

ความแตกต่างของ CD8⁺ T lymphocytes ที่จำเพาะต่อ Nef และ Gag
ในการควบคุมการติดเชื้อ HIV-1 ในผู้ป่วยคนไทย

นางสาวชุตีมา กิตติธนานุกูล

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**THE DIFFERENCE OF NEF AND GAG-SPECIFIC CD8⁺ T LYMPHOCYTE
RESPONSES IN CONTROL OF HIV-1 INFECTION IN THAI PATIENTS**

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ศูนย์วิทยทรัพยากร

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ชุตินา กิตติชนานุกูล: ความแตกต่างของ CD8⁺ T lymphocytes ที่จำเพาะต่อ Nef และ Gag ในการควบคุมการติดเชื้อ HIV-1 ในผู้ป่วยคนไทย (The Difference of Nef and Gag-specific CD8⁺T Lymphocyte Responses in Control of HIV-1 Infection in Thai Patients.) อาจารย์ที่ปรึกษา: อ. นพ. ดร. ปกรณ์ หงส์สุด; 185 หน้า. ISBN 974-53-2656-9

นักวิทยาศาสตร์เชื่อว่า CD8⁺ T lymphocytes มีบทบาทสำคัญในการควบคุมการติดเชื้อเอชไอวี แต่ความจำเพาะของการตอบสนองที่ต่างกันของ CD8⁺ T lymphocytes อาจส่งผลต่อประสิทธิภาพในการควบคุมระดับเชื้อเอชไอวี ดังนั้นเราจึงทำการศึกษาความจำเพาะในการตอบสนองของ CD8⁺ T lymphocytes ต่อเชื้อเอชไอวีในกลุ่มคนที่มีความสามารถควบคุมการติดเชื้อได้ต่างกัน การศึกษานี้ทำการทดสอบหาการตอบสนองของ CD8⁺ T lymphocytes ต่อ Nef และ Gag ในผู้ติดเชื้อจำนวน 35 คนที่มีปริมาณไวรัสแตกต่างกัน ตลอดจนคู่สามีภรรยาที่มีการสัมผัสเชื้อหลายครั้งแต่ยังคงไม่ติดเชื้อจำนวน 11 คนและผู้ที่ติดเชื้อจำนวน 9 คนด้วยวิธี ELISpot assay และทำการศึกษากลไกของ escape mutation ในผู้ที่ไม่พบการตอบสนอง

ผลการศึกษาพบว่าเปปไทด์ที่พบการตอบสนองสูงสุดและมีความแรงสูงสุด ได้แก่ Nef ตามด้วย p24 Gag p17 Gag และ p2p7p1p6 Gag ตามลำดับ โดยมีผู้ติดเชื้อที่ตอบสนองต่อ Nef protein จำนวน 30 คน (85.7%) และผู้ตอบสนองต่อ Gag protein จำนวน 16 คน (45.7%) อย่างไรก็ตามไม่พบความสัมพันธ์ระหว่างความแรงและความกว้างในการตอบสนองกับปริมาณไวรัส นอกจากนี้ยังไม่พบความแตกต่างของการตอบสนองระหว่างกลุ่มผู้ติดเชื้อแต่ละกลุ่มซึ่งมีปริมาณไวรัสที่แตกต่างกัน ในผู้ติดเชื้อที่ไม่พบการตอบสนองพบว่ามี mutation ในบริเวณ epitope หรือในส่วน flanking region ส่วนการศึกษาในกลุ่มคู่สามีภรรยาที่ฝ่ายหนึ่งติดเชื้อแต่อีกฝ่ายหนึ่งไม่ติดเชื้อพบว่ามีความจำเพาะของการตอบสนองที่ต่างกกัน โดยผู้ไม่ติดเชื้อตอบสนองต่อ Nef 7 Nef 8 และ Nef 9 ผู้ติดเชื้อตอบสนองต่อ Nef 9 Nef 14 และ Nef 15 ดังนั้น Nef 7 และ Nef 8 อาจมีบทบาทสำคัญในการป้องกันการติดเชื้อเอชไอวี

สาขาวิชา จุลชีววิทยาทางการแพทย์
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ลายมือชื่อนิติ.....ชุตินา กิตติชนานุกูล.....
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CD8⁺ T lymphocytes are believed to play an important role in the control of Human Immunodeficiency Virus (HIV) infection. Recent studies showed that specificities of CD8⁺ T lymphocytes might have an effect on efficiency of protective immune response. We therefore screened HIV-1-specific CD8⁺ T lymphocytes in 35 HIV-1-seropositive Thais with different level of HIV RNA and in 11 high risk HIV-1-seronegative Thais and their HIV-1-seropositive partners using of Nef and Gag peptides based gamma interferon-enzyme-linked immunospot (ELISpot) assay. In addition, we analysed the amino acid sequences of relevant HIV proteins in non-responsive subjects to study HIV escape mutation.

The results demonstrated that the most frequently recognised peptides and the strongest responses were located in Nef, followed by p24 Gag, p17 Gag, and p2p7p1p6 Gag, respectively. There are 30 subjects (85.7%) responded against Nef protein and there are 16 subjects (45.7%) responded against Gag protein. However, neither the breadth nor the magnitude of HIV-1-specific CD8⁺ T lymphocyte responses correlated with plasma viral load. In subgroup analysis, there was no significant difference of responses among individuals with different HIV RNA load. In subjects whose the responses could not be detected had amino acid mutations either within epitope or in the flanking region. However, in case of the discordant couples, we found the different antigenicity recognition against Nef protein. One high risk HIV-1-seronegative subject had vigorous responses against Nef 7, Nef 8, and Nef 9 whilst their partner responded against Nef 9, Nef 14, and Nef 15. These results may indicate that the CD8⁺ T cells specific for Nef 7 and Nef 8 peptides play a critical role in control of HIV-1 infection.

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CONTENTS

	Page
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	xii
LIST OF FIGURES.....	xiv
ABBREVIATIONS.....	xvii
CHAPTER	
I INTRODUCTION.....	1
II OBJECTIVES.....	3
III LITERATURE REVIEW.....	4
Discovery of HIV-1.....	4
Origin of HIV.....	5
Structure and molecular biology of HIV.....	7
The structural proteins.....	9
1. Gag.....	9
2. Pol.....	11
3. Env protein (p88) gp160.....	12
The regulatory proteins.....	13
1. Tat.....	13
2. Rev.....	13
The accessory proteins.....	14
1. Nef.....	14
2. Vif.....	14
3. Vpr.....	15
4. Vpu.....	15
HIV life cycle.....	15

CHAPTER	Page
Binding.....	15
Entry.....	16
Uncoating and reverse transcription.....	17
Nuclear entry.....	17
Integration and transcription.....	17
Translation and assembly and budding.....	18
The genetic diversity of HIV-1.....	18
The genetic diversity of HIV-1 in Thailand.....	20
The immune responses.....	23
Innate immunity.....	23
Adaptive immunity.....	23
The immune responses to viral infection.....	24
Mechanisms of cytotoxic T lymphocytes (CTL) mediated cytolysis.....	25
The structure of MHC I.....	25
Cytolytic pathway for antigen processing and presentation.....	28
HIV-1 specific CD8 ⁺ T lymphocyte responses.....	32
Cytotoxic T-Lymphocyte (CTL) assays.....	33
1. Limiting dilution assay (LDA).....	33
2. Interferon gamma (IFN- γ) ELISpot assay.....	34
3. Intracellular cytokine staining (ICS).....	35
4. Cytotoxic T-Lymphocyte (CTL) assay.....	35
5. MHC tetramer binding assay.....	36
HIV viral load assays.....	36
1. The HIV-1 Quantiplex (bDNA) assay.....	36
2. The AMPLICOR HIV-1 MONITOR assay.....	37
3. The NucliSens HIV-1 assay.....	39
IV MATERIALS AND METHODS.....	41
Materials	
Part I Population and subjects.....	41

CHAPTER

Page

Study groups and sample size determination.....	41
Part II Specimen collections and processing.....	41
1. Specimen collection.....	41
2. Specimen processing.....	41
3. HLA typing.....	42
Methods	
Part I Peptide preparation.....	43
1. Peptide selection.....	43
2. Design of peptide matrices.....	43
Part II Study of CD8 ⁺ T lymphocyte responses.....	49
1. Peptide-based IFN- γ ELISpot assay.....	49
2. CD8 ⁺ T lymphocyte depletion assay.....	50
3. Cultured ELISpot assay.....	50
Part III HIV DNA preparation.....	51
1. PHA blast.....	51
2. Dried packed cells preparation.....	51
3. HIV DNA extraction.....	51
4. Quantitation the amount of extracted DNA.....	51
Part IV Producing PCR product.....	52
1. Nef gene amplification.....	52
2. Gag gene amplification.....	52
Part V HIV DNA cloning.....	54
1. PCR purification.....	54
2. Competent cells preparation.....	54
3. Cloning Nef gene into pCR [®] 2.1.....	54
4. Transforming Nef gene into competent cells.....	55
5. Cloning Gag gene into pCR [®] 2.1.....	55
6. Transforming Gag gene into competent cells.....	56
7. Plasmid extraction.....	56

CHAPTER

Page

	Part VI HIV DNA sequencing.....	58
V	RESULTS.....	59
	1. Demographic data.....	59
	1.1 HIV-1-seropositive Thais.....	59
	1.2 High risk HIV-1-seronegative Thais and their partners.....	64
	1.3 Low risk HIV-1-seronegative controls.....	67
	2. HIV-1-specific CD8 ⁺ T cell responses.....	70
	2.1 HIV-1-specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais	70
	2.1.1 The frequencies of HIV-1-specific CD8 ⁺ T lymphocyte recognition in HIV-1-seropositive Thais	73
	2.1.2 Breadth and magnitude of HIV-1-specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais	74
	2.1.3 Correlation between viral load and Nef and Gag- specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais.....	81
	2.1.4 The frequencies of HIV-1-specific CD8 ⁺ T lymphocyte recognition at different viral load quartiles.....	86
	2.1.5 Breadth and magnitude of HIV-1-specific CD8 ⁺ T lymphocyte responses at different viral load quartiles.....	88
	2.2 HIV-1-specific CD8 ⁺ T lymphocyte responses in high risk HIV-1- seronegative Thais and their partners.....	96
	2.2.1 HIV-1-specific CD8 ⁺ T lymphocyte responses in high risk HIV-1-seronegative Thais after augmentation.....	104
	2.3 HIV-1-specific CD8 ⁺ T lymphocyte responses in HIV-1- seronegative controls.....	106
	3. HIV-1-specific T cell responses following CD8 ⁺ T lymphocyte depletion.....	108
	4. Cloning and sequencing of <i>nef</i>	110
	4.1 Sensitivity of PCR assay	110

CHAPTER	Page
4.2 Amplification of <i>nef</i>	112
4.3 Cloning of <i>nef</i>	117
4.4 Detection of <i>nef</i> inserts	118
4.5 Sequencing of <i>nef</i>	120
5. Cloning and sequencing of <i>gag</i>	136
5.1 Sensitivity of PCR assay	137
5.2 Amplification of <i>gag</i>	139
5.3 Cloning of <i>gag</i>	143
5.4 Detection of <i>gag</i> inserts	144
5.5 Sequencing of <i>gag</i>	146
VI DISCUSSION.....	160
REFERENCES.....	164
APPENDICES.....	178
Appendix I.....	179
Appendix II.....	181
Appendix III.....	184
BIOGRAPHY.....	185

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Table	Page
1. Full-genome sequences of HIV-1.....	21
2. Example of peptide matrix setup for Nef ^a	44
3. Peptides marix setup for Nef and Gag.....	45
4. Nef peptide sequences (HIV-1 CRF01_AE strain CM240)	46
5. Gag peptide sequences (HIV-1 subtype A strain 92UG037).....	47
6. Method of quartiles calculation.....	60
7. Range of viral load quartiles.....	61
8. Clinical information of HIV-1-seropositive Thais.....	62
9. HLA typing of HIV-1-seropositive Thais.....	63
10. Clinical information of high risk HIV-1-seronegative Thais.....	65
11. Clinical information of HIV-1-seropositive partners.....	66
12. Clinical information low risk HIV-1-seronegative controls.....	68
13. The summary and the average of the clinical information of overall donors.....	69
14. The modified matrix system for 21 Nef peptides.....	72
15. HIV-1-specific CD8 ⁺ T lymphocyte responses to Gag in HIV-1-seropositive Thais.....	75
16. HIV-1-specific CD8 ⁺ T lymphocyte responses to Nef in HIV-1-seropositive Thais.....	77
17. HIV-1-specific CD8 ⁺ T lymphocyte responses to Gag in high risk HIV-1-seronegative Thais and their partners.....	98
18. HIV-1-specific CD8 ⁺ T lymphocyte responses to Gag in high risk HIV-1-seronegative Thais and their partners.....	100
19. HIV-1-specific CD8 ⁺ T lymphocyte responses after depletion of CD8 ⁺ T lymphocyte by immunomagnatic beads.....	109
20. Possible epitopes in amino acid sequences of the subject NKP based on anchor residues.....	124
21. Possible epitopes in amino acid sequences of the subject PMH based on anchor residues.....	126

Table

Page

22. Possible epitopes in amino acid sequences of the subject TKH based on anchor residues.....	129
23. Possible epitopes in amino acid sequences of the subject NKP based on anchor residues.....	150



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

Figure	Page
1. Drawing of the HIV virion.....	7
2. Electron micrograph of HIV virion.....	8
3. Genomic organization of HIV-1 and HIV-2.....	9
4. HIV life cycle.....	18
5. CRF01_AE Reference strain : CM240 Subtypes : A, E.....	22
6. Geographic distribution of HIV-1 types, groups, subtypes, and CRF.....	22
7. Subtype distributions represent the frequency in the HIV Database and not the population.....	22
8. Innate and adaptive immunity against viral infection.....	24
9. Drawing of HLA class I molecule.....	27
10. Location and organization of HLA complex.....	27
11. Ribbon model of the tertiary structure of HLA class I peptide-binding module.....	28
12. Proteasome composition.....	30
13. Formation of the immunoproteasome.....	31
14. processing and presentation of viral antigen to CD8 ⁺ T lymphocyte through MHC Class molecules.....	31
15. Example of Nef and Gag-specific CD8 ⁺ T lymphocyte responses of subject MWT in preliminary study.....	71
16. Percentage of HIV-1-seropositive Thais recognizing Nef and Gag peptides.....	78
17. Frequency of recognition of the individual protein and protein subunits in HIV-1-seropositive Thais.....	79
18. Comparison of breadth and magnitude between protein and protein subunits in HIV-1-seropositive Thais.....	80
19. Correlation between viral load and breadth and magnitude of the total HIV-1-specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais	82
20. Correlation between viral load and breadth and magnitude of the total Nef-specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais	83

Figure	Page
21. Correlation between viral load and breadth and magnitude of the total Gag-specific CD8 ⁺ T lymphocyte responses in HIV-1-seropositive Thais	84
22. Correlation between viral load and breadth and magnitude of CD8 ⁺ T lymphocyte responses against Nef 9 in HIV-1-seropositive Thais	85
23. Comparison of frequency of recognition between viral load quartiles.....	87
24. Comparison of breadth of the total HIV-1-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	89
25. Comparison of breadth of the total Nef-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	90
26. Comparison of breadth of the total Gag-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	91
27. Comparison of magnitude of the total HIV-1-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	92
28. Comparison of magnitude of the total Nef-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	93
29. Comparison of magnitude of the total Gag-specific CD8 ⁺ T lymphocyte responses between the different viral load quartiles.....	94
30. Comparison of magnitude of CD8 ⁺ T lymphocyte responses against Nef 9 between the different viral load quartiles.....	95
31. Percentage of HIV-1-seropositive partners recognizing Nef and Gag.....	101
32. Frequency of recognition of the individual protein and protein subunits in HIV-1-seropositive partners.....	102
33. Comparison of breadth and magnitude between Nef and Gag proteins in HIV-1-seropositive partners.....	103
34. HIV-1-specific CD8 ⁺ T lymphocyte responses to Nef and Gag proteins in subject ASP.....	105
35. HIV-1-specific CD8 ⁺ T lymphocyte responses in low risk HIV-1-seronegative controls.....	107
36. Sensitivity of the nested PCR assay.....	111

Figure	Page
37. The position of outer and inner primers in HIV-1 subtype A/E reference strain (U54771).....	113
38. Gel electrophoresis of amplified <i>nef</i> products from proviral DNA of subject NKP, PMH, TKH, VMK, and VPT.....	114
39. Gel electrophoresis of amplified <i>nef</i> products from proviral DNA of subject TKH, VMK, and VPT.....	115
40. The position of outer and inner primers in HIV-1 subtype A/E reference strain (U54771).....	116
41. The representative amplified <i>nef</i> products from the <i>nef</i> -inserted clones analysed by PCR assay.....	119
42. Alignment of Nef amino acid sequences.....	123
43. Possible HLA-A24 and B*4002-restricted epitopes in Nef amino acid sequences of the subject NKP based on anchor residues.....	127
44. Possible HLA-A24, B44, and B27-restricted epitopes in Nef amino acid sequences of the subject PMH based on anchor residues.....	131
45. Possible HLA-A*1101 and A*0207-restricted epitopes in Nef amino acid sequences of the subject TKH based on anchor residues.....	134
46. Sensitivity of the semi-nested PCR assay.....	138
47. The position of outer and inner primers in HIV-1 subtype A/E reference strain (U54771).....	140
48. Gel electrophoresis of amplified <i>gag</i> products from proviral DNA of subject NKP, VMK, and VPT.....	141
49. Gel electrophoresis of amplified <i>gag</i> products from proviral DNA of subject VPT.....	142
50. Gel electrophoresis of amplified <i>gag</i> products from the <i>gag</i> -inserted clones analysed by PCR assay.....	145
51. Alignment of Gag amino acid sequences.....	147
52. Possible HLA-A24 and B*4002-restricted epitopes in Gag amino acid sequences of the subject NKP based on anchor residues.....	156

ABBREVIATIONS

aa	amino acid
Ab	Antibody
ACD	Acid citric dextrose
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
APC	Antigen presenting cell
bdNA	branched DNA
β_2m	beta 2 microglobulin
BLCL	B lymphoblastoid cell line
bp	Base pair
CA	Capsid
Ca^{2+}	calcium 2 ⁺
$CaCl_2$	Calcium Chloride
CD	Cluster of differentiation
CMI	Cell mediated immunity
CO_2	Carbon dioxide
CRF	Circulating recombinant form
^{51}Cr	Chromium-51
CTL	Cytotoxic T lymphocyte
CTLp	Cytotoxic T lymphocyte precursor
cu.mm.	cubic millimeter
$^{\circ}C$	degree celsius
DDW	Double-deionized distilled water
DMSO	Dimethyl sulphoxide
DNA	Deoxy nucleic acid
dNTP	Deoxyribonucleotide triphosphate
Dw	Distilled water
EDTA	Ethylenediamine tetraacetic acid
ELISpot	Enzyme-linked immunospot

Env	Envelope
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
Gag	Group-specific antigen
gp	glycoprotein
Group M	Major group
Group N	New group
Group O	Outlier group
HEPS	Highly Exposed but Persistently seronegative persons
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMI	Humoral immunity
HRP	Horseradish peroxidase
ICS	Intracellular cytokine staining
IFN- α	Interferon alpha
IFN- β	Interferon beta
IFN- γ	Interferon gamma
IL	interleukin
IL-2	interleukin 2
IL-7	interleukin 7
IN	Integrase
INr	Initiator
IVDU	Intravenous drug user
kb	kilobase
kD	kilodalton
KS	Kaposi's sarcoma
LDA	Limiting dilution assay
LTNP	Long-term nonprogressor
LTR	Long terminal repeat
MA	Matrix
mg	milligram

MgCl ₂	Magnesium chloride
MHC	Major histocompatibility complex
MIP-1 α	Macrophage inflammatory protein-1 alpha
MIP-1 β	Macrophage inflammatory protein-1 beta
ml	milliliter
mM	millimolar
mRNA	messenger ribosomal nucleic acid
μ g	microgram
μ l	microliter
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NC	Nucleocapsid
Nef	Negative factor
NES	Nuclear export signal
NK cell	Natural killer cell
ng	nanogram
nm	nanometer
OD	Optical density
p	protein
PA28	Proteasome activator
PAMPs	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline
PCP	<i>Pneumocystis carinii</i> pneumonia
PCR	polymerase chain reaction
PHA	Phytohemagglutinin
PIC	Preintegration complex
pmole	picomole
Pol	Polymerase
PR	Protease
PRR	Pattern recognition receptors

qs	quantitation standard
RANTES	Regulated upon activation, normal T expressed and secreted
Rev	Regulatory of expression of viral protein
RNA	Ribonucleic acid
RNase H	Ribonuclease H
RNAP II	RNA polymerase II
rpm	round per minute
RPMI 1640	Rosewell park memorial institute formular 1640
RRE	Rev-responsive element
RT	Reverse Transcriptase
SAIDS	Simian AIDS
SCID	Severe combinded immunodeficiency
SEB	Staphylococcal enterotoxin B
SFU	Spot-forming unit
SIV	Simian immunodeficiency virus
SR	Spontaneous release
TAP	Transporter associated with processing
TAP 1	Transporter associated with antigen 1
TAP 2	Transporter associated with antigen 2
T cell	Thymus-derived lymphocyte
Tat	Transactivator of transcription
TAR	Transactivation response element
TCR	T cell receptor
Th cell	Helper T cell
TM	Transmembrane
TNF- α	Tumour necrosis factor alpha
TR	Total release
Tris	Tris-(hydroxymethyl)-aminoethane
Vif	Viral infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U