

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	xi
LIST OF FIGURES	xv
ABBREVIATIONS	xviii
I. INTRODUCTION	1
II. LITERATURE REVIEW	
A. History of <i>P. marneffei</i>	4
B. Epidemiology: animal and soil studies	7
C. Morphological and cultural aspects	
1. Mycelial phase	8
2. Dimorphism and yeast like phase	8
3. Histopathology	8
D. Clinical manifestation	9
E. Laboratory diagnosis	10
F. Antifungal therapy of penicilliosis marneffei	11
G. Antigen and antibody detection of <i>P. marneffei</i>	12
H. HIV	
1. The structural of HIV	15
2. The life cycle of HIV	15
3. Immune response to HIV	
3.1 Human response to HIV	17
3.1.1 General characteristics	17
3.1.2 Neutralizing antibodies	18
3.2 Cell-mediated cytotoxicity to HIV	20
4. Mechanisms of CD4 ⁺ T cell killing	21
5. Functional abnormalities of CD4 ⁺ T cell and opportunistic infection	23

	PAGE
III. MATERIALS AND METHODS	
A. Subjects	
1. HIV negative donors	29
2. Patients	29
B. Blood collection and mononuclear leukocyte preparation	30
C. Preparation of crude sonicated <i>P. marneffei</i> antigen	30
D. Purification procedure	31
E. In vitro lymphocyte transformation test	
1. Determination of the suboptimal and optimal concentration of PHA-P	32
2. Determination of the optimal concentration of PBMC	32
3. Determination of the optimal concentration of PHA-P and PBMC by block titration method	33
4. Determination of the suboptimal and optimal concentration of crude sonicated <i>P. marneffei</i> antigen	33
5. Determination of the suboptimal and optimal concentration of PPD	34
6. Determination of the suboptimal and optimal concentration of crude sonicated <i>P. marneffei</i> antigen after gel filtration	34
7. Determination of the ability of lymphocytes transformation in AIDS patients stimulated with PHA-P	35
8. Determination of the ability of lymphocytes transformation in AIDS patients stimulated with crude sonicated <i>P. marneffei</i> antigen	35
9. Determination of the ability of lymphocytes transformation in AIDS patients stimulated with PPD	36
10. Identification of the active fraction of <i>P. marneffei</i> antigen after gel filtration	36
11. Determination of plasma from AIDS patient with <i>P. marneffei</i> infection inhibit proliferation of normal T cell	37
12. Determination of plasma from AIDS patient with <i>P. marneffei</i> infection inhibit proliferation of normal T cell	38

	PAGE
IV. RESULTTS	
In vitro lymphocyte transformation test	
1. Determiation of the suboptimal and optimal concentration of PHA-P	39
2. Determiation of the optimal concentration of PBMC	39
3. Determiation of the optimal concentration of PHA-P and PBMC by block titration method	45
4. Determiation of the suboptimal and optimal concentration of crude sonicated <i>P. marneffei</i> antigen	45
5. Determiation of the suboptimal and optimal concentration of PPD	50
6. Determiation of the suboptimal and optimal concentration of crude sonicated <i>P. marneffei</i> antigen after gel filtration	53
7. Determiation of the ability of lymphocytes transformation in AIDS patients stimulated with PHA-P	57
8. Determiation of the ability of lymphocytes transformation in AIDS patients stimulated with crude sonicated <i>P. marneffei</i> antigen	65
9. Determiation of the ability of lymphocytes transformation in AIDS patients stimulated with PPD	73
10. Identification of the active fraction of <i>P. marneffei</i> antigen after gel filtration	81
11. Determiation of plasma from AIDS patient with <i>P. marneffei</i> infection inhibit proliferation of normal T cell	84
12. Determiation of plasma from AIDS patient with <i>P. marneffei</i> infection inhibit proliferation of normal T cell	85
V. DISCUSSION	95
VI. SUMMARY	107
VII. REFERENCES	110
VIII. APPENDIX	125
IX. CURRICULUM VITAE	128

LIST OF TABLES

TABLE	PAGE
1a ³ H-thymidine incorporation of PBMC stimulated with various concentrations of PHA-P (0 to 8 µg/ml)	40
1b ³ H-thymidine incorporation of PBMC stimulated with various concentrations of PHA-P (0 to 1 µg/ml)	40
2 ³ H-thymidine incorporation by various concentrations of PBMC stimulated with PHA-P	43
3 The block titration to show the ability of various concentrations of PHA-P to induce PBMC proliferation at different concentration of PBMC	46
4 ³ H-thymidine incorporation by PBMC from HIV negative donors stimulated with various concentrations of crude sonicated <i>P. marneffei</i> antigen	48
5 ³ H-thymidine incorporation by PBMC from HIV negative donors stimulated with various concentrations of PPD	51
6 ³ H-thymidine incorporation by HIV negative PBMC after stimulated with various concentrations of crude sonicated <i>P. marneffei</i> or the fractions of <i>P. marneffei</i> antigen from gelfiltration	55
7 The suboptimal and optimal concentration (0.125 and 1.0 µg/ml, respectively) of PHA-P induced proliferation of PBMC from HIV negative donors	58

TABLE	PAGE
8 The suboptimal and optimal concentration (0.125 and 1.0 µg/ml, respectively) of PHA-P induced proliferation of PBMC from asymptomatic HIV infected individuals	59
9 The suboptimal and optimal concentration (0.125 and 1.0 µg/ml, respectively) of PHA-P induced proliferation of PBMC from AIDS patients without <i>P. marneffei</i> infection	60
10 The suboptimal and optimal concentration (0.125 and 1.0 µg/ml, respectively) of PHA-P induced proliferation of PBMC from AIDS patients with <i>P. marneffei</i> infection	61
11 The suboptimal and optimal concentration (0.125 and 1.0 µg/ml, respectively) of PHA-P induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection	62
12 The suboptimal and optimal concentration (0.32 and 2.56 µg/ml, respectively) of crude sonicated <i>P. marneffei</i> antigen induced proliferation of PBMC from HIV negative donors	66
13 The suboptimal and optimal concentration (0.32 and 2.56 µg/ml, respectively) of crude sonicated <i>P. marneffei</i> antigen induced proliferation of PBMC from asymptomatic HIV infected individuals	67

TABLE	PAGE
14 The suboptimal and optimal concentration (0.32 and 2.56 µg/ml, respectively) of crude sonicated <i>P. marneffei</i> antigen induced proliferation of PBMC from AIDS patients without <i>P. marneffei</i> infection	68
15 The suboptimal and optimal concentration (0.32 and 2.56 µg/ml, respectively) of crude sonicated <i>P. marneffei</i> antigen induced proliferation of PBMC from AIDS patients with <i>P. marneffei</i> infection	69
16 The suboptimal and optimal concentration (0.32 and 2.56 µg/ml, respectively) of crude sonicated <i>P. marneffei</i> antigen induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection	70
17 The suboptimal and optimal concentration (0.3125 and 10.0 µg/ml, respectively) of PPD induced proliferation of PBMC from HIV negative donors	74
18 The suboptimal and optimal concentration (0.3125 and 10.0 µg/ml, respectively) of PPD induced proliferation of PBMC from asymptomatic HIV infected individuals	75
19 The suboptimal and optimal concentration (0.3125 and 10.0 µg/ml, respectively) of PPD induced proliferation of PBMC from AIDS patients without <i>P. marneffei</i> infection	76
20 The suboptimal and optimal concentration (0.3125 and 10.0 µg/ml, respectively) of PPD induced proliferation of PBMC from AIDS patients with <i>P. marneffei</i> infection	77

TABLE	PAGE
21 The suboptimal and optimal concentration (0.3125 and 10.0 µg/ml, respectively) of PPD induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without with <i>P. marneffe</i> i infection	78
22 ³ H-thymidine incorporation by PBMC from HIV negative donors after stimulated with the fraction protein of <i>P. marneffe</i> i antigen	82
23 Inhibitory effect of the individual plasma (10%) from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection ³ H-thymidine incorporation by suboptimal concentration of PHA-P (0.125 µg/ml) stimulated normal PBMC	86
24 Inhibitory effect of the individual plasma (10%) from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection on ³ H-thymidine incorporation by optimal concentration of PHA-P (1.0 µg/ml) stimulated normal PBMC	87
25 The correlation of the concentration and inhibitory effect plasma from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection on ³ H-thymidine incorporation by suboptimal concentration of PHA-P (0.125 µg/ml) stimulated normal PBMC	91
26 The correlation of the concentration and inhibitory effect plasma from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection on ³ H-thymidine incorporation by optimal concentration of PHA-P (1.0 µg/ml) stimulated normal PBMC	92

LIST OF FIGURES

FIGURE		PAGE
1a	³ H-thymidine incorporation of PBMC stimulation with various concentrations of PHA-P (0 to 8 µg/ml)	41
1b	³ H-thymidine incorporation of PBMC stimulation with various concentrations of PHA-P (0 to 1 µg/ml)	42
2	³ H-thymidine incorporation by various concentrations of PBMC stimulated with PHA-P	44
3	The block titration to show the ability of various concentrations of PHA-P to induce PBMC proliferation at different concentration of PBMC	47
4	³ H-thymidine incorporation of PBMC stimulation with various concentrations of crude sonicated <i>P. marneffe</i> antigen	49
5	³ H-thymidine incorporation of PBMC stimulation with various concentrations of PPD	52
6	Sephadex G-100 column fraction of <i>P. marneffe</i> antigen	54
7	³ H-thymidine incorporation by HIV negative PBMC after stimulation with various concentrations of crude sonicated <i>P. marneffe</i> antigen or the fractions of <i>P. marneffe</i> antigen from gel filtration	56
8	The suboptimal concentration of PHA-P (0.125 µg/ml.) induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> infection in the presence of fetal bovine serum	63

FIGURE	PAGE
9 The optimal concentration of PHA-P (1.0 $\mu\text{g/ml}$.) induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection in the presence of fetal bovine serum	64
10 The suboptimal concentration of crude sonicated <i>P. marneffe</i> i antigen (0.32 $\mu\text{g/well}$) induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection in the presence of fetal bovine serum	71
11 The optimal concentration of crude sonicated <i>P. marneffe</i> i antigen (2.56 $\mu\text{g/well}$) induced proliferation of PBMC from HIVnegative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection in the presence of fetal bovine serum	72
12 The suboptimal concentration of PPD (0.3125 $\mu\text{g/ml}$) induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection in the presence of fetal bovine serum	79
13 The optimal concentration of PPD (10.0 $\mu\text{g/ml}$) induced proliferation of PBMC from HIV negative donors, asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffe</i> i infection in the presence of fetal bovine serum	80
14 ^3H -thymidine incorporation by PBMC from HIV negative donors after stimulation with crude sonicated <i>P. marneffe</i> i or the fractions of <i>P. marneffe</i> i antigen from gel filtration	83

FIGURE	PAGE
15 Inhibitory effect of the individual plasma (10%) from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection. ³ H-thymidine incorporation by suboptimal concentration of PHA-P (0.125 µg/ml.) stimulated of normal PBMC	88
16 Inhibitory effect of the individual plasma (10%) from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection. ³ H-thymidine incorporation by optimal concentration of PHA-P (1.0 µg/ml) stimulated of normal PBMC	89
17 The correlation of the concentration and inhibitory effect of plasma from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection on ³ H-thymidine incorporation of suboptimal concentration (0.125 µg/ml) stimulated normal PBMC	93
18 The correlation of the concentration and inhibitory effect of plasma from asymptomatic HIV infected individuals, AIDS patients without and with <i>P. marneffei</i> infection on ³ H-thymidine incorporation of optimal concentration (1.0 µg/ml) stimulated normal PBMC	94

ABBREVIATIONS

Ab	antibody
ADCC	antibody-dependent cell mediated cytotoxicity
Ag	antigen
AIDS	acquired immunodeficiency syndrome
BHA	brain heart infusion agar
BHI	brain heart infusion
°C	degree Celsius
CF	complement fixation
CMI	cell-mediated immunity
cpm	count per minute
CTLs	cytolytic T lymphocytes
EB-A1	monoclonal antibody against Aspergillus galactomannan
ELISA	enzyme-linked immunosorbent assay
FCS	fetal calf serum
x g	gravity
h	hour
HCl	hydrochloric acid
HIV	human immunodeficiency virus
HMI	humoral immunity
ID	immunodiffusion
IFAT	indirect immunofluorescent antibody test
IFN	interferon

Igs	immunoglobulins
IL	interleukin
lbs	pounds
kD	kilodalton
mAb	monoclonal antibody
mg	milligram
ml	milliliter
mM	millimolar
min	minute
NaCl	sodium chloride
NSS	normal saline solution
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffer saline
PHA	phytohemagglutinin
PPD	purified protein derivative
POPOP	dimethyl POPOP
PPO	2,5-diphenyloxazole
SDA	saboraud dextrose agar
μ Ci	microcurie
μ g	microgram
α	alpha
β	beta
γ	gamma