HLA-B*35:05 is a protective allele with a unique structure amongst CRF01_AE-infected Thais.

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Abstract

Background:
Most of CTL epitopes and their restricted HLA alleles information in Los Alamos database have been derived from subtype B-infected Caucasians, and from subtype C-infected Africans. However, studies from south-east Asia, where about 1.5 million patients are living with HIV,and where about 90% of them are infected with subtype CRF01_AE, remain sparse.
Although HLA-B*57 is a protective allele amongst Africans and Caucasians, its prevalence amongst Asians is lower than other ethnic groups.

Methodology:
1) 557 HIV-1 CRF01_AE-infected Thais were recruited, and their HLA type and viral load associations were statistically analyzed.
2) *In silico* molecular dynamics was used to evaluate the effect of HLA structure variants on epitope binding.
3) Recently HLA-B*35-restricted epitope Gag 253-262 NPPIPVGDIY, which D260E mutation causing HLA-epitope binding instability and consequent viral load increase was reported. Mutation at this site was statistically analyzed amongst HLA-B*35:05 positives and HLA-B*35:01 positives.

Results:
1) HLA-B*35:05 was identified as the most protective allele (p=0.003 and q=0.17), along with HLA-B*57:01 (p=0.044 and q=0.31).
2) Structurally HLA-B*35:05 belongs to PY, however, analysis of 3D HLA structure and molecular dynamics showed that Ser (S) at 97 in HLA-B*35:05 led to (1) less flexibility in the groove, and (2) shorter distances between the α-helixes compared to the other PY, HLA-B*35:01, suggesting fewer peptide variants will be able to bind to the groove, leading to induction of stronger virus-specific immunity by HLA-B*35:05.
3) In the viral sequence at Gag 253-262 NY10, mutations were significantly identified amongst HLA-B*35:01 positives. However, there was no significant mutations amongst HLA-B*35:05 positives in this site.

Conclusions:
Our data indicates the existence of a protective effect of HLA-B*57 across ethnic groups and highlights HLA-B*35:05 as an allele uniquely protective in subtype CRF01_AE-infected Thais, due to its divergence from conventional PY structural sequences at the peptide-binding groove.
Identification of unique protective alleles in each endemic area provides the opportunity to better define the nature of HLA-mediated immune control and will be valuable for CTL vaccine development.

Background

- Genome wide association study (GWAS) reported class I HLA allele as the best contribution factor for HIV viral control.
- About 1,500 of CTL epitopes including 250 best-defined epitopes and their restricting HLA allele data have been accumulated in the Los Alamos database.
- However, most of these have been derived from subtype B-infected Caucasians, and from subtype C-infected Africans. Studies from south-east Asia, where about 1.5 million patients are living with HIV, and where about 90% of them are infected with subtype CRF01_AE, remain sparse.
- Although HLA-B*57 is a protective allele amongst Caucasians and Africans, its prevalence amongst Asians is lower than other ethnic groups.

Our objective here is to identify protective class I HLA alleles amongst HIV-1 CRF01_AE-infected Thais.

Methodology

Subjects:
557 treatment naïve HIV-1 CRF01_AE-infected patients from Lampang hospital, Thailand.
- 300 female and 257 male
- Med. age 32 years old (range 15 - 63)



Data:
- CD4+ T cell count at enrollement: Med. 86 counts/ul (range 0 - 1,191)
- Viral load: Med. 5.26 log copies/ml (range 2.60 - 6.72)
- Gag sequence of 216 patients from our previous report (Gesprasert G et al. PLoS ONE 2010)

***In silico* molecular dynamics:**
- 3D structure template: 2CIK (HLA-B*35:01)
- 3D structure construction of HLA-B*35:05 by MOE® (CCG, Canada)
- Distance measurament between α-helixes of residues 74 Tyr (Y) and 147 Trp (W), which are adjacent with residue 97, by NAMD.

Statistical analysis:
- Viral load difference amongst class I HLA alleles
- Viral load difference amongst supertype B07 comprising alleles
- Distance difference of α-helixes between HLA-B*35:01 and HLA-B*35:05
- D260E frequency difference between HLA-B*35:01 positives and HLA-B*35:05 positives

Results

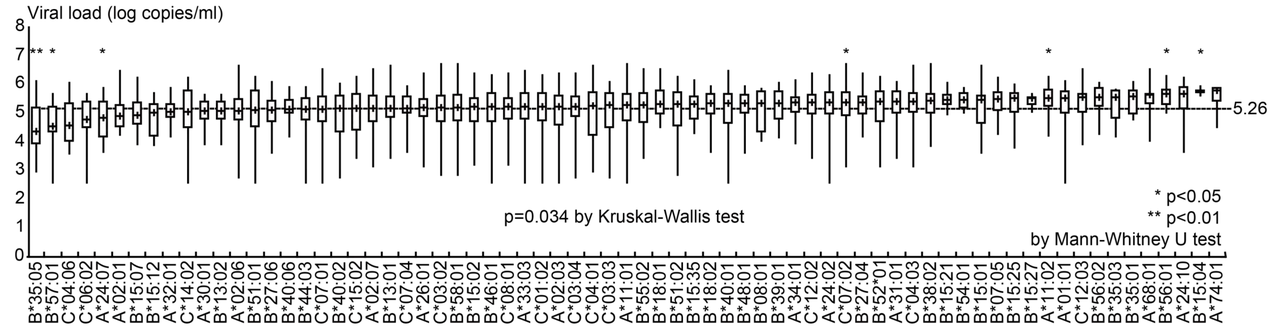


Figure 1. Class I HLA allele expression and viral load association.
The association between the 71 class HLA alleles expressed by >3 individuals and viral load was analyzed using the Kruskal-Wallis test. A Mann-Whitney U test was performed to compare viral load in subjects with and without the alleles. Including false discovery rate analysis, HLA-B*35:05 was identified as the most protective allele (p=0.0030 and q=0.17), along with HLA-B*57:01 (p=0.044 and q=0.31).

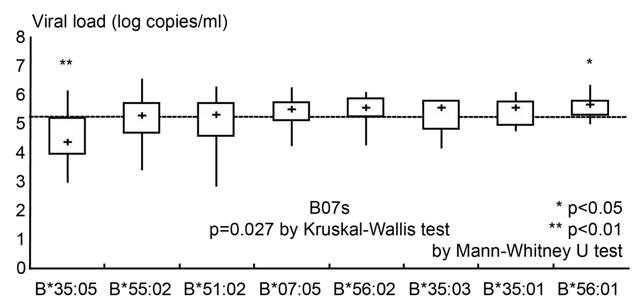


Figure 2. Viral load difference amongst HLA supertype comprising alleles.
The viral load difference amongst supertype B07 comprising alleles was analyzed using the Kruskal-Wallis test. A Mann-Whitney U test was performed to compare viral load in subjects with and without the alleles. Including false discovery rate analysis, HLA-B*35:05 was identified as the protective allele (p=0.0030 and q=0.021), and HLA-B*56:01 as the susceptible allele (p=0.011 and q=0.037).

	Residue				
HLA	94	95	97	114	116
B*35:01 (PY)	I	I	R	D	S
B*35:03 (Px)	-	-	-	N	F
B*35:05 (PY)	T	L	S	-	-

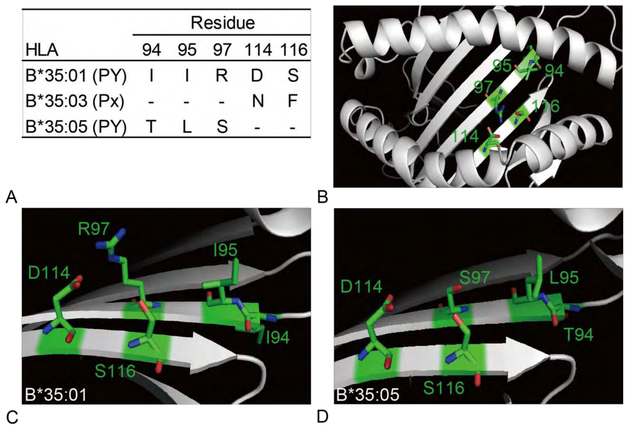
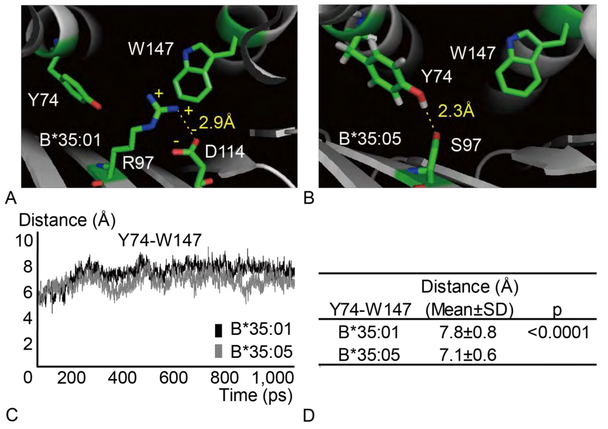


Figure 3. HLA sequence differences at residues 94, 95, 97, 114, and 116 amongst HLA-B*35 alleles, and 3D crystal structure difference between HLA-B*35:01 and HLA-B*35:05.
A hyphen (-) indicates the concordant sequence with HLA-B*35:01 at each residue (A). HLA sequence difference sites listed in (A) are shown. View from above (B), side of HLA-B*35:01 (C) and HLA-B*35:05 (D) are shown.



	Distance (Å)	
Y74-W147	(Mean±SD)	p
B*35:01	7.8±0.8	<0.0001
B*35:05	7.1±0.6	

Figure 4. Sequence interactions with residue 97, and distance analysis between α-helix structures by *in silico* program NAMD.
Salt bridge between R97 and D114 in HLA-B*35:01 (A), and hydrogen bond between Y74 and S97 in HLA-B*35:05 with hydrogen figure in Y74 (B) are shown. Distance between α-helix structures of Y74 and W147 was analyzed by *in silico* molecular dynamics program of NAMD (C), with significant shorter distance in HLA-B*35:05 by Student's t-test (D).

ID	A	A	B	B	C	C	NNPPIPVGDIY
206	A*02:03	A*11:02	B*18:02	**B*35:01**	C*04:01	C*07:04	D - - - V - - - E - -
381	A*11:01	A*33:03	B*15:02	**B*35:01**	C*04:01	C*08:01	S - - - - - - - - L -
453	A*24:02	A*24:02	B*15:25	**B*35:01**	C*07:02	C*12:03	S - - - - - - - - - -
1349	A*02:03	A*11:01	**B*35:01**	B*38:02	C*03:03	C*07:02	S - - - - - - - E F -
2097	A*24:02	A*31:01	**B*35:01**	B*46:01	C*01:02	C*04:01	S - - - - - - - E - -
115	A*02:06	A*11:01	**B*35:05**	B*46:01	C*01:02	C*04:01	- - - - - - - - - - -
1013	A*02:03	A*11:01	**B*35:05**	B*52:01	C*04:01	C*07:01	S - - - - - - - - - -
1497	A*02:01	A*11:01	B*13:01	**B*35:05**	C*04:01	C*04:06	S - - - - - - - - - -
1651	A*02:06	A*11:01	**B*35:05**	B*40:06	C*04:01	C*08:01	- - - - - - - - - - -

Table 1. Sequence difference at the epitope Gag p24 253-262 NPPIPVGDIY (NY10) between HLA-B*35:01 positives and HLA-B*35:05 positives.
Sequence difference at the epitope Gag p24 253-262 NPPIPVGDIY (NY10) with its flanking N252 between HLA-B*35:01 positives and HLA-B*35:05 positives amongst 216 patients from our previous report are shown. Significant D260E mutation amongst HLA-B*35:01 positives (p=0.00012 by Fisher's exact test, OR 104, and 95% CI 12-868), but not amongst HLA-B*35:05 positives, was identified.

Conclusions

These data indicate the existence of a protective effect of HLA-B*57 across ethnic groups and highlights HLA-B*35:05 as an allele uniquely protective in subtype CRF01_AE-infected Thais. The divergence of HLA-B*35:05 from conventional HLA-B*35-PY structural sequences at the peptide-binding groove is consistent with previous studies that have identified HLA residue 97 as strongly influential in shaping HLA impact on immune control of HIV, and that a more restricted peptide binding motif may be associated with improved control.

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