Selection of higher avidity HLA-restricted T cell responses as a viral adaptation strategy

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Introduction

Loss of immune reactivity due to HIV mutational escape is well described. Data generated from a large population-based study (n>800) suggested that certain CD8 T cell epitopes are created *as a result of* HIV adaptation and are associated with enhanced viral replication. Here we sought to investigate the HLA-restricted T-cell responses associated with seven such adaptations which we refer to here as "neo-epitopes" (**Table 1**).

Table 1

HLA allele	Published epitope	Location	p-value*	Substitution
HLA-A*0301	Pol (424-432) QIYPGIKVR	Pol 432 (p9)	6.36E-44	K423R
HLA-A*0201	Gag (77-85) SLYNTVATL	Gag 83 (p7)	0.0007	V83A*
HLA-A*0201	Vpr (59-67) AIIRILQQL	Vpr 60 (p2)	0.0004	L60I*
HLA-B*0702	Gag (355-363) GPGHKARVL	Gag 357 (p3)	7.63E-13	S357G
HLA-B*0702	Nef (128-137)TPGPGVRYPL	Nef 133 (p6)	0.0005	I133V
HLA-B*4201	Nef (128-137)TPGPGVRYPL	Nef 133 (p6)	0.0018	I133V*
HLA-B*1503	Nef (183-191) WRFDSRLAF	Nef 184 (p2)	1.43E-13	K184R
HLA-C*0702	Nef (105-115) KRQEILDLWVY	Nef 108 (p4)	0.0009	D108E
HLA-B*4402	Pol 724-734 QEEHEKYHSNW	Pol 729 (p6)	7.73E-06	R729K

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Aim

To demonstrate and characterise CD8 T cell responses to T cell epitopes generated from HIV adaptation (*adapted* epitopes / "neo epitopes") in HLA-A*0201, A*0301, B*0702, B*1503, B*4402, C*0702 restricted individuals from the WA-HIV cohort.

Study Group and Methods

214 cryopreserved PBMC samples from 125 HIV-infected patients were screened by IFN-γ ELISpot assay for responses to non-adapted (wild type) and adapted (escaped / "neo") epitopes (**Table 1**, **Figure 1**). Samples with detectable IFN-γ responses (>50 spot forming units/million cells after background subtraction) to both epitopes were further characterised using decreasing peptide dilutions in the ELISpot assay (functional affinity). Comparisons were made with IFN-γ responses to previously published peptides where HLA driven adaptation leads to reduction or abrogation of the T cell response as in "classical" immune escape (HLA-B*0801 restricted Nef 90-97 FLKEKGGL and FLKGKGGL, and HLA-B*5701 restricted Int 123-132 STTVKAACWW and STAVKAACWW). Peptide specific CD8+ T cell lines were cultured to determine cytokine profile and TCR Vβeta repertoire (Flow Cytometry). Cytotoxicity was determined using Chromium release. Multiple contemporaneous plasma (HIV viral sequencing) and PBMC (T cell responses) samples from a single patient (day 13 post infection - day 466) were investigated for evidence of HLA restricted T cell driven HIV adaptation.

Results

Detectable IFN-γ responses to both adapted and non-adapted peptide pairs were observed in 21% (46/214) of samples. Marginally increased frequencies of IFN-γ responses to adapted epitopes compared with non-adapted epitopes (79[5-370] vs 46[5-198] median[range] spots/well) were observed in a subset of 22 patients with detectable viral load (**Fig 2**). This is in contrast to reduced/abrogated T-cell-stimulated IFN-γ responses detected in samples to adapted peptides of classical escape epitopes (7 vs 40 median spots/well for non-adapted and adapted epitopes respectively) see **Fig 3a, 3b**.

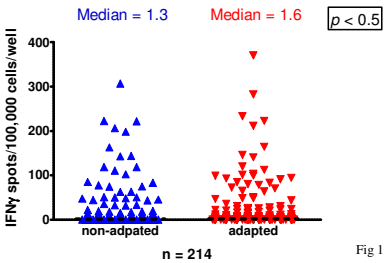

Fig 1

Acknowledgements. We thank the participants in the WA HIV Cohort study, Staff in the departments of Clinical Immunology and Immunogenetics and Haematology, Royal Perth Hospital. The work was supported by NH&MRC (Grant 384702), NIAID (ROI A1060460), and The Bill and Melinda Gates Foundation

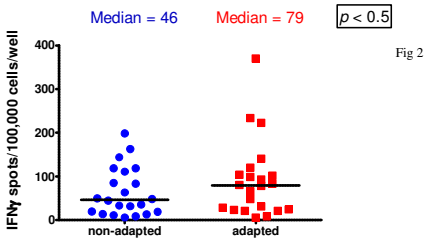

Fig 2

Figures 1 and 2. Counts were analysed on the square root scale to accommodate heterogeneity of variance. Signed ranks of the paired differences in means for adapted vs non-adapted cases were analysed by mixed models incorporating repeated measures on the same individuals. While the counts for adapted were slightly higher than for non-adapted there was no significant difference overall (n=214, p=0.15), or among those with detectable viral load (n=22, p=0.12).

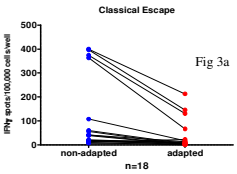

Fig 3a

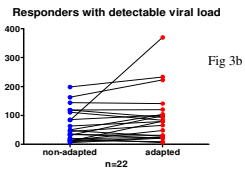

Fig 3b

Fig 3a and b show IFN-γ spots/well at 2μg/ml peptide dilution plotted for non-adapted and adapted peptide pairs for classical epitopes (3a) and neo-epitope pairs (3b).

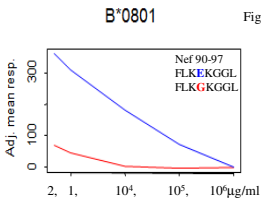

Fig 4a

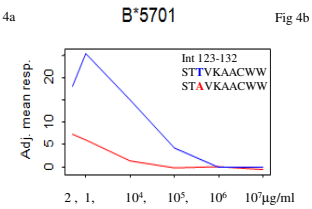

Fig 4b

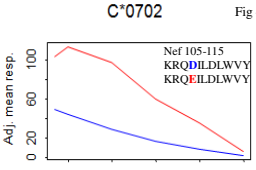

Fig 4c

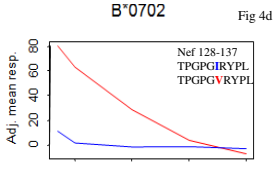

Fig 4d

Figure 4 shows functional avidity plots of classical epitopes HLA-B*0801, HLA-B*5701 (Fig 4a, b) and "neo-epitope" pairs HLA-C*0702 and B*0702 (Fig 4 c, d). IFN-γ spots / well (minus the background) are plotted against peptide concentration in μg/ml.

Functional Avidity

Adapted epitope specific CD8 T cells display increased responsiveness and produce higher levels of IFN-γ compared with non-adapted epitope specific CD8 T-cells for similar peptide concentrations. Ratios of adapted to non-adapted IFN-γ spots/well were relatively constant across dilutions (p>0.7) for classical (n=18) and neo-epitope cases (n=46) when restricted to cases with >50 spot forming units / million cells. From mixed models accommodating multiple measurements per individual, the estimated average ratios were 1.6 (0.19) for neo-epitopes and 0.6 (0.38) for classic escape epitopes (**p<0.05**).

Flow cytometry

The increased IFN-γ production and functional affinity generated by adapted peptide-stimulated CD8 T-cells in the ELISpot assay was not explained by differences between central memory or effector memory peptide specific dual IFN-γ/IL-2 or single IL-2 producing cells in HLA-B*0702 (**Fig 6 a, b**) or HLA-C*0702 (**Fig 6 c, d**) restricted cell lines. Indeed HLA-B*0702 restricted central and effector memory T-cells from adapted CTLs produced similar frequencies of IFN-γ producing cells (41(14-67) vs 56(43-63) median (range) for central memory and effector memory % of IFN-γ producing cells respectively).

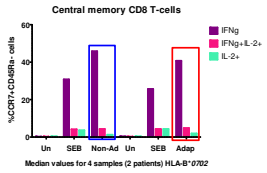

Fig 5a

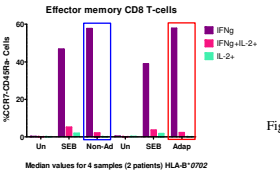

Fig 5b

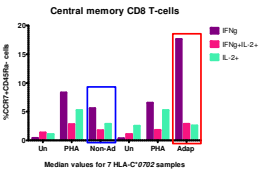

Fig 5c

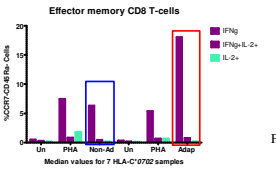

Fig 5d

Figure 5 shows % of IFN-γ positive central and effector memory cell subsets from HLA-B*0702 (5a, b) and HLA-C*0702 (5c, d) restricted patients.

Cytotoxicity and TCR Vbeta Repertoire

We observed cytotoxicity by adapted CTLs for non-adapted peptide pulsed target cells CTLs in some experiments, which may account for increased prevalence of adapted virus in plasma of HLA restricted individuals, however cytotoxicity data was not consistent. Differences between adapted and non-adapted CTLs was not accounted for by differences in TCR Vbeta repertoire in CD8 T-cell lines

Longitudinal case

Fig 6 shows HIV Nef protein viral sequencing data from six consecutive time points post HIV transmission in a single patient aligned with HXB2 clade B. The change from Lysine (K) in position 3 of the HLA-B*1503 sequence to Arginine (R) by day 382 associates with loss of HIV viral control (70,000 to 245,471 HIV RNA copies/ml). The patient commenced ART between days 382 and day 466 post infection.

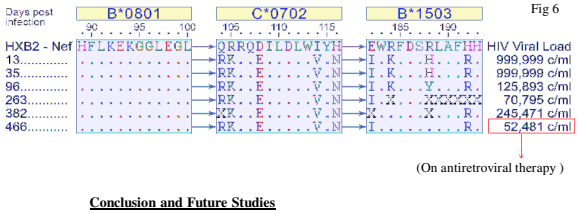

Fig 6

Conclusion and Future Studies

Here we demonstrate HLA-driven changes in HIV epitopes which elicit enhanced T cell responses rather than reduced T cell recognition as classically described for immune escape. As these "neo epitopes" appear to be the result rather than the cause of HIV adaptation they may be deleterious for antiviral control if included in vaccines. Future studies will explore cytotoxic functionality of these CTLs through epitope specific cloning.