The association of Gag-dependent replication capacities with clinical outcome of HIV-1 infection in a treatment-naïve Japanese Cohort

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Introduction

Studies indicated a vital role of the breath and magnitude of CTL responses to Gag in the control of HIV infection. An association between CTL escape mutations in Gag and reduced HIV replication has also been implicated previously, suggesting that a severe fitness cost imposed by escape mutations could have a significant impact on the disease progression. In agreement with this hypothesis, previous studies reported that HLA-associated escape mutations in Gag impair viral replication capacity in Caucasian and African cohorts, and viral fitness is significantly reduced in elite controllers with protective HLA alleles, including B*57:01.

However, protective alleles well-studied in Caucasian/ Africa cohorts, including B*57:01, are virtually absent in Japan, and the impact of HLA-mediated immune pressure on viral fitness and its influence on HIV-1 pathogenesis has not been defined at the population level. In this study, we investigated HLA-associated changes in Gag-dependent viral replication capacity in treatment-naive HIV$^+$ Japanese patients at a population level.

Methods

<Replication Capacity> Gag-mediated-replication capacities (RCs) were examined by ① generating chimeric HIV-1_{NL4-3} encoding plasma-derived *gag-protease* (Figure 1) from 327 treatment-naive Japanese patients who are chronically infected with HIV-1 subtype B (Table 1). ② *gag-protease* PCR fragments were electroporated into a CEM T-cell line engineered to carry LTR-GFP, CXCR4, and CCR5 (CEM-GXR, a generous gift from T. Miura and M.A. Brockman), together with linearized pNL4-3 which lacks *gag-protease* (*delta gag-pro* NL4-3, a generous gift from Z.L. Brumme and T. Miura) to ③ produce viral stocks. Stock titers were determined by infecting CEM-GXR and measuring GFP$^+$ cells, using flow cytometry. To determine RCs, ④ CEM-GXR cells were infected with chimeric viruses at a multiplicity of infection of 0.03 on day 2, and the frequency of GFP-expressing cells was monitored for a week. RC was calculated as the natural-log slope of the percentage of GFP-expressing cells during the exponential viral spread and normalized against the mean value obtained with wild-type NL4-3 (*i.e.* NL4-3 RC = 1.00). Virus titration and RC experiments were done in triplicate.

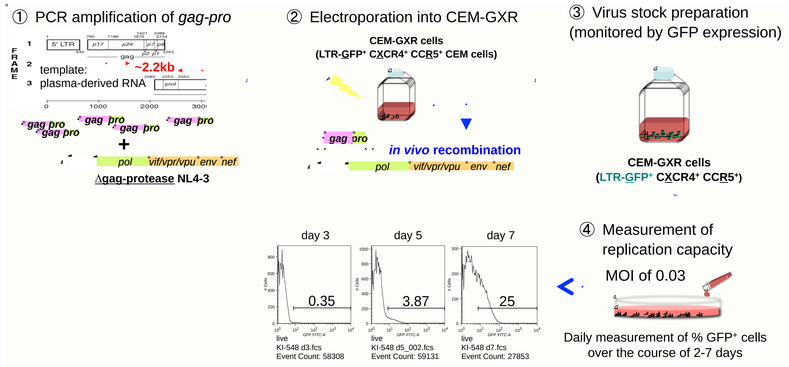


Table 1. Treatment-Naïve Cohort Information

	minimum	maximum	mean	median
Viral Load (copies/ml)	39	4,800,000	177,600	33,000
CD4 counts (cells/μl)	3	852	299	305
Total number of patients	327			

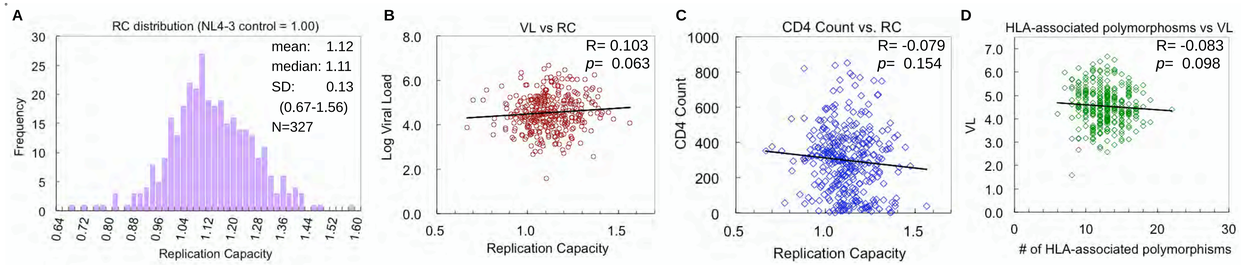


Figure 1. Gag-mediated HIV-1 replication capacities and clinical parameters in a Japanese population. **(A)** Replication capacities (RCs) of chimeric viruses carrying patient-derived *gag-protease* were determined *in vitro* by infecting CEM-GXR as in Fig. 1. The values were normalized against mean growth rate of wild-type NL4-3. The samples were measured in triplicate during the exponential phase of viral growth. RC values smaller or larger than 1.00 indicate slower or faster replication than wild-type NL4-3, respectively. (B, C) Replication capacities of chimeric viruses carrying patient-derived Gag-Pro were plotted against clinical plasma viral load (B) and CD4 counts (C) at the time of sample collection. (D) An overall number of HLA-induced amino acid changes in Gag was plotted against plasma viral load. Spearman's rank correlation test was used for statistical analysis.

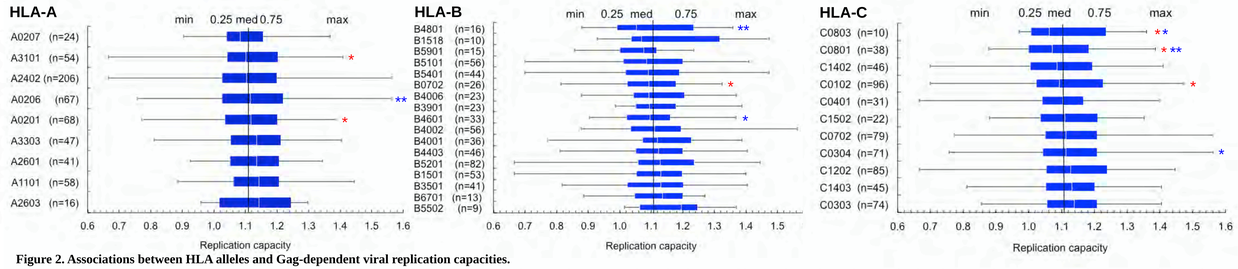


Figure 2. Associations between HLA alleles and Gag-dependent viral replication capacities.
In each graph, the median (white line), minimum and maximum values (whiskers), and interquartile range (box) were indicated. The vertical lines indicate the median RC for the whole population. Asterisks show statistically significant associations between viral load and replication capacity (red asterisks) and CD4 and replication capacity (blue) determined by Pearson's correlation tests. Two asterisks: p-value < 0.01; one asterisk: p-value < 0.05

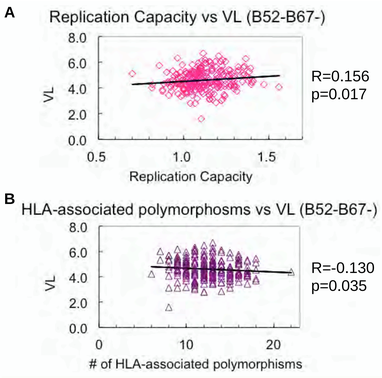


Figure 3. Associations of viral load with replication capacity and the number of HLA-associated amino acid changes in viral sequences.
The associations with replication capacity (A) and HLA-associated polymorphisms (B) were determined by Spearman's rank correlation test for patients who do not carry protective HLA-B5201 and -B6701 alleles.

Table 3. Changes in replication capacities caused by amino acid polymorphisms detected in the Gag-Protease region of viruses from patients' plasma (patients without protective HLA alleles).

B52- B67- population	position	# of subject change +	# of subject change -	Average RC change +	Average RC change -	Mann-[1] Whitney P-value	with a.a. changes RC changes	consensus	a.a. changes
Gag	79	147	71	1.10	1.16	0.0081	decreased	Y	non Y
	218	58	160	1.16	1.11	0.0268	increased	V	non V
	228	12	206	1.05	1.13	0.0158	decreased	M	L
	286	115	103	1.10	1.14	0.0302	decreased	R	non R
	357	159	59	1.11	1.16	0.0077	decreased	G	non S
Protease	19	195	21	1.13	1.07	0.0456	increased	L	(L)

[1]Mann-Whitney test was used for statistical analyses.

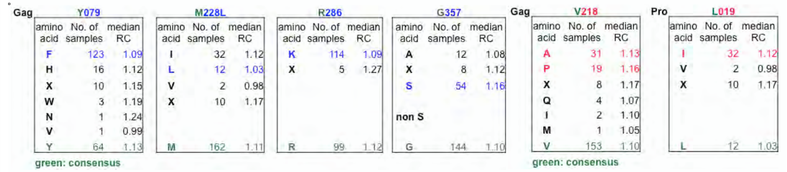


Table 2. Associations between HLA alleles and clinical parameters

HLA allele	HLA + or -[1]	VL or CD4	R^2	p value[2]
A0201	+	VL	0.29	< 0.05
A0206	+	CD4	-0.32	< 0.01
A3101	+	VL	0.31	< 0.05
B0702	+	VL	0.40	< 0.05
B1501	-	CD4	-0.12	< 0.05
B3901	-	VL	0.13	< 0.05
B4601	+	CD4	-0.40	< 0.05
B4801	+	CD4	-0.73	< 0.01
B5101	-	CD4	-0.12	< 0.05
B5201	-	VL	0.15	< 0.05
		CD4	-0.18	< 0.01
B5901	-	VL	0.11	< 0.05
B6701	-	VL	0.12	< 0.05
C0102	+	CD4	-0.25	< 0.05
C0303	-	CD4	-0.13	< 0.05
C0304	+	CD4	-0.25	< 0.05
C0401	-	CD4	-0.13	< 0.05
C0702	-	VL	0.15	< 0.05
C0801	+	VL	0.34	< 0.05
		CD4	-0.49	< 0.01
C0803	+	VL	0.70	< 0.05
		CD4	-0.68	< 0.01
C1202	-	VL	0.16	< 0.01
		CD4	-0.19	< 0.01
C1403	-	VL	0.15	< 0.05

[1]HLA+ and HLA- indicate subjects with (+) and without (-) the particular HLA allele, respectively.
[2]Spearman's rank correlation test was used to determine correlations.

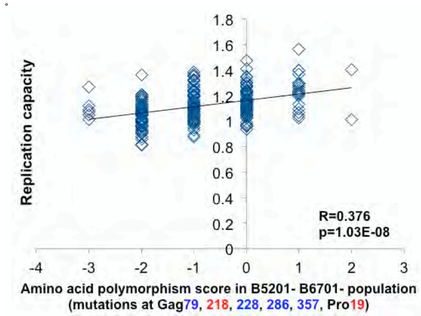


Figure 4. The impact of amino acid polymorphisms on replication capacity.
The x- and y-axis indicate mutation scores in patients and replication capacities, respectively. Mutation scores were calculated by giving -1 for each changes associated with decreased replication capacities, *i.e.* at Gag 79, 228, 286, and 357, and +1 for a polymorphism at Gag 218 and Protease 19, both of which were associated with increased replication capacities. According to this calculation, if a patient has Gag 218 (+1) and Pro 19 (+1) mutations only, the score is +2 for the patient. If a patient has Gag 79, 218, and 228, the score is -1.

Conclusions

- In the absence of protective HLA alleles, Gag replication capacities correlated with viral load in treatment–naïve Japanese individuals who are chronically infected with HIV-1 subtype B, suggesting the impact of Gag replication capacities in the absence of protective immune pressure.
- Amino acid polymorphisms at six locations in Gag-Protease were negatively or positively correlated with VL in the patient group without protective B52/B67 alleles.
- Thus, the mechanism of HLA-induced changes in Gag replication capacities and their impact on clinical outcomes may be different in the Japanese population compared to African/Caucasian cohorts.