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Frequencies of Gag-restricted T-cell escape "footprints" differ across HIV-1 clades A1 and D chronically infected Ugandans irrespective of host HLA B alleles☆

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ABSTRACT

Objective(s): We evaluated relationships between critical Gag T-cell escape mutations and concomitant T-cell responses to determine whether HLA-restricted Gag mutations that confer protection, occur at similar rates in a population infected with mixed HIV-1 clades A1 and D viruses.
Methods: Assessment of Gag selective pressure, and adaptive T-cell functions to KAFSPEVIPMF (KF11), ISPRTLNAW (ISW9) and TSTLQEQIGW (TW10) Gag epitopes were combined with host HLA to assess correlations with rates of critical epitope escape mutations in clades A1- ($n=23$) and D- ($n=21$) infected, untreated subjects. Infecting clades and selection pressure were determined from the gag sequences.
Results: Overall, Gag escape mutations A163X in KF11 were detected in 61% (14/23) A1- infected compared to 5% (1/21) in D-infected subjects ($p=0.00015$). Gag mutations I147X in the ISW9 epitope were seen in 43%: (10/23) clade A compared to 5%: (1/21) clade D infected subjects, $p=0.007$, Fisher's Exact test. Both mutations were more frequent in clade A1 infection. Frequencies of the measured epitope-specific T-cell responses were comparable across clades. Peptide binding affinities for the restricting HLA alleles did not differ across clades. Overall, selection pressure on the Gag protein was significantly greater in clade A than in clade D sequences.
Conclusions: These findings imply that HIV-1 vaccine strategies designed to target structurally constrained T-cell epitopes may be further challenged by clade-driven outcomes in specific HLA-restricted Gag epitopes. Equally, the data are line with slower HIV-1 disease progression in clade A infection; and raise hope that increased selective pressure on Gag may be protective irrespective of host HLA alleles.

1. Introduction

HIV-1 has evolved into distinct clades that influence disease outcome differently; for example, infection with HIV-1 clade D results in faster disease progression than infection with clade A [1–3]. Contrasting outcomes are partly linked to differences in the quality of induced virus-specific T-cell responses [4–6]. Protectiveness of the induced T-cell responses is partly linked to the superior secretion of HIV-specific Perforin, polyfunctionality of the responses [5,7–9] and greater targeting of Gag [5,10,11]. The Gag p24 region is highly immunogenic, but structurally constrained. Immune pressure on Gag p24 epitopes yields critical epitope escape mutations [11–13] that impair virus replication, affording survival advantage to infected hosts [5,10,14] and vaccine recipients [15]. Host HLA B alleles exert the greatest influence on HIV-1 disease outcome; HLA B*57 and B*5801 alleles are associated with slower disease progression [16–18]. Of the HLA alleles studied in Caucasian, African, Asian, and Hispanic populations to date, HLA B*57 imparts the greatest impact on virological control [19]; this outcome is largely

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Table 1
Characteristics of the study populations and their infecting viruses. This table illustrates characteristics of the study participants. Sequence variations in key Gag epitopes KAFSPEVIPMF (KF11) residues 162–172; ISPRTLNAW (ISW9), residues 147–155 and TSTLQEQIGW (TW10) residues 240–249 are compared. Horizontal boxes highlight peptides that elicited IFN-γ response in that patient. Spot Forming Units per million PBMCs are indicated for cases where interferon (IFN)-γ responses were detected. HLA alleles known to present KF11, ISW9 and TW10 epitopes are highlighted in bold. Induced Spot forming units are highlighted in bold for two cases where IFN-γ responses were maintained despite the presence of an A163X escape mutation.

				ISW9 Epitope		KF11 Epitope		TW10 Epitope		
	Amino acid mutation			A146X & I147X		A163X & S165N		T242X & G248X		
	Clade			A I S P R T L N A W		K A F S P E V I P M F		T S T L Q E Q I G W		
	Cons of Cons			. L		. . . . .		. . . . .		
	Cons A1			. L		. . . . .		. . . P		
	Cons D	RNA copies/mL	CD4	. . . . . SFU		. . . . . SFU		. . . . . SFU		HLA B Allele
UG003	A1	378000	521	. V 0		 0		. . . P . . . . A . 0		B*5802,B*1801
UG005	A1	73600	444	S L 0		 0		. . . T . . . . A . 0		B*1801,B*8101
UG010	A1	378000	521	. V 125		 0		 0		B*1510, B*1402
UG012	A1	314000	442	. V 0		 0		ND 0		B*0801, B*4901
UG018	A1	15400	578	. L 0		. G 0		. . . P 0		**B*5801**, B5802
UG020	A1	36100	457	. L 0		. G 0		. . . P 0		**B*5801**, B*1503
UG025	A1	158000	528	. L 0		. G 0		. . . P . . . . A . 0		B*4201, B*15
UG026	A1	3140	656	N W 515		. V **4095**		 0		**B*5703, B*5801**
UG029	A1	647	402	? V 0		 0		. . . P . . . . A . 0		B*0801,B*1510/18/15
UG033	A1	10500	485	 0		 820		 0		B*1503, B*8101
UG034	A1	36600	456	G V 0		R G 0		. . . P 0		B*0801,B*5802
UG040	A1	2280	361	. L 0		R G 0		. . . P 0		B*5802,B*4501
UG046	A1	12700	510	P L 0		. G 1875		. . . T 0		B*3501
UG047	A1	52400	467	. L 0		R G 0		. . . P . . . V . 0		B*1402,B*0702
UG051	A1	200	1166	. V 0		 0		. . . P . . . A . 0		B*1302,B*1503
UG052	A1	488	591	P F 0		. G **2105**		. . . T 0		B*5301, **B*5703**
UG053	A1	700	312	? ? 0		 720		. . . P . . . . A . 0		B*4403, B*1503
UG054	A1	572000	512	G V 0		. G 0		. . . P . . . V . 0		B*5301, B*1402
UG055	A1	30300	586	. V 190		. G 0		. . . P 0		B*5802, B*4901
UG058	A1	1870	1046	. L 0		R G 0		. . . P 0		B*3901, B*4501
UG060	A1	5540	551	N L 0		 0		. . N I 0		B*1401, **B*5704**
UG061	A1	5740	493	. L 0		R G 0		. . . P 0		B*0801,B*5802
UG065	A1	4240	363	. L 0		. G . N 0		. . . T . . . . A . 0		B*0702, B*4901
UG002	D	10600	497	N L 0		 0		. . . . . A . 0		N/A
UG006	D	27200	816	. L 3593		 0		. . . H . . . L Q . 0		**B*5702,** B*1510
UG007	D	11600	581	P L 130		 3810		. . N 0		**B*5702, B*5703**
UG009	D	15700	640	. . . . .		. . . . .		. . . . . A . 0		B*5301, B*1402
UG015	D	2660	840	. L 0		 0		. . . . . A . 0		B*5301,B*8101
UG017	D	178000	480	 110		R S . N 360		. . . . . T . 0		B*1503, B*0702
UG019	D	13100	432	P 0		 0		 0		B*4901,B*1402
UG021	D	169000	371	. L . . K P . . . 0		 0		 0		**B*5801,** B*0702
UG022	D	12700	1089	P 0		 0		 0		B*0801, B*1510
UG023	D	162000	431	 0		 0		. . . . . A . 0		B*1503, B*1510
UG024	D	1900	497	 0		 0		 0		B*5802, B*4201
UG027	D	551000	357	. L 0		 5615		. . . . . A . 0		B*0705,B*4501
UG031	D	42200	579	P 0		 0		. . . . . A . 0		B*1402, B*8101
UG032	D	2070	851	. L . . . . H . . 0		 0		. . N . . . . . A . 0		N/A
UG035	D	3010	487	. . . . . H . . 0		 645		. . S 0		B*1801,B*1503
UG041	D	6070	538	 0		 0		. . S 0		B*3910, B*1801
UG042	D	699000	650	. L 0		 0		. . . . . A . 0		B*1510/1518,B*4501
UG043	D	294000	592	 0		 0		. . N 0		B*5301, *1510/1518
UG044	D	200	727	 0		 940		 420		B*4403, B*1503
UG056	D	6600	600	V M 0		I . . . . I 0		 0		B*5301, B*1402
UG059	D	27000	495	. L 0		. . . . D 0		. . S 0		B*4403,B*5301

achieved through T-cell targeting of Gag TSTLQEQIAW ('TW10', Gag 240–249), KAFSPEVIPMF ('KF11', Gag 162–172) epitopes [20].

Gag-associated protection is partly achieved through targeting the conserved and highly constrained KAFSPEVIPMF (KF11), ISPRTLNAW (ISW9) and TSTLQEQIGW (TW10) [11,12,21] Gag p24 epitopes; yielding critical escape mutations that reduce virus replication [11–13,21]. The TW10 response dominates in acute HIV infection of HLA B*57 and B*5801 subjects yielding T242N mutations that are associated with lower viral loads over time [22,23]. Reversion of the transmitted T242N to wild-type sequence implies that this mutation affects virus fitness [24]. The KF11 epitope sequence is identical in both clades A and D consensus sequences. In subjects with presenting alleles, chronic infection is dominated by the HLA-B*57-restricted KF11 response and consequent A146X escape mutations that impair virus replicative ability [12,13,24]. Effects of the A163X are partially compensated for by a subsequent S165N substitution [25].

HLA-restricted imprints in structurally compromised epitopes would be expected to follow predicted patterns in subjects with the same presenting alleles; however, this has not always been the case [26,27]. It is not clear how concurrent T-cell responses are attributable to this outcome. Here, we combined adaptive T-cell responses, host HLA alleles and the KF11, ISW9 and TW10 epitope sequences to evaluate how frequencies of critically relevant epitope escape correlate with concurrent T-cell responses across clades A and D infection among subjects living in the same environment.

2. Methods

2.1. Study population and evaluation of immune responses

HIV-1 infected, therapy-naïve subjects were recruited for a cross sectional evaluation. Participant plasma viral loads (HIV RNA copies per ml), CD4+ T-cell counts (cells/μl) and HLA alleles were determined as previously described [10]. Infecting clades, estimation of selection pressure and KF11, ISW9 and TW10 epitope diversities were determined from the *gag* sequences. Cryopreserved peripheral blood mononuclear cells (PBMC) from 44 subjects were initially evaluated for IFN-γ response to KF11, ISW9 and TW10, Table 1. Sixteen subjects were further assessed for simultaneous secretion of IFN-γ, IL-2, TNF-α and Perforin in response to KF11, ISW9 and TW10, using intracellular cytokine staining assay. Selection for flowcytometry evaluations was based on cell availability, presence of A163G mutations in the KF11epitope and/or possession of HLA B*57 or B*5801 alleles. Uganda Virus Research Institute Ethics Review Board and the Uganda National Council of Science and Technology reviewed and approved this study. All subjects provided written informed consent for collection and subsequent evaluation of their specimens.

2.2. Estimation of synonymous (dS) and non-synonymous (dN) rates

Selective pressure was computed from the rates of non-synonymous (dN) and synonymous (dS) substitutions. The