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HIV-1 Gag Evolution in Recently Infected HLA-B*57 Patients with Low Level Viremia

Christine M. Durand[*,1], **Karen A. O'Connell**[*,1], **Linda G. Apuzzo**[2], **Susan J. Langan**[2], **Hejab Imteyaz**[2], **Aima A. Ahonkhai**[1,@], **Christina M. Ceccato**[1], **Thomas M. Williams**[3], **Joseph B. Margolick**[2], and **Joel N. Blankson**[1,#]

[1]Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore MD

[2]Department of Molecular Microbiology and Immunolgy, Bloomberg School of Public Health, Johns Hopkins University, Baltimore MD

[3]Department of Pathology, School of Medicine and Tricore Reference Laboratories, University of New Mexico, Albuquerque, New Mexico

Summary

We studied viral evolution in five HLA-B*57 patients recently infected with HIV-1. Escape mutations in HLA-B*57-restricted Gag epitopes were present at study entry in all patients but were not associated with significant increases in viremia. Conversely, no new escape mutations in HLA-B*57-restricted epitopes or known compensatory mutations were detected in patients who experienced significant increases in viremia. Thus the development of escape mutations alone does not determine virologic outcome in recently infected HLA-B*57 patients.

The HLA-B*57 allele is overrepresented in patients who naturally control HIV-1 infection [1]. Although $CD8^+$ T cell responses to immunodominant HLA-B*57-restricted epitopes in HIV-1 epitopes are thought to play a significant role in the suppression of viral replication, escape mutations are present in HLA-B*57-restricted epitopes in virtually all HLA-B*57 individuals [1] and thus it is not clear how control is maintained. Recent studies have shown that escape mutations develop shortly after infection [2] and two HLA-B*5701 patients [3]and an HLA-B*5801 patient [4] were shown to maintain exceptionally low viral loads despite the development of TW10 and IW9 Gag mutations during primary infection. However, all three patients were followed for less than 9 months and thus the long term effect of these mutations on the level of viremia is unknown.

We present here longitudinal sequence analysis from 5 recently infected treatment naïve HLA-B*57 patients (Clinical trial registry # NCT00106171). Early infection, defined as seroconversion in the past year, was confirmed by history and by the de-tuned assay [5]. All patients were infected with clade B isolates, and at the time of study entry had a median viral load of 217 copies/ml (range: 171–742 copies/ml). The patients were followed for a median of 23 months (range 13 to 36 months). Sequence analysis of plasma virus was

[#]Corresponding author: Joel Blankson, Broadway Research Bldg, Rm 880, Johns Hopkins University School of Medicine, 722 N. Broadway, Baltimore, MD 21205, Phone: 410-955-7757, Fax: 443-287-6218, jblanks@jhmi.edu.
[*]Authors contributed equally to the study.
[@]Current address: Division of Infectious Disease, Massachusetts General Hospital, Boston, MA

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performed on at least two independent near full length *gag* clones amplified at each time point. We specifically tracked escape mutations in well described HLA-B*57-restricted Gag epitopes as well as previously described compensatory mutations in the cyclophilin A binding loop [6]. Reverse transcriptase and protease genes were also sequenced to rule out drug resistance mutations that could have affected viral fitness. Subject RH103 was noted to have the M184V mutation; no primary drug resistance mutations were seen in the other patients.

As shown in Table 1, subjects RH113, RH103, and X01 all had low viral loads despite having mutations in the TW10 and QW9 epitopes in plasma virus at time of study entry (month 0). Evolution occurred in the IW9 epitope in subjects RH113 and X01 and in the TW10 epitope in all 3 subjects. In addition, isolates from subject RH 103, who was also positive for the protective HLA-B*27 allele, developed the L268M escape mutation in the immunodominant HLA-B*27 restricted epitope KK10 at month 28. In spite of the development of these new mutations in HLA-B*57-restricted Gag epitopes, all three patients maintained low viral loads for at least 13 months.

Subjects RH014 and RH062 had significant increases in viremia during the period of study. As shown in Table 1, subject RH014 had a mixture of plasma viruses, including some isolates with wild type sequence at the earliest time point. Virus with T242N and G248N mutations in TW10 and the I147L mutation in the IW9 epitope was predominant at month 7. The A146P mutation that effects processing of IW9 was detected 3 months later. As with the three prior subjects, no significant changes in his viral load were noted in spite of these mutations. However, at month 36, the patient was noted to have a more than 1 log increase in his viral load. No new mutations were found in HLA-B*57-restricted epitopes and no new compensatory mutations that restore the fitness of the T242N mutation [6] were found at this time point.

The first plasma clones amplified from subject RH062 had the A146P mutation as well as a P149 mutation in the IW9 epitope. He also had the G248 mutation in the TW10 epitope and the E312D mutation in the QW9 epitope. In spite of this, his viral load at enrollment was just 171 copies/ml and it reached a nadir of 84 copies/ml 3 months later. However his viral load increased to 1039 copies at month 8, and to 3156 copies/ml at month 21. Sequence analysis at this time point revealed no new mutation in HLA-B*57-restricted epitopes and no new compensatory mutations.

Taken together these data suggest that escape mutations in HLA-B*57-restricted epitopes alone do not explain virologic outcome. A similar finding was reported in a longitudinal study of 5 HLA-B*5703 patients recently infected with clade C virus [7]. However the median viral load after virologic escape was 6,784 copies/ml, which is more than a log higher than the median viral load of 405 copies/ml after escape in our cohort. Thus our data emphasizes the fact that remarkable virologic control can be achieved in early infection even in the presence of multiple escape mutations. More importantly, in the clade C cohort, development of mutations at position 163 of the KF11 epitope was associated with an increase in viral load [7,8]. This finding does not explain disease progression in HLA-B*57 positive patients infected with Clade B virus as mutations at position A163 are rarely seen in Clade B isolates [8] and the majority of chronic progressors maintain wild type sequence at the KF11 epitope [9,10]. Two patients in our cohort developed viral loads that were more than a log higher than the nadir values. Neither evolution in HLA-B*57-restricted epitopes nor the development of known compensatory mutations could explain the increase in viremia in either case. The data presented here stand in contrast to our earlier case report of an HLA-B*5703 patient who maintained a viral load of < 50 copies/ml for a year before ultimately progressing to a viral load of 13,000 copies/ml [11]. In that study, full viral

genome sequence analysis before and after progression implicated mutations in the TW10 epitope as the cause of virologic breakthrough. This current study suggests that in some cases, evolution in HLA-B*57-restricted epitopes alone does not explain virologic escape. Three subjects maintained low viral loads for more than 11 months despite accruing multiple escape mutations in HLA-B*57-restricted epitopes whereas two subjects developed an increase in viremia in the absence of new mutations in HLA-B*57-restricted epitopes or known compensatory mutations. Factors such as reduced fitness of escape variants [6,12–17] and de novo CTL responses [18–20] to escape variants are probably important in the restriction of viral replication. A clearer understanding of the variables involved in the maintenance of virologic control and in some cases, the loss of control in untreated HIV infected HLA-B*57 patients, may lead to the development of effective HIV-1 vaccines.

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Table 1

Sequence of four HLA-B*57 restricted epitopes at different time points. Time is shown as the month after enrollment. Substitutions outside HLA-B*57 restricted epitopes are shown and the month at which they were first detected is noted in parentheses. A lower case letter denotes a mixture of sequences at the residue. Reversion to consensus clade B sequence is denoted by an asterisk. Residue 223 is a compensatory mutation for TW10 escape mutations. The other two associated residues in the cyclophilin A binding loop (H219, M228) that have been shown to serve as compensatory mutations had wild type sequence at all time points for all five subjects.

Pt	Month	VL	IW9	KF11	TW10	QW9	Comp
			(147–155)	(162–172)	(240–249)	(308–316)	
			AISPRTLNAWV	KAFSPEVIPMF	TSTLQEQIGW	QASQEVKNW	
RH113	0	217	----------	----------	--------A-	--T------	I223Q
(B*5703)			----------	----------	--------T-	--T------	I223Q
	6	857	P---------	----------	--N-----A-	--T------	I223Q
	11	868	P---------	----------	--N-----A-	--T------	I223Q
	23	431	P---------	----------	--N-----A-	--T------	I223Q
Other substitutions: k361R*(6), G357S (23), S427T* (23)							
			AISPRTLNAWV	KAFSPEVIPMF	TSTLQEQIGW	QASQEVKNW	
RH103	0	742	----------	----------	-----D-VA-	--T------	I223V
(B*5701)	10	1255	----------	----------	--N----VA-	--T------	I223V
	16	1208	----------	----------	--N----VA-	--T------	I223V
	28	1396	----------	----------	--N----VA-	--T------	I223V
			-L---------	----------	--N----VA-	--T------	I223V
			----------	----------	--N-----A-	--T------	I223V
Other substitutions: V191I(10), L268M (28), G357S (28), S381G*(10) F383Y(10), K387r*(16) R436K*(10)							
			AISPRTLNAWV	KAFSPEVIPMF	TSTLQEQIGW	QASQEVKNW	
X01	0	190	----------	----------	--------E-	----D----	I223V
(B*5702)	1	<50	----------	----------	--------D-	----D----	I223V
	13	405	-L---------	----------	----R---D-	----D----	I223V
Other substitutions: L34I*(13), D207E*(13), R411K* (13)							
			AISPRTLNAWV	KAFSPEVIPMF	TSTLQEQIGW	QASQEVKNW	
RH014	0	571	----------	----------	----------	---------	I223V
(B*5703)			----------	----------	--------N-	---------	I223V
			----------	----------	--N-----N-	---------	I223V

Pt	Month	VL	IW9	KF11	TW10	QW9	Comp
	7	97	-L--------	----------	--N-----N-	--------	I223V
	10	295	PL--------	----------	--N-----N-	--------	I223V
	36	3093	PL--------	----------	--N-----N-	--------	I223V
Other substitutions: K91r*(7), R95K*(36), K335r(36), S348a(36), F383y(36), K397r(36), T427p(36), R429k(36)							
			AISPRTLNAWV	KAFSPEVIPMF	TSTLQEQIGW	QASQEVKNW	
RH062	0	171	P--A------	----------	--------D-	---D---	I224V
(B*5701)	21	3156	P--A------	----------	--------D-	---D---	I224V
Other substitutions: q136R(21)							