

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	I
ABSTRACT	II
LIST OF TABLES	IX
LIST OF FIGURES	X
ABBREVIATIONS	XI
 I. INTRODUCTION	1
 II. LITERATURE REVIEWS	3
A. Leukocyte surface molecules	3
B. Monoclonal antibody	6
1. Principle of the monoclonal antibody technology	6
2. Monoclonal antibodies are powerful tools for the characterization of leukocyte surface antigens	6
C. Cell lines	10
 III. MATERIALS AND METHODS	12
1. Reagents	12
2. Antibodies	12
3. Cell lines	13
4. Isolation of peripheral blood mononuclear cells	14
5. Isolation of peripheral blood granulocytes	14
6. Isolation of leukocytes from leukemic whole blood	14
7. Indirect Immunofluorescence analysis	15
8. Detection of 1B2 molecule on the cell surface of stimulated PBMC	15
9. Optimization of the antigen and mitogen concentrations for PBMC stimulation	16
10. Assay of the effect of 1B2 mAb on the proliferation of activated PBMC	16
11. Assay of the effect of 1B2 mAb on the proliferation of U-937 and K-562	16

12. Assay for the cytotoxicity of 1B2 mAb	17
13. SDS-PAGE and Western blotting	17
14. Optimization of conditions for a chemiluminescent technique	17
15. Preparation of U-937 lysate	18
16. Immunoblotting for the detection of 1B2 mAb-binding protein	19
IV. RESULTS	20
1. Expression of 1B2 molecules on haematopoietic cell lines	20
2. Expression of 1B2 molecules on non-haematopoietic cell lines	20
3. Expression of 1B2 molecules on peripheral blood cells	24
4. Expression of 1B2 molecules on activated PBMC	24
5. Expression of 1B2 molecules on leukemic blood cells	27
6. The 1B2 molecules involved in cellular proliferation	38
7. Optimization of conditions for immunoblotting	59
8. Determination of the molecular weight of 1B2 molecules on U-937	59
V. DISCUSSION	62
VI. SUMMARY	70
VII. REFERENCES	71
VIII. APPENDIX	81
IX. CURRICULUM VITAE	92

LIST OF TABLES

TABLE		PAGE
1	Specifications of all the cell lines used in this study	13
2	Expression of 1B2 molecules on haematopoietic cell lines	21
3	Expression of 1B2 molecules on non-haematopoietic cell lines	21
4	Expression of 1B2 molecules on peripheral blood cells	25
5	Expression of 1B2 molecules on PHA activated PBMC	28
6	Expression of 1B2 molecules on ConA activated PBMC	30
7	Expression of 1B2 molecules on PPD activated PBMC	32
8	Expression of 1B2 molecules on rTNF α activated PBMC	34
9	Expression of 1B2 molecules on rGM-CSF activated PBMC	36
10	Expression of 1B2 molecules on leukemic blood cells	39
11	Effect of 1B2 mAb on proliferation of activated PBMC	43
12	Inhibitory effect of 1B2 mAb on the proliferation of U-937	46
13	Inhibitory effect of 1B2 mAb on the proliferation of K-562	49
14	Inhibitory effect of control mAb on the proliferation of U-937	52
15	Inhibitory effect of control mAb on the proliferation of K-562	55
16	Assay for the cytotoxicity of 1B2 mAb	58

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

LIST OF FIGURES

FIGURE	PAGE
1 Photographs of cell lines given positive reaction with 1B2 mAb	22
2 Expression of 1B2 molecules on haematopoietic cell lines	23
3 Expression of 1B2 molecules on peripheral blood cells	26
4 Expression of 1B2 molecules on PHA activated PBMC	29
5 Expression of 1B2 molecules on ConA activated PBMC	31
6 Expression of 1B2 molecules on PPD activated PBMC	33
7 Expression of 1B2 molecules on rTNF α activated PBMC	35
8 Expression of 1B2 molecules on rGM-CSF activated PBMC	37
9 Determination of the suboptimal concentration of PHA	40
10 Determination of the suboptimal concentration of ConA	41
11 Determination of the suboptimal concentration of PPD	42
12 Effect of 1B2 mAb on proliferation of activated PBMC	44
13 Inhibitory effect of 1B2 mAb on the proliferation of U-937 at 5 hours incubation	47
14 Inhibitory effect of 1B2 mAb on the proliferation of U-937 at 3 hours incubation	48
15 Inhibitory effect of 1B2 mAb on the proliferation of K-562 at 5 hours incubation	50
16 Inhibitory effect of 1B2 mAb on the proliferation of K-562 at 3 hours incubation	51
17 Effect of control mAb on the proliferation of U-937 at 5 hours incubation	53
18 Effect of control mAb on the proliferation of U-937 at 3 hours incubation	54
19 Effect of control mAb on the proliferation of K-562 at 5 hours incubation	56
20 Effect of control mAb on the proliferation of K-562 at 3 hours incubation	57
21 Optimization of conditions for immunoblotting	60
22 Determination of the molecular weight of 1B2 molecule on U-937	61

ABBREVIATIONS

APS	ammonium persulphate
Ab	antibody
BCG	Bacille Calmette Gue'rin
ConA	concanavalin A
cm ²	square centimeter
CTL	cytotoxic T lymphocyte
c.p.m.	count per minute
°C	degree celcius
DNP	dinitrophenylene
EDTA	ethylene diamine tetraacetic acid
FCS	fetal calf serum
HCl	hydrochloric acid
IgG	immunoglobulin G
IgA	immunoglobulin A
IgM	immunoglobulin M
Igs	immunoglobulins
IL	interleukin
KCl	potassium chloride
KHCO ₃	potassium bicarbonate
KH ₂ PO ₄	potassium dihydrogen phosphate
MHC	major histocompatibility complex
mAb	monoclonal antibody
mA	milli ampare
mM	milli molar
NK	natural killer
NaCl	sodium chloride
Na ₂ HPO ₄	disodium hydrogen phosphate
NaHCO ₃	sodium bicarbonate
NH ₄ Cl	ammonium chloride
NP-40	nonidet p-40
PBS	phosphate buffered saline
PBMC	peripheral blood mononuclear cell
PMSF	phenylmethylsulphonyl fluoride
PBSTween	0.05%Tween-20 in PBS
PPD	purified protein derivative
PHA	phytohemagglutinin

PPO	2,5-diphenyloxazole
POPOP	dimethyl POPOP
rTNF	recombinant Tumor Necrosis Factor
rGM-CSF	recombinant granulocyte-macrophage colony stimulating factor
rpm	revolution per minute
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS polyarylamide gel electrophoresis
TEMED	N,N,N,N-tetramethylenediamine
μ	micro
κ	kappa
α	alpha
β	beta
γ	gamma
μ l	microliter
μ g	microgram
μ M	micromolar
μ m	micrometer
μ Ci	microcurie
ng	nanogram

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved