

## CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT IN THAI	iv
ABSTRACT IN ENGLISH	viii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xx
STATEMENTS OF ORIGINALITY IN THAI	xxvi
STATEMENTS OF ORIGINALITY IN ENGLISH	xxvii
CHAPTER 1 Introduction	1
1.1 Statement and significance of problem	1
1.2 Objectives of study	2
1.3 Education/application advantages	2
1.4 Literature reviews	2
1.4.1. Biology of viruses	2
1) Human immunodeficiency virus (HIV)	2
2) Influenza virus	4
3) Hepatitis B virus (HBV)	5
1.4.2. Immunity against viral infections in healthy population	7
1) Immunity against influenza virus infection	7
1.1) Innate immunity against influenza virus infection	7
1.2) Adaptive immunity against influenza virus infection	11

2)	Immunity against HBV infection	16
2.1)	Innate immunity against HBV infection	16
2.2)	Adaptive immunity against HBV infection	16
1.4.3.	Immunity against viral infection in HIV+ individuals	19
1)	Immunity against influenza virus infection in HIV+ individuals	20
2)	Immunity against HBV infection in HIV+ individuals	21
1.4.4.	Immune response against vaccination in healthy individuals	21
1)	Immunity against influenza virus vaccination	21
2)	Immunity against HBV vaccination	23
1.4.5.	Immune response against vaccination in HIV+ individuals	23
1)	Immunity against influenza virus vaccination	23
2)	Immunity against HBV vaccination	24
CHAPTER 2	Materials and methods	25
2.1	Human subject research ethics	25
2.2	Blood collection	25
2.3	Isolation of peripheral blood mononuclear cells (PBMCs) by gradient centrifugation	25
2.4	Cell stimulations	26
2.5	Determination of cytokine-secreting T cells and granzyme-producing T cells (CD107a+) in response to specific antigen by intracellular cytokine staining (ICS) technique	26
2.6	Flow-cytometric analysis	27
2.7	Statistical analysis	30
CHAPTER 3	Investigation of Cellular Immune Responses after 2009 H1N1 Influenza A Vaccination in HIV-infected Adults	31

3.1	Introduction	31
3.2	Methods	32
3.2.1.	Vaccination and blood collection	32
3.2.2.	Study population	32
3.2.3.	Isolation of PBMCs by gradient centrifugation	33
3.2.4.	Cell stimulation	33
3.2.5.	Determination of cytokine-secreting T cells and granzyme-producing T cells (CD107a+) in response to specific antigen by intracellular cytokine staining (ICS) technique	33
3.2.6.	Characterization of antigen-specific T cell phenotypes and subpopulations using cell surface staining technique	34
3.3	Results	34
3.3.1.	Cytokine production and CD107a expression of CD4 and CD8 T cells	34
3.3.2.	Cytokine production and CD107a-expression of memory CD4 and CD8 T cells	42
3.3.3.	Activation of 2009 H1N1 influenza A-specific memory T cell subpopulations	46
3.3.4.	Expression of co-stimulatory molecules CD28 and activation marker CD69 on T cells	49
3.3.5.	Expression of T-cell trafficking molecules	52
3.4	Discussion	54
CHAPTER 4 Investigation of Conserved Epitopes-Specific Memory CD8 T cells Responses in HIV+ Children after 2009 H1N1 Influenza A Vaccination		58
4.1	Introduction	58
4.2	Methods	59
4.2.1.	Vaccination and blood collection	59
4.2.2.	Study populations	59

4.2.3. Isolation of PBMCs by gradient centrifugation	60
4.2.4. Cell stimulations	60
4.2.5. Determination of cytokine-secreting T cells in response to specific antigen by ICS technique	61
4.3 Results	61
4.4 Discussion	66
CHAPTER 5 Cellular Immune Responses after Different Hepatitis B Vaccination Regimen in HIV-infected Individuals	68
5.1 Introduction	68
5.2 Methods	69
5.2.1. Vaccination and blood collection	69
5.2.2. Study population	70
5.2.3. Isolation of PBMCs by gradient centrifugation	71
5.2.4. Cell stimulation	71
5.2.5. Determination of cytokine-secreting T cells in response to specific antigen by ICS technique	71
5.3 Results	73
5.3.1. Cytokine production and CD107a expression of CD4 and CD8 T cells	73
5.3.2. Cytokine production and CD107a expression of CD4 and CD8 memory T cells	81
5.4 Discussion	87
CHAPTER 6 Conclusions	89
REFERENCES	91
LIST OF PUBLICATIONS	127
APPENDIX 128	
CURRICULUM VITAE	140

## LIST OF TABLES

	Page
<b>Table 3.1</b> Base line characteristic and vaccine response rate	33
<b>Table 3.2</b> Fold increase of cellular immune response in HIV-infected and healthy individuals after 2009 H1N1 influenza vaccine antigen stimulation <i>in vitro</i>	37
<b>Table 4.1</b> Baseline characteristic and vaccine response	60
<b>Table 4.2</b> HLA-restricted amino acid sequences of H1N1 influenza A epitopes obtained from the immune epitope database and analysis resource (IEDB) website (www.iedb.org).	61
<b>Table 4.3</b> The median and interquartile range of % cytokine producing CD8+ memory T cells in HIV+ children after pooled peptide stimulation <i>in vitro</i> .	63
<b>Table 5.1</b> Vaccination schedule of recombinant HBsAg vaccine in this study	70
<b>Table 5.2</b> Baseline demographics and clinical characteristics of participants by vaccination regimen.	72

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright © by Chiang Mai University  
All rights reserved

## LIST OF FIGURES

	Page
Figure 1.1. Schematic diagram of HIV-1 virus	3
Figure 1.2. Schematic diagram of an influenza A virion	5
Figure 1.3. Schematic diagram of HBV virion and subviral particles	6
Figure 1.4. Innate sensing of influenza virus infection via TLR3 (A) and TLR7 (B).	8
Figure 1.5. Innate sensing of influenza virus infection via RIG-I and NLRs-pyrin domain-containing 3.	9
Figure 2.1. Analysis of cytokine-producing and CD107a-expressing T cells	.28
Figure 2.2. Analysis of cytokine-producing and CD107a-expressing memory T cells..	29
Figure 3.1. Fold increase of cytokine-producing and CD107a-expressing T cells.	36
Figure 3.2. Fold increase of cytokine-producing and CD107a-expressing T cells in response to PHA stimulation	39
Figure 3.3. Fold increase of cytokine-producing and CD107a-expressing T cells between HIV+ with regard to absolute CD4+ T cell counts of 350 cells/mm <sup>3</sup> .	40
Figure 3.4. Fold increase of cytokine-producing and CD107a-expressing T cells between HIV+ with regard to absolute CD4+ T cell counts of ≤ 200, 201-500 and > 500 cells/mm <sup>3</sup>	41
Figure 3.5. Flow cytometry of cytokine producing memory T cells.	43
Figure 3.6. Fold increase of cytokine-producing and CD107a-expressing memory T cells between HIV+ groups with regard to absolute CD4+ T cell counts of 350 cells/mm <sup>3</sup> .	44

<b>Figure 3.7.</b> Fold increase of cytokine-producing and CD107a-expressing memory T cells between HIV+ groups with regard to absolute CD4+ T cell counts of $\leq 200$ , 201-500 and $> 500$ cells/mm <sup>3</sup> .	45
<b>Figure 3.8.</b> Increases of memory T cell subpopulations in response to 2009 H1N1 influenza A vaccine antigen.	48
<b>Figure 3.9.</b> Expression of activation markers of T cells after stimulation with 2009 H1N1 influenza A vaccine antigen.	50
<b>Figure 3.10.</b> Fold increase of inhibitory molecule-expressing total and memory T cells.	51
<b>Figure 3.11.</b> Expression of trafficking molecules on T cells after stimulation with 2009 H1N1 influenza A antigen	53
<b>Figure 4.1.</b> Cytokine production and CD107a expression of memory CD8+ T cells.	64
<b>Figure 4.2.</b> Cytokine production and CD107a expression of total CD8+ T cells at D56.	65
<b>Figure 5.1.</b> Fold increases of cytokine-producing and CD107a-expressing T cells in healthy control individuals.	74
<b>Figure 5.2.</b> Fold increases of cytokine-producing and CD107a-expressing T cells in the standard dose group of HIV+ individuals.	75
<b>Figure 5.3.</b> Fold increases of cytokine-producing and CD107a-expressing T cells in the four doses group of HIV+ individuals	76
<b>Figure 5.4.</b> Fold increases of cytokine-producing and CD107a-expressing T cells in the four double doses group of HIV+ individuals	77
<b>Figure 5.5.</b> Fold increases of cytokine-producing and CD107a-expressing T cells between study groups	79

- Figure 5.6.** Fold increases of cytokine-producing and CD107a-expressing T cells between groups with regards to serological responsiveness to the vaccine. 80
- Figure 5.7.** Fold increases of cytokine-producing and CD107a-expressing memory T cells in HIV- individuals. 82
- Figure 5.8.** Fold increases of cytokine-producing and CD107a-expressing memory T cells in the standard dose group of HIV+ individuals. 83
- Figure 5.9.** Fold increases of cytokine-producing and CD107a-expressing memory T cells in the four doses group of HIV+ individuals 84
- Figure 5.10.** Fold increases of cytokine-producing and CD107a-expressing memory T cells in the four double doses group of HIV+ individuals 85
- Figure 5.11.** Fold increases of cytokine-producing and CD107a-expressing memory T cells between study groups 86

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved



## LIST OF ABBREVIATIONS

ACD	Acid Citrate Dextrose
ACK	Ammonium-Chloride-Potassium
ADCC	Antibody-Dependent Cellular Cytotoxicity
AIDS	Acquired Immunodeficiency Syndrome
Abs	Antibodies
APCs	Antigen Presenting Cells
ART	Antiretroviral Therapy
ATP	Adenosine Triphosphate
ASCs	Antibody-Secreting Cells
CCR5	Chemokine (C-C motif) Receptor 5
CMI	Cell-Mediated Immune
CMU	Chiang Mai University
CMV	Cytomegalovirus
CTL	Cytotoxic T Lymphocyte
CXCR3	Chemokine (C-X-C motif) Receptor 3
DCs	Dendritic Cells
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DR	Death Receptor
dsDNA	Double Stranded Deoxyribonucleic Acid

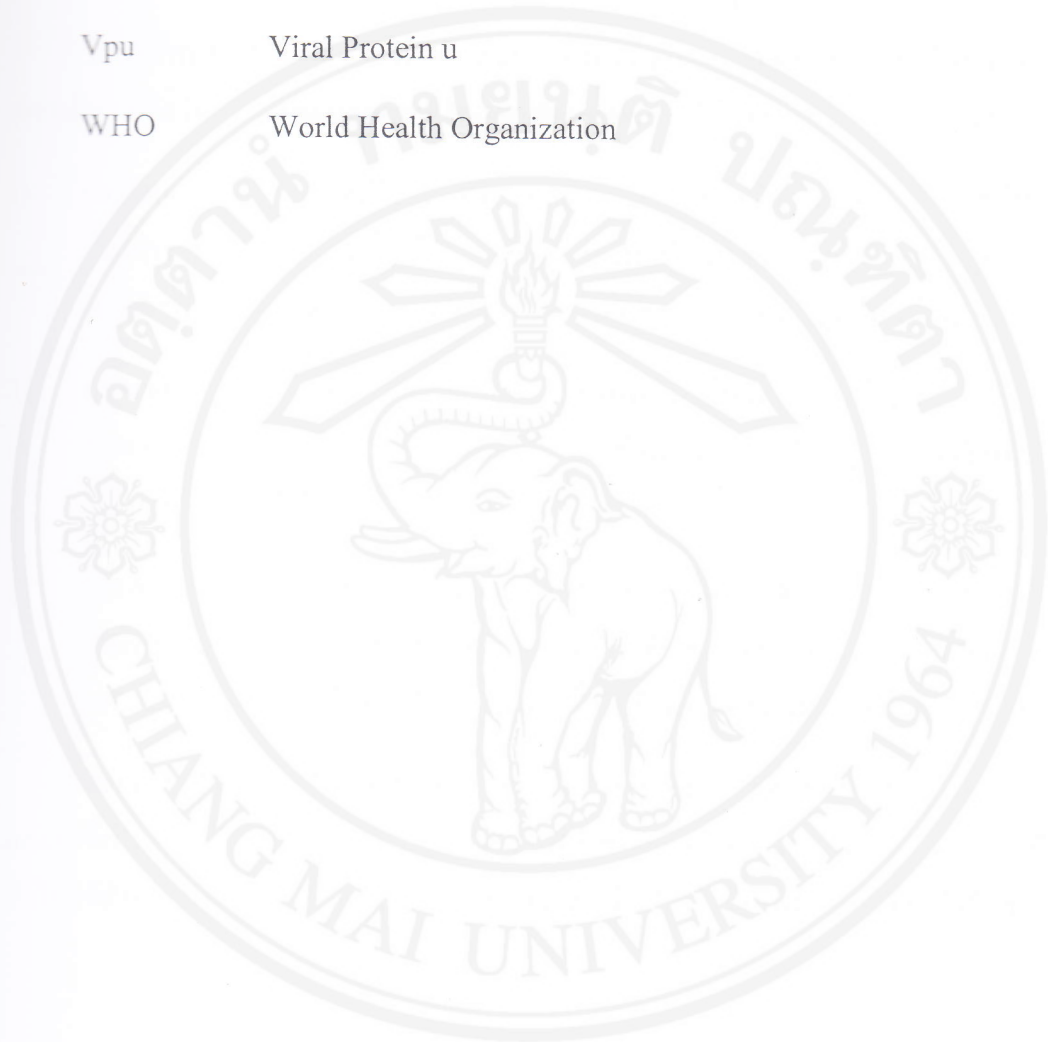
Env	Envelope
FASL	Fas Ligand
FBS	Fetal Bovine Serum
FcRL-4	Fc-Receptor-Like-4
GC	Germinal Centre
gp	Glycoprotein
HA	Haemagglutinin
HAART	Highly Active Antiretroviral Therapy
HAI	Haemagglutination Inhibition
HBcAg	Hepatitis B core Antigen
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSCs	Hepatic Stellate Cells
ICOS1	Inducible T-Cell Co-Stimulator
ICS	Intracellular Cytokine Staining
IEDB	Immune Epitope Database and Analysis Resource
IFNs	Interferons
Igs	Immunoglobulins
IL	Interleukin
IN	Integrase

IRF	IFN-Regulatory Factor
ISGs	IFN-Stimulated Genes
IU	International Unit
KCs	Kupffer Cells
LAIV	Live - Attenuated Influenza Vaccines
LAMPs	Lysosomal-associated Membrane Proteins
L-protein	Large Protein
LSEC	Liver Sinusoidal Endothelial Cells
M1	Matrix Protein 1
M2	Matrix Protein 2
MAbs	Monoclonal Antibodies
MAVS	Mitochondrial Antiviral Signalling Protein
MCPs	Monocyte Chemoattractant Proteins
MDA	Melanoma Differentiation Associate Gene
MHC	Major Histocompatibility Complex
MIPs	Macrophage Inflammatory Proteins
MOPH	Ministry of Public Health
M-protein	Medium Protein
mRNA	Messenger Ribonucleic Acid
NA	Neuraminidase
NCRs	Natural Cytotoxicity Receptors
Nef	Negative Effector
NF- $\kappa$ B	Nuclear Factor - Kappa B

NK	Natural Killer Cell
NKG	Natural Killer Cell Group
NKT	Natural Killer T Cell
NLRs	Nucleotide-binding Oligomerization Domain -Like Receptors
NLRP3	Nucleotide-binding Oligomerization Domain -Like Receptors – Pyrin Domain-containing 3
NP	Nucleoprotein
NSP1	Non-Structural Protein1
NSP2	Non-Structural Protein2
NEP	Nuclear Export Protein
NOD	Nucleotide-binding Oligomerization Domain
NPCs	Non-Parenchymal Cells
OAS	Oligoadenylate Synthetases
PA	Polymerase Acidic Protein
PAMP	Pathogen Associated Marker Pattern
PB1	Polymerase Basic Protein 1
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffer Saline
PD1	Programmed Cell Death Protein 1
pDCs	Plasmacytoid DCs
PHA	Phytohaemagglutinin
PMNs	Polymorphonuclear Cells
Pol	Polymerase

PR	Viral Protease
PRRs	Pattern Recognition Receptors
RBCs	Red Blood Cells
Rev	Regulator of Virion
RIG	Retinoic Acid - Inducible Gene
RIHES	Research Institute for Health Sciences
RLRs	Retinoic Acid - Inducible Gene-I-Like Receptors
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
S-protein	Small Protein
ssRNA	Single Stranded Ribonucleic Acid
Tat	Transactivator
T <sub>CM</sub>	Central Memory T Cell
TCR	T Cell Receptor
T <sub>EM</sub>	Effector Memory T Cell
T <sub>fh</sub>	Follicular T Helper Cell
TGF	Transforming Growth Factor
Th	Helper T Cell
TIV	Trivalent Inactivated Influenza Vaccines
TLRs	Toll-like Receptors
TNF	Tumour Necrosis Factor
TRAIL	Tumour Necrosis Factor -related Apoptosis-Inducing Ligand
Treg	Regulatory T Cell

Vif	Viral Infectivity Factor
VLA	Very Late Antigen
Vpr	Viral Proteins r
Vpu	Viral Protein u
WHO	World Health Organization



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright© by Chiang Mai University  
 All rights reserved

## ข้อความแห่งการริเริ่ม

วิทยานิพนธ์นี้ได้นำเสนอการตอบสนองของภูมิคุ้มกันชนิดเซลล์ต่อวัคซีนไข้หวัดใหญ่ 2009 เอช1เอ็น1 หรือวัคซีนไวรัสตับอักเสบบีในกลุ่มผู้ติดเชื้อเอชไอวี โดยนำเสนอเป็น 3 หัวข้อ

- 1) การแสดงออกบนผิวเซลล์ในระดับที่ต่ำของตัวบ่งชี้การกระตุ้น และตัวรับทีโมไคน์ที่เกี่ยวข้องกับการบอกตำแหน่งในเนื้อเยื่อที่มีการอักเสบบนเม็ดเลือดขาวชนิดทีเซลล์ที่ถูกกระตุ้นด้วยแอนติเจนของไข้หวัดใหญ่ 2009 เอช1เอ็น1 ในหลอดทดลองของกลุ่มผู้ติดเชื้อเอชไอวี
- 2) การขาดแคลนในการชักนำให้เกิดการตอบสนองต่อแอนติเจนบริเวณที่ไม่มีมีการเปลี่ยนแปลงที่จำเพาะกับเซลล์เม็ดเลือดขาวชนิดซีดีแปดทีเซลล์แบบเมมโมรี ในกลุ่มเด็กติดเชื้อเอชไอวีหลังได้รับวัคซีนไข้หวัดใหญ่ 2009 เอช1เอ็น1
- 3) การสร้างทีเอ็นเอฟ-แอลฟาในระดับต่ำของเซลล์เม็ดเลือดขาวชนิดซีดีสี่ทีเซลล์ต่อการตอบสนองต่อการกระตุ้นในหลอดทดลองด้วยโปรตีนเชื่อมต่อบริเวณผิวของไวรัสตับอักเสบบีในกลุ่มผู้ติดเชื้อเอชไอวีหลังได้รับวัคซีนต่อเชื้อไวรัสตับอักเสบบีมาตรฐานจำนวน 3 ครั้ง

ผลงานที่ได้นำเสนอในวิทยานิพนธ์นี้เกิดจากองค์ความรู้และความศรัทธาของข้าพเจ้า ซึ่งเป็นผลงานที่เป็นต้นฉบับที่เกิดจากการทำงานวิจัยของข้าพเจ้า โดยผลงานทั้งหมดหรือบางส่วนยังไม่เคยนำไปใช้สำหรับการขออนุมัติปริญญา ณ มหาวิทยาลัยเชียงใหม่แห่งนี้หรือมหาวิทยาลัยอื่นๆ

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright © by Chiang Mai University  
All rights reserved

## STATEMENTS OF ORIGINALITY

Cellular immune responses of HIV-infected individuals after vaccination with 2009 H1N1 influenza A or HBV vaccine are proposed in this thesis in order of three topics:

1. Low expression of activation marker and chemokine receptors that associate with localization in inflammatory tissues on memory T cells after 2009 H1N1 influenza A antigen stimulation *in vitro* following H1N1 vaccination of HIV-infected individuals.
2. Lack of induction of conserved epitope-specific memory CD8 T cell responses in HIV+ northern Thai children after 2009 H1N1 influenza A vaccination.
3. Low TNF- $\alpha$  production of CD4+ T cells in response to recombinant HBsAg stimulation *in vitro* following standard 3 doses HBV vaccination of HIV-infected individuals.

The work presented in this thesis is to my knowledge and belief, original and my own work that has not been submitted, either in whole or in part, for a degree at this or any other university.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved