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Effect of KIR2D-mediated immunity on clinical outcome among CRF01_AE-infected Thais.

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Abstract

Background:
Class I human leukocyte antigen (HLA) molecule contributes to the immune control of HIV through antigen presentation to both cytotoxic T lymphocytes (CTL) and natural killer cells (NK). CTL contribution to HIV control through antigen presentation by protective HLA alleles, such as HLA-B*57 and HLA-B*27, has been studied well previously. However, reports on the NK cell contribution, with the exception of the protective effect of HLA-Bw4 and its ligand killer immunoglobulin-like receptor (KIR) 3DL1 and 3DS1 in HIV disease, are limited. In particular, studies about HLA-C-KIR2D on HIV control remain sparse.

Methodology:
209 HIV-1 CRF01_AE-infected treatment-naïve Thais were recruited, and the effects of HLA-C-KIR2D on clinical outcome were statistically analyzed.

Results:
1) HLA-B*46:01, which worked as an HLA-C1 allele binding the same type of ligands despite being an HLA-B allele, was identified as the most predominant HLA-B allele (29% in the population frequency).
2) In the analysis of the viral set point difference among HLA-C1-positive individuals, subjects with KIR2DL3 had significantly higher set point compared to the subjects without KIR2DL3 (median 4.8 vs 4.2 log copies/ml, p=0.033 by Mann-Whitney U-test).
3) In multivariate analysis, the number of HLA-C1-KIR2DL3 associations (ranging from subjects having no HLA-C1-KIR2DL3 associations to subjects having up to four combinations; the KIR2DL3-positive individuals with HLA-B*46:01 homozygous and HLA-C1 allele homozygous) was identified as a significant viral load predictor (β=0.13, p=0.039 by linear regression model).
4) In longitudinal analysis, higher mortality rate was identified among the subjects with higher number of HLA-C1-KIR2DL3 associations (p=0.002 by log rank test; aHR=2.0, 95% CI 1.3-3.2, p=0.004 by Cox hazard model).
5) However, no advantage of HLA-Bw4-KIR3DL1/S1 on clinical outcome was found in this cohort.

Conclusions:
In this study, the effects of HLA-C and KIR2D interaction with a focus on the effect of the detrimental HLA-C1-KIR2DL3 association, on clinical outcome among HIV-1 CRF01_AE-infected Thais were investigated. These findings will impact the existence of the unique anti-HIV innate immune pressure and viral adaptation to such pressure in each endemic area.

Background

- Genome wide association study (GWAS) reported class I HLA allele as the best contribution factor for HIV viral control, and that interacts with both cytotoxic T lymphocytes (CTLs) through their T cell receptors (TCR), and natural killer (NK) cells through their killer immunoglobulin-like receptor (KIR).

- Compared to CTLs, the contribution of NK cells to HIV viral control *in vivo* is less clear.

- Whether there is an association between KIR2D-HLA-C receptor-ligand interactions and HIV viral control or clinical outcome, as has been demonstrated for KIR3DL1/S1-HLA-Bw4 interactions and HIV viral load, has not been fully investigated.

Our objective here is to identify the effect of KIR2D receptors and their interaction with their HLA-C ligands on viral control in an HIV-infected cohort from South-east Asia, where HIV-1 CRF01_AE is the dominant circulating subtype of virus.

Methodology

Subjects and data:
209 treatment naïve HIV-1 CRF01_AE-infected patients from Lampang hospital, Thailand.
- 159 female and 50 male
- Med. age 31 years old (IQR 27-36)
- CD4+ T cell count at enrollement: Med. 396 /ul (IQR 286-519)
- Viral load at enrollment: Med. 4.7 log copies/ml (IQR 4.2-5.2)
- KIR2DL1+ 205 (98%)
- KIR2DL2+ 78 (37%)
- KIR2DL3+ 204 (98%)
- KIR2DS1+ 90 (43%)
- KIR2DS2+ 80 (38%)
- KIR3DL1+ 203 (97%)
- KIR3DS1+ 78 (37%)



Statistical analysis:
- The effect of KIR-HLA receptor-ligand combinations on VL was tested with Mann-Whitney U tests, and with linear regression model for multivaraite analysis.
- A log rank test was performed for the longitudinal analysis of survival rates, then a Cox hazard model for multivariate analysis was applied.

Results

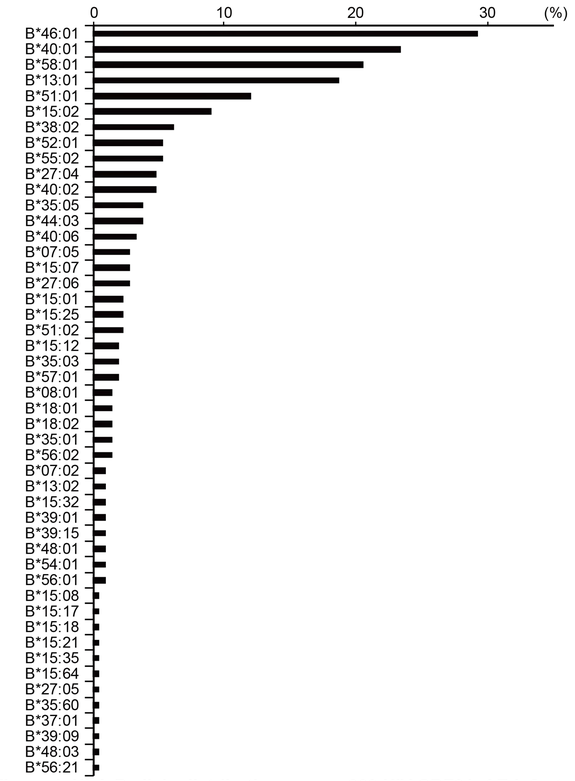


Figure 1. HLA-B allele distribution among 209 HIV CRF01_AE-infected patients. Population frequency is shown.

	VL (log copies/ml) (Median (IQR) (Num.)) KIR+	KIR-	p (U-test)
HLA-C1+ n=205			
KIR2DL2	4.8 (4.2-5.2) (77)	4.7 (4.2-5.2) (128)	0.74
KIR2DL3	4.8 (4.2-5.2) (200)	4.2 (4.2-4.3) (5)	0.033
KIR2DS2	4.7 (4.2-5.2) (79)	4.7 (4.2-5.2) (126)	0.75
HLA-C2+ n=45			
KIR2DL1	4.3 (3.9-4.8) (45)	-	-
KIR2DS1	4.2 (3.7-4.8) (17)	4.5 (4.1-4.9) (28)	0.14
HLA-B*46:01+ n=61			
KIR2DL2	5.0 (4.5-5.4) (23)	4.6 (4.0-4.9) (38)	0.017
KIR2DL3	4.7 (4.2-5.1) (60)	4.4 (4.4-4.4) (1)	0.50
KIR2DS2	4.8 (4.4-5.4) (25)	4.6 (4.1-5.0) (36)	0.13
HLA-Bw4+ n=135			
KIR3DL1	4.7 (4.2-5.2) (133)	4.4 (4.0-4.8) (2)	0.68
KIR3DS1	4.5 (4.1-5.1) (50)	4.8 (4.4-5.2) (85)	0.23
HLA-Bw4 80I+ n=81			
KIR3DL1	4.5 (4.1-5.2) (81)	-	-
KIR3DS1	4.4 (4.2-4.9) (26)	4.6 (4.1-5.2) (55)	0.47
HLA-C1/C1+ n=164			
KIR2DL2	4.9 (4.4-5.3) (61)	4.7 (4.3-5.2) (103)	0.48
KIR2DL3	4.8 (4.3-5.3) (161)	4.2 (3.8-4.3) (3)	0.049
KIR2DS2	4.8 (4.4-5.2) (63)	4.8 (4.3-5.2) (101)	0.92
HLA-C2/C2+ n=4			
KIR2DL1	3.9 (3.7-4.0) (4)	-	-
KIR2DS1	3.7 (3.6-3.9) (2)	3.9 (3.8-3.9) (2)	1
HLA-B*46:01/B*46:01+ n=7			
KIR2DL2	6.1 (6.0-6.1) (2)	4.6 (4.2-4.7) (5)	0.053
KIR2DL3	4.7 (4.4-5.4) (7)	-	-
KIR2DS2	6.1 (6.0-6.1) (2)	4.6 (4.2-4.7) (5)	0.053

Table 1. Effect of KIR-HLA receptor-ligand combinations on viral load. IQR: Inter quartile range.

	n=209 β	p	n=187 β	p
The number of KIR2DL2-ligand HLA association	0.15	0.15	0.13	0.25
The number of KIR2DL3-ligand HLA association	0.13	0.039	0.15	0.038
The number of KIR2DS2-ligand HLA association	-0.07	0.52	-0.08	0.49
CD4+ T cell counts (/ul)	-0.33	<0.001	-0.31	<0.001
Age	-0.09	0.14	-0.09	0.17
Sex (female)	-0.30	<0.001	-0.27	<0.001

Table 2. Multivariate linear regression model analysis of viral load, among whole 209 subjects, and among 187 subjects without homozygosity in HLA-B*46:01 and HLA-C1 comprising alleles.
β: Standarized coefficient

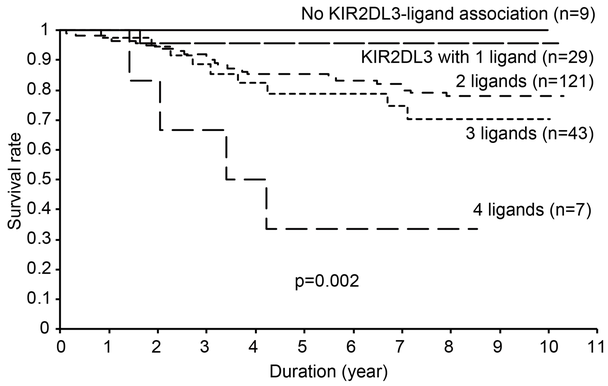


Figure 2. The number of KIR2DL3-ligand HLA associations and survival rate analysis, by log rank test. Survival rate analysis among the groups of KIR2DL3-ligand HLA allele number (from no KIR2DL3-HLA-C1 association group up to four association group of KIR2DL3 positive with HLA-C1 homozygous and HLA-B*46:01 homozygous) by log rank test is shown.

	HR (95% CI)	p	aHR (95% CI)	p
The num. of KIR2DL2-lig. HLA assoc.	1.1 (0.8-1.5)	0.51		
The num. of KIR2DL3-lig. HLA assoc.	2.1 (1.4-3.3)	<0.001	2.0 (1.3-3.2)	0.004
The num. of KIR2DS2-lig. HLA assoc.	1.1 (0.8-1.5)	0.54		
CD4+ T cell counts (/ul)	0.9 (0.9-0.9)	0.004	0.9 (0.9-0.9)	0.022
Viral load (log copies/ml)	3.0 (1.8-5.1)	<0.001	2.0 (1.2-3.3)	0.009
Age	1.1 (1.03-1.1)	0.002	1.1 (1.02-1.1)	0.007
Sex (Female)	0.5 (0.2-0.9)	0.029	0.6 (0.3-1.3)	0.20

Table 3. The number of KIR2D ligands and survival rate analysis, by Cox hazard model. Variables with p<0.1 in a univariate analysis were reanalysed in a multivariate analysis.

Conclusions

In conclusion, this is the study to investigate the effect of the expression of KIR2D and their HLA ligands on viral control in an HIV-1 CRF01_AE-infected Asian population. We have found that the KIR2DL3-HLA-C1 receptor-ligand combination is disadvantageous in this population and the number of KIR2DL3-HLA-C1 allele interactions is a significant predictor of VL. Furthermore, our data highlights regional differences in the frequency and distribution of KIR allotypes and their ligands, which in turn affects the NK cell response, and consequently, the selection pressure exerted on HIV in each endemic area.
The identification of uniquely protective and susceptible KIR-HLA receptor-ligand combinations in each endemic area will provide the opportunity to better define the nature of HLA-associated immune control of HIV.

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