Early changes in the CD8 T cell immunodominance hierarchy in primary HIV infection prior to seroconversion

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Western Australian HIV Cohort Study

Introduction:

Identification of the earliest CD8 T cell responses against HIV may help select or exclude critical viral targets for inclusion in an HIV vaccine. Here we describe the dynamics of the earliest detected HIV-specific CD8 T cell responses and changes in the HIV population sequences encoding the targeted epitopes in a 43 year old female who presented 27 days after a known date of HIV sexual transmission with a CD4 T cell count of 225 cells/μL and an HIV RNA viral load of >1 million copies/mL, in Fiebig stage II, with acute clade C HIV-infection. The patient was heterozygous for the delta 32 CCR5 gene and expressed the HLA-alleles:

HLA-A*01, A*02, B*07, B*08, C*07

Materials and Methods:

T cell responses

Cryopreserved peripheral blood mononuclear cells collected on days 27, 34, 42, 48, 90, 118 and 223 post-transmission (PT) were evaluated for HIV-specific CD8 T cells responses by IFNγ ELISpot against 64 HLA class 1 restricted HIV peptides (48 known + 16 novel peptides) based on the patient's HLA class 1 alleles. The IFNγ ELISpot assay was performed as shown (Fig 1) and as previously described (1). ELISpot responses were defined as positive if > 50 spots/million cells (SFU) after background subtraction.

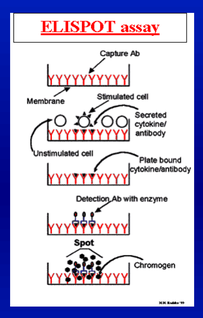

Figure 1: Cartoon of ELISpot assay

HIV sequencing

Full length HIV genome sequencing of Gag, Pol, Nef and Envelope proteins was performed using 454 deep pyro sequencing (Roche FLX) on six longitudinal plasma samples collected on days 27, 34, 42, 48, 90 and 118 PT.

Results

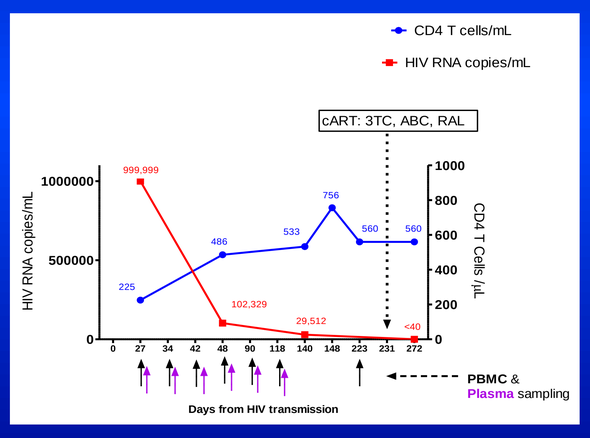

Figure 2

Figure 2: The clinical progression of the patient is illustrated here from initial presentation to day 272 post HIV sexual transmission. cART = Combination antiretroviral therapy, 3TC = Lamivudine , ABC = Abacavir, RAL = Raltegravir

Day 23 - p24antigen+, antibody negative by Western Blot (**Fiebig stage 11**)
Day 27 - p24 antigen+, Viral RNA > 1 million copies/mL
Day 34 - p24 antigen+, gp160 ab+, p24ab+ western blot indeterminate group 4 (**Fiebig stage IV**)
Day 118 - p24 antigen + Western Blot positive with p31+ (**Fiebig stage V1**)

Clinical Progression

At first presentation 23 days after likely date of HIV transmission from a known donor, p24 antigen was positive but p24 antibody was negative by ELISA and Western blot. HIV RNA was detected in plasma by day 27 PT and the first PBMC sample was collected at this point. The ELISA became reactive and the Western blot indeterminate (gp160+, p24+) when samples were tested a week later, suggesting Fiebig stage IV. The Western blot tested positive three months later (including p31+) on day 118 and the patient was classified as Fiebig stage VI. The patient commenced treatment on day 231 when the CD4 T cell count dropped from 756 to 560 cells/μL. The HIV RNA was undetectable (<40 HIV RNA copies/mL) when tested on day 272 PT.

HLA class 1 restricted HIV specific IFNγ T cell responses

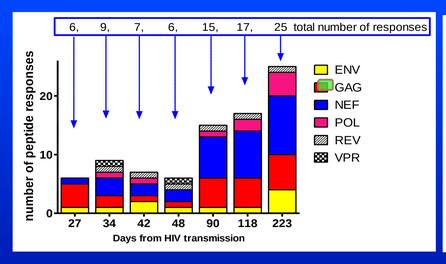

Figure 3a

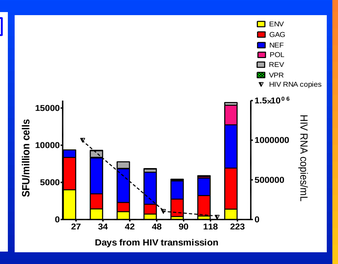

Figure 3b

Figure 3 shows the frequency of peptide responses detected at each time point (3a) and the total magnitude of peptide-induced IFNγ responses detected at each time point (3b) with contributions of each HIV protein indicated by colour : Gag (red), Pol (pink), Nef (blue), Env (yellow), Rev (stripe) and Vpr (black & green squares). IFNγ responses against variants of known peptides were excluded from this analysis.

HIV-specific IFNγ responses were detected against 58% (37/64: 29 known [including 7 variants] + 8 novel peptides) of the HLA-restricted epitopes tested over all time points evaluated across Gag (9/15), Nef (15/20), Pol (5/12), Env (5/13) and Vpr-Vpu-Rev (2/4). Six HIV peptides (variants excluded) in Gag (4), Env (1) and Nef (1) induced IFNγ responses on day 27 post HIV transmission when the patient's CD4 T cell count was 224 cells/μL with plasma HIV RNA of $>10^6$ copies/mL and prior to any detectable p24 antibody. The breath of the IFNγ responses broadened to 25 on day 223PT (Fig 3a) at a time when the CD4 T cell count decreased to 560 from 756 cells/μL, whilst viral load was declining. The overall magnitude of the IFNγ response increased by day 223 PT (Fig 3b) whilst individual peptide-induced IFNγ responses appeared to decline (Fig 4). This may be due to the increased T cell targeting of Nef epitopes from one peptide on day 27 to 8 peptides by day 223 PT. The first 6 peptide-induced IFNγ responses were therefore investigated (Fig 5)

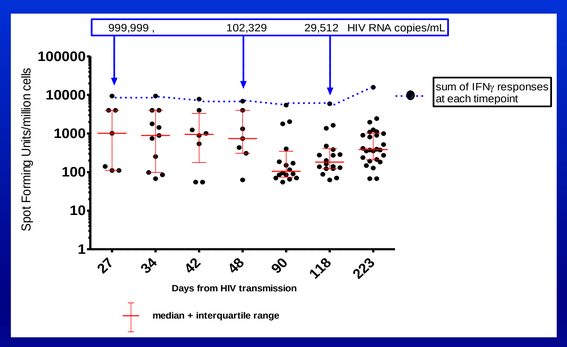

Figure 4

Figure 4: The breath of the HIV specific T cell response increased from 6 responses on day 27 to 25 responses by day 223 PT. The median magnitude of individual peptide responses appeared to decline from day 27 to day 223. HIV viral load is indicated in the blue text box at the top of the plot. Median and inter quartile range is indicated by the red bars.

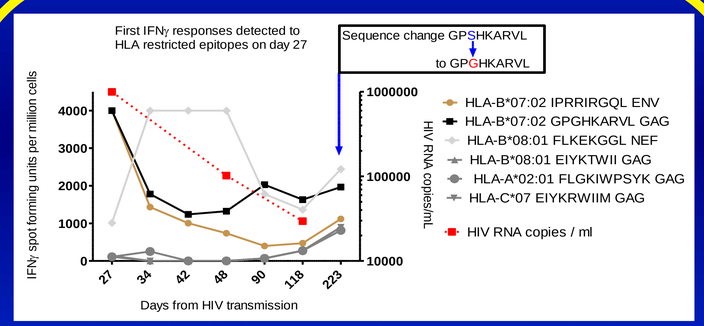

Figure 5 shows the fate of the first six HIV-specific IFNγ responses detected on day 27 over time to sampling on day 223. HIV Viral load is indicated by red symbols.

Immunodominant responses to the HLA-B*07:02 restricted Env IIRRIRQGL (IL9) epitope and the B*07:02 Gag GPGHKARVL (GL9) epitope (>4000 SFU) were observed on day 27 PT with a detectable but weaker response observed to HLA-B*08 Nef FLKEKGGL (FL8) epitope (1010 SFU). The magnitude of these responses declined over subsequent time points to 1117 and 1967 SFU respectively when tested on day 223 coincident with a 2 log fall in the plasma viral load after antibody seroconversion. The Nef FL8 epitope attained immunodominance by day 34 (>4000 SFU) which was maintained until after day 48 when it too declined.

HIV deep sequencing

Analysis of Gag, Pol, Nef and Env sequences showed late sequence change in 2 of 25 targeted epitopes: an amino acid change from Serine (S) to Glycine (G) by day 118 in the B*07:02 restricted Gag GP**G**HKARVL (GL9) epitope (Fig 5a) and a change from Glutamic acid (E) to Lysine (K) by day 90 in position 1 of the C*07 restricted Nef **K**RQDILDLWVY epitope (Fig 5b). There was limited sequence change in the remainder of the genome (Table 1)

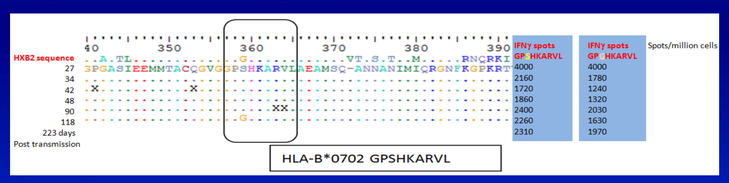

Figure 5a

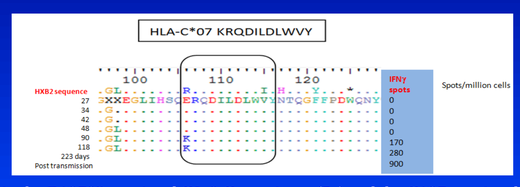

Figure 5b

Figure 5 Sequence change in the B*0702 restricted GL 9 Gag epitope (5a) and the C*07 restricted KY11 Nef epitope with IFNγ responses shown in blue boxes.

HIV protein	Amino acid change within patient HLA restricted epitope	Amino acid change outside HLA-restricted epitopes
Gag	1- day 118- HLA-B*0702 GPSHKARVL-GPGHKARVL	1 - day 118, not maintained, not within a known class 1 epitope
Pol	0	1 - day 48, not maintained, not within a known class 1 epitope
Nef	1 - day 90 HLA -C*07 ERQDILDLWVY-KRQDILDLWVY	0
Env	0	2 – day 34, 1 – day 42, none maintained, not within a known class 1 epitope

Table 1: The incidence of full amino acid sequence change across Nef, Pol, Gag and Env across 6 time points.

Summary and Conclusions

Changes in early CD8 T cell immunodominance hierarchy were apparent in this case with known date of transmission and testing prior to development of the antibody response. Env and Gag epitopes were the earliest targets of T cell responses. Subsequent broadening of the CD8 T cell response was observed but was not associated with prominent mutational escape (only 2 of 25 targeted epitopes) evident by deep parallel sequencing. In addition, there was no evidence of donor reversion in epitopes not restricted by the patient's HLA alleles, suggesting that donor virus may have been already well adapted to the patient's CD8 T cell immune response.

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References: 1 Keane NM, Roberts SG and Almeida CA *et al* Immunol Cell Biol. 2012 90(2):224-234

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