Identification of multiple novel HIV-specific T-cell epitopes using HLA-associated HIV polymorphisms

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INTRODUCTION

Cytotoxic CD8 T-cells (CTLs) recognise antigenic peptides that are presented in the context of class I human leukocyte antigens (HLA). The high degree of polymorphism in the HLA molecules enables the binding of peptides from a number of infectious pathogens. The human immunodeficiency virus type-1 (HIV-1) selects for mutations in CTL epitopes which can affect binding to the HLA molecule, recognition by the T-cell receptor (TCR) or intracellular processing; and as a consequence, the virus evades detection by the immune system[1]. However, the pathways of viral escape can be predicted by the HLA-alleles carried in an individual. At a population level, knowledge of the most frequently occurring alleles could therefore be used to predict targets of CTLs that are immunologically relevant *in vivo*[2].

We have previously investigated HIV adaptation in response to immune pressure in a cohort of 800 anti-retroviral naïve individuals from two geographically distinct populations (United States of America and Western Australia) predominantly infected with the subtype B virus[3]. Using whole genome HIV sequencing and high resolution HLA genotyping, a consensus sequence was generated and scanned with the 'Epi-pred' epitope prediction programme to identify putative *in vivo* selection of CD8 T-cell targes (non-adapted) as well as HLA-adapted variants capable of inducing a *de novo* immune response (adapted epitopes). Here we investigate the immunologic relevance of the predicted HIV-HLA associations e*x vivo* in a subset of individuals from the USA cohort.

METHODS

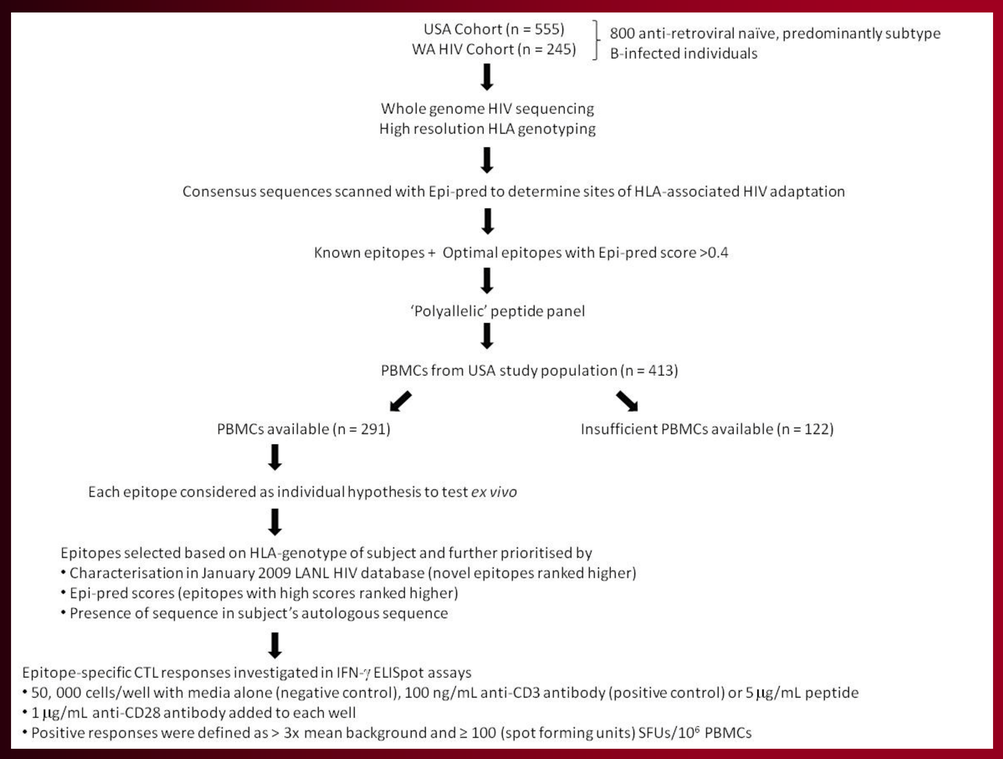

Figure 1 Summary of epitope identification, selection and IFN-γ ELISpot assay

Potential sites of HIV-adaptation to immune pressure were identified using the 'Epi-pred' prediction programme in a cohort of 800 anti-retroviral naïve individuals. IFN-γ responses to the predicted epitopes were investigated in a subset of these individuals with the ELISpot assay.

RESULTS

IFN-γ responses were detected in 267 of the 291 samples with sufficient PBMCs to test the predictions. Amongst the 267 individuals, 54.3% were classified as responders (at least one response ≥100 SFUs/10^6 PBMCs) with an average response of 353 SFUs/10^6 PBMCs.

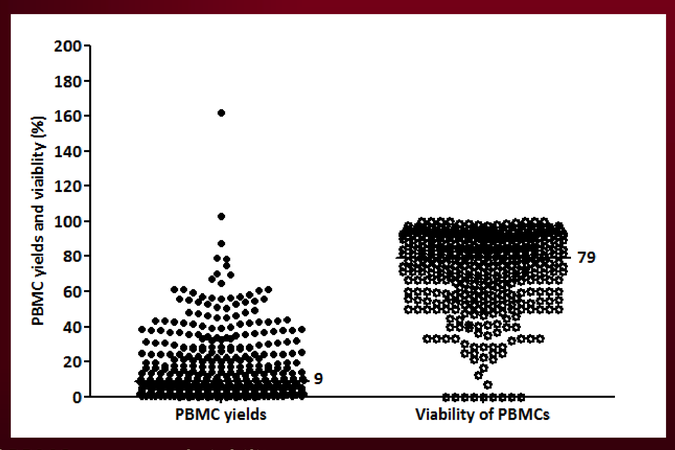

Figure 2 Comparison of PBMC counts and viability

Frozen PBMCs were counted on the Vi-Cell XR after an overnight rest in 10 mL of 10% HI-FCS/RPMI at 37 C. The PBMC yields recovered after thawing were low (median = 9%) but the majority of samples had a high proportion of viable cells (median = 79%).

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RESULTS

An epitope was considered immunogenic if it was tested in more than five individuals and was reactive in greater then 40% of the subjects tested. Using these criteria, ten epitopes with novel HLA-restrictions were identified primarily from the Nef region of the virus.

HLA	Protein	Epitope	Number of individuals tested	Positive responses (%)	Median SFUs/10^6 PBMCs (IQR range)
*B*57*	Gag	RLRPGGKKKY	7	42.86	60 (0 – 280)
*Cw*0701*	Gag	EIYKRWIIM	14	64.29	320 (0 - 475)
*Cw*1701*	Gag	TPQDLNTML	6	66.67	1810 (375 – 2855)
*B*2705*	Pol	KRKGGIGGY	7	85.71	400 (170 - 550)
*A*03*	Nef	SVVGWPAVR	19	57.89	220 (0 - 550)
*B*40*	Nef	MDDPEKEVL	7	42.86	0 (0 - 640)
*Cw*0401/Cw*0602*	Nef	PRYPLTFGW	19/7	47.37/42.86	40 (0 - 300)/0 (0 - 480)
*Cw*08*	Nef	QVPVRPMTYK	11	54.55	320 (0 - 740)
*B*1402/Cw*0802*	Nef	QRQDILDLW	6/7	50/42.86	210 (0 - 630)/0 (0 - 560)
*A*3201*	Tat	NCYCKQCCF	6	50	60 (0 - 300)

Table 1 Novel epitopes identified ex vivo

The epitope prediction programme, 'Epi-pred', also identified sites of potential viral escape in CTL epitopes. Response to the adapted epitopes together with the non-adapted epitope showed complex patterns of escape.

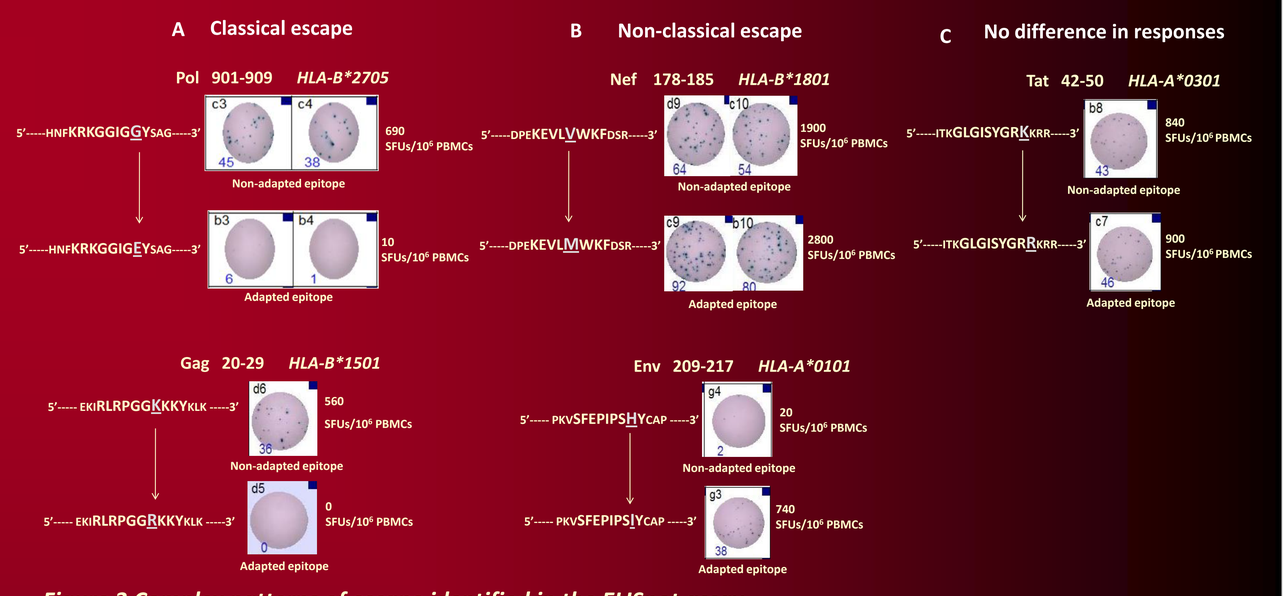

Figure 3 Complex patterns of escape identified in the ELISpot assay

The creation of a variant resulted in the abrogation of the response as shown in A. Non-classical patterns of escape were also identified wherein the adapted epitope elicited higher (B) or similar (C) IFN-γ responses in comparison to the non-adapted epitope. The epitope used in the ELISpot assay is highlighted in bold; the non-adapted and adapted amino acid residues are underlined.

CONCLUSIONS

Of the HIV-HLA associations identified, 475 were tested in the IFN-γ ELISpot assay using a set of criteria that took into consideration the limited cellular material available. Ten novel epitopes were identified where the predicted HLA-restriction matched the genotype of the reacting PBMC. In most instances, the creation of an escape variant led to the loss of reactivity indicative of a 'classical' immune response. However, some adapted epitopes elicited higher or equivalent IFN–γ responses compared to the non-adapted epitope, suggesting that the HIV escape may drive the creation of epitopes that produce ineffectual immune responses that benefit the virus. These results therefore provide new insights into CD8 T-cell responses against the virus and have implications for HIV vaccine design.

REFERENCES

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2. Brumme ZL, John M, Carlson JM, *et al*. 2009. *PLoS One*, **4**; e6687.
3. John M, Heckerman D, James I, *et al*. Submitted.

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