecules	3
B. Cell surface receptors and signal transduction	3
C. Cell adhesion molecules (CAMs)	5
D. M6 molecule	7
E. Monoclonal antibodies	8
1. The monoclonal antibody technology in theory	8
2. Monoclonal antibodies are powerful immunochemical tools for the characterization of leukocyte surface antigens	11
F. Expression of cloned genes in cultured mammalian cells	13
 III. MATERIALS AND METHODS	 16
1. Reagents	16
2. Antibodies	17
3. Cell lines	18
4. DNA	19
5. Production of monoclonal antibodies to M6 molecule	20
5.1 Immunization of mouse	20
5.2 Production of hybridomas	20
5.2.1 Preparation of myeloma cells for fusion	20
5.2.2 Preparation of splenocytes for fusion	21
5.2.3 Preparation of splenocyte feeder	21
5.2.4 Fusion	22

5.3 Screening of hybridomas	22
5.3.1 Indirect Immunofluorescence analysis	23
5.3.2 Screening of hybridoma by COS cell expression system	23
5.3.2.1 Preparation of M6 DNA	23
5.3.2.1.1 Preparation of competent bacteria	23
5.3.2.1.2 Transformation of competent <i>E. coli</i> by M6 DNA	24
5.3.2.1.3 Screening of transformed bacterial colonies	24
5.3.2.1.3.1 Isolation of plasmid DNA from transformed bacteria	24
5.3.2.1.3.2 Restriction analysis of plasmid DNA	25
5.3.2.1.3.3 Large scale preparation of M6 DNA	26
5.3.2.2 Transfection of M6 DNA into COS cells by DEAE-dextran method	27
5.3.2.3 Screening of hybridoma produced anti-M6 monoclonal antibodies	28
5.4 Single-cell cloning by limiting dilution	28
5.5 Freezing and thawing of hybridoma and myeloma lines	29
5.6 Production of anti-M6 monoclonal antibodies	29
5.6.1 Collection of tissue culture supernatant	29
5.6.2 Collection of ascitic fluid	29
6. Determination of the isotype of monoclonal antibodies	30
7. Detection of M6 antigen on the cell surface of white blood cells	30
7.1 Isolation of peripheral blood mononuclear cells	30
7.2 Isolation of peripheral blood granulocytes	31
7.3 Detection of M6 antigen on leukocyte surface	31
8. Detection of M6 antigen on the cell surface of stimulated PBMC	31
9. Detection of M6 antigen on the cell surface of haematopoietic cell lines	31
10. Functional analysis of M6 molecule on the proliferation of haematopoietic cell lines	32
11. Optimization of the mitogen concentrations for PBMC stimulation	32
12. Functional analysis of M6 molecule on the proliferation of activated PBMC	32

IV. RESULTS	34
1. Production of Anti-M6 monoclonal antibodies	34
1.1. Preparation of M6 DNA and vector DNA	34
1.2. Immunization and cell fusion	37
1.3. Screening of hybridoma	37
1.4. Cloning of positive hybridoma by limiting dilution	39
1.5. Production of culture supernatant and ascitic fluid	39
2. Determination of the isotype of monoclonal antibodies	40
3. Expression of M6 molecule on haematopoietic cell lines	40
4. Expression of M6 molecule on peripheral blood leukocytes	40
4.1 Peripheral blood mononuclear cells (PBMC)	40
4.2 Granulocytes	46
5. Expression of M6 molecule on activated PBMC	49
6. The M6 molecule involved in cellular proliferation	53
6.1 Peripheral blood mononuclear cells (PBMC)	53
6.2 Haematopoietic cell lines	57
V. DISCUSSION	62
VI. SUMMARY	69
VII. REFERENCES	70
VIII. APPENDIX	77
IX. CURRICULUM VITAE	87

## LIST OF TABLES

TABLE		PAGE
1	Specification of all human haematopoietic cell lines used in this study	18
2	Specification of all animal cell lines used in this study	19
3	Expression of M6 molecule on peripheral blood mononuclear cells	44
4	Expression of M6 molecule on granulocytes	47
5	Effect of anti-M6 mAb (1B9 and 2G11) on proliferation of PHA activated PBMC	55
6	Effects of incubation times and concentrations of the K-562 cell line in the proliferation assay	58
7	Inhibitory effect of anti-M6 mAb on the proliferation of K-562 cell line	59
8	Inhibitory effect of anti-M6 mAb on the proliferation of Molt-4 cell line	59

# LIST OF FIGURES

FIGURE		PAGE
1	Basic protocol for derivation of monoclonal antibodies from hybridoma	10
2	Restriction analysis of M6 DNA and vector DNA isolated from transformed <i>E. coli</i> by plasmid miniprep	35
3	Photograph of M6 DNA transfected COS cells reacted with anti-M6 monoclonal antibody	36
4	Restriction analysis of M6 DNA and vector DNA isolated from transformed <i>E. coli</i> by Cesium Chloride-Ethidium bromide gradient ultracentrifugation	38
5	Determination of the isotype of anti-M6 mAb by capture ELISA	41
6	Expression of M6 molecule on haematopoietic cell lines	42
7	Photographs of cell lines given positive reaction with anti-M6 mAb	43
8	Expression of M6 molecule on peripheral blood mononuclear cells	45
9	Expression of M6 molecule on granulocytes	48
10	Expression of M6 molecule on PHA activated PBMC	50
11	Expression of M6 molecule on PPD activated PBMC	52
12	Determination of the suboptimal concentration of PHA for PBMC stimulation	54
13	Inhibitory effect of anti-M6 mAb on the proliferation of PHA activated PBMC	56
14	Inhibitory effect of anti-M6 mAb on the proliferation of K-562 cell line at 5 hours incubation	60
15	Inhibitory effect of anti-M6 mAb on the proliferation of Molt-4 cell line at 5 hours incubation	61

## ABBREVIATIONS

Ab	Antibody
BSA	bovine serum albumin
CD	cluster of differentiation
cm <sup>2</sup>	square centimeter
c.p.m.	count per minute
°C	degree celcius
CTL	cytotoxic T lymphocyte
DMSO	dimethyl sulphoxide
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorter
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
HAT	hypoxanthine aminopterin and thymidine
HGPRT	hypoxanthine guanine phosphoribosyltransferase
HCl	hydrochloric acid
IF	immunofluorescence
IgG	immunoglobulin G
IgM	immunoglobulin M
Igs	immunoglobulins
IL	interleukin
IMDM	Iscoe's Modified Dulbecco's Medium
kDa	kilodalton
KCl	potassium chloride
KHCO <sub>3</sub>	potassium bicarbonate
KH <sub>2</sub> PO <sub>4</sub>	potassium dihydrogen phosphate
MEM	minimal essential medium
MHC	major histocompatibility complex
mAb	monoclonal antibody
mM	milli molar
NK	natural killer
NaCl	sodium chloride
Na <sub>2</sub> HPO <sub>4</sub>	disodium hydrogen phosphate
NaHCO <sub>3</sub>	sodium bicarbonate
NH <sub>4</sub> Cl	ammonium chloride
OD	optical density

PBS	phosphate buffered saline
PBMC	peripheral blood mononuclear cell
PBSTween	Tween-20 in PBS
PEG	polyethylene glycol
PHA	phytohemagglutinin
PPD	purified protein derivative
rpm	revolution per minute
SDS	sodium dodecyl sulphate
SRBC	sheep red blood cells
$\mu$	micro
$\kappa$	kappa
$\alpha$	alpha
$\beta$	beta
$\gamma$	gamma
$\mu$ l	microliter
$\mu$ g	microgram
$\mu$ M	micromolar
$\mu$ m	micrometre
$\mu$ Ci	microcurie
ng	nanogram