

การตอบสนองของ HLA-A*1101 restricted HIV-specific CD8+ T lymphocyte

ในผู้ติดเชื้อ HIV ที่ไม่มีอาการ



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HLA-A*1101-RESTRICTED HIV-SPECIFIC CD8+ T LYMPHOCYTE RESPONSES IN
ASYMPTOMATIC HIV-INFECTED PATIENTS



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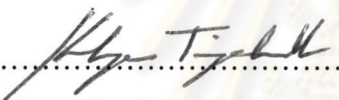
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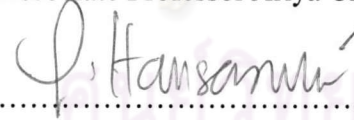
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Human immunodeficiency virus type1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) พบว่ามีบทบาทสำคัญในการควบคุมการติดเชื้อไวรัส HIV การวิจัยนี้จึงทำการศึกษาการตอบสนองของ CTL ที่จำเพาะกับ HLA-A11 ซึ่งเป็น HLA class I allele ที่พบมากที่สุดจากประชากรของประเทศ และหาความสัมพันธ์ระหว่างการตอบสนองของ CTL ดังกล่าวและปริมาณไวรัส นอกจากนี้ยังทำการศึกษาลำดับ amino acid ในผู้ติดเชื้อที่ไม่มีอาการตอบสนอง หรือมีการผันแปรของการตอบสนองต่อ immunodominant epitope เพื่อศึกษากลไก escape mutation ของเชื้อ HIV การวิจัยนี้ทำการทดสอบ HLA-A11-restricted epitope เป็นจำนวนทั้งสิ้น 17 ชิ้น ในผู้ติดเชื้อ HIV-1 ที่มี HLA-A11 จำนวน 18 คน โดยวิธี ELISpot assay จากการวิจัยพบว่า โปรตีน Nef (QVPLRPMTYK และ GAFDLSFFLK peptide) เป็นโปรตีนที่กระตุ้น T cell ได้ดีที่สุด (18/18) รองลงมาได้แก่ โปรตีน Pol (16/18) Env (6/18) และ Gag (4/18) ตามลำดับ และไม่พบว่ามีความสัมพันธ์ระหว่างการตอบสนองของ CTL กับปริมาณไวรัส ในผู้ป่วยส่วนใหญ่ ส่วนการศึกษาลำดับของ amino acid ในผู้ติดเชื้อที่มีความผิดปกติของการตอบสนองต่อ immunodominant Nef epitope พบว่ามีกลไกการเกิด mutation ในบริเวณ epitope หรือในส่วน flanking region แสดงว่าการกลายพันธุ์ดังกล่าวอาจเป็นสาเหตุสำคัญของการไม่ตอบสนองต่อ epitopes เหล่านี้ ผลจากการศึกษานี้ยืนยันว่า Nef เป็น immunodominant protein และเหมาะสมที่จะเป็นส่วนประกอบที่สำคัญในการพัฒนาวัคซีนสำหรับทดสอบในประเทศไทยต่อไป

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Human immunodeficiency virus type1 (HIV-1)-specific cytotoxic T lymphocytes (CTL) play a major role in the controlling HIV infection. In the present study, we analysed HIV-specific HLA-A11-restricted CTL responses in asymptomatic HIV-infected Thai patients which HLA-A11 is the most common HLA class I allele in Thailand. We also established the correlation between HIV viral load and CTL responses against the immunodominant epitope, and analysed the amino acid sequences in some patients who had fluctuation or absence of responses against the immunodominant epitopes to study HIV immune escape. In this experiment, seventeen of HLA-A11-restricted epitopes were analysed by the ELISpot assay in the eighteen HLA-A11 positive HIV-infected patients. The results showed that Nef protein (QVPLRPMTYK and GAFDLSFFLK peptides) was the most immunodominant (18/18) protein followed by Pol (16/18), Env (6/18) and Gag (4/18). There was no correlation between HIV viral load and the CTL responses against immunodominant epitope in most patients. Most patients who showed no responses against immunodominant epitopes had amino acid mutation either within epitope or in the flanking region. This study supported the immunodominance of Nef protein which was conserved even in the presence of strong selective pressure. This protein is therefore should be included in the HIV vaccine construct.

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ABBREVIATIONS

aa	Amino acid
AIDS	Acquired Immunodeficiency Syndrome
APC	Antigen presenting cell
β_2m	beta 2 microglobulin
BLCL	B lymphoblastoid cell line
bp	Base pair
CA	Capsid
Ca^{2+}	calcium 2+
CD	Cluster of differentiation
CO_2	Carbon dioxide
CRF	Circulating recombinant form
Cr-51	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
FBS	Fetal bovine serum
Gag	Group-specific antigen
gp	Glycoprotein

Group M	Major group
Group N	New group
Group O	Outlier group
h	hour
HEPS	Highly Exposed but Persistently seronegative persons
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IFN- γ	Interferon gamma
IL	interleukin
IL-2	interleukin 2
IL-7	interleukin 7
IN	Integrase
kb	kilobase
kD	kilodalton
LTNP	Long-term nonprogressor
LTRs	Long terminal repeats
MA	Matrix
mg	milligram
MgCl ₂	Magnesium chloride
MHC	Major histocompatibility complex
min	minute
MIP	Macrophage inflammatory protein
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

NK cell	Natural killer cell
ng	Nanogram
nm	Nanometer
OD	Optical density
p	Protein
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline
PCR	polymerase chain reaction
PHA	Phytohemagglutinin
pmole	Picomole
Pol	Polymerase
PR	Protease
RANTES	Regulated upon activation, normal T expressed and secreted
Rev	Regulatory of expression of viral protein
RNA	Ribonucleic acid
RNaseH	Ribonuclease H
rpm	round per minute
RPMI 1640	Rosewell park memorial institute formular 1640
RRE	Rev-responsive element
RT	Reverse Transcriptase
SFU	Spot-forming unit
SIV	Simian immunodeficiency virus
SR	Spontaneous release
TAP	Transporter associated with processing
T cell	Thymus-derived lymphocyte
Tat	Transactivator of transcription
TCR	T cell receptor
Th cell	Helper T cell
TNF	Tumour necrosis factor
TR	Total release
Tris	Tris-(hydroxymethyl)-aminoethane

Vif	Viral infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U



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จุฬาลงกรณ์มหาวิทยาลัย