

Quantitative T Cell Repertoire Spectratype Analysis Reveals Evidence of Persistent T Cell Receptor Perturbation in Aviremic Perinatally-Infected Adolescents and Adults

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BACKGROUND

The thymus is the main site for T-cell production and plays an important role in HIV-1 immunopathogenesis. Since the introduction of effective ART regimens, survival has markedly increased in pediatric patients, particularly due to immune reconstitution in treated patients. It has also been suggested that thymopoiesis is improved by treatment but the true extent and contribution of this phenomenon is not well understood. We studied youths with perinatally-acquired HIV-1 infection who had developed prior significant immunodeficiency with subsequently effective ART, to examine the long term consequences of uncontrolled infection during early childhood on thymopoiesis. The naive T cell repertoire was assessed with a novel quantitative spectratyping approach. The results were analyzed in the context of thymic CT scans, TREC analyses, blood CD4 T cell counts, and HIV-1-specific ELISpot reactivity, to examine factors that may affect thymopoiesis and long-term immunologic outcome.

METHODS AND MATERIALS

Study subjects: 43 Perinatally HIV-1 infected young adults (13-23 years old) were recruited. All patients were receiving effective combination ART with plasma HIV-1 RNA levels < 400 copies/mL at the time of enrollment. 10 of these were assessed by quantitative TCR-Vβ spectratyping analysis.

28 adolescents and young adults were recruited as controls. Among these subjects, 10 HIV-1-infected (14-21 years old, infected during adolescence or older) and 6 age-matched uninfected patients were chosen for TCR-Vβ spectratyping. The 10 infected subjects all had undetectable viremia (<50 RNA copies/mL). Informed consent for the study's participants was obtained in accordance with protocols approved by the Institutional Review Board at University of California, Los Angeles.

FACS analysis and isolation of naive CD4⁺ and CD8⁺ T-cells: PBMC were stained with anti-CD4 Pacific Blue (BD Pharmingen), anti-CD8 APC-Cy7TM (BD Pharmingen), anti-CD45RA FITC (BD Pharmingen), anti-CD28 APC (BD Pharmingen) and anti-CD31 PE (BD Pharmingen) and sorted on a FACS DIVA fluorescent-activated cell sorter (Becton Dickinson) into naive CD4⁺ T-cell (CD4⁺/CD45RA⁻/CD31⁻) and CD8⁺ T-cell (CD8⁺/CD45RA⁻/CD28⁺) populations. Dead cells were excluded by 7AAD and aliquots of sorted cells were reanalyzed for purity (routinely >99%).

Signal joint T cell receptor excision circles (TREC) measurements, IFN-γ ELISpot assay for HIV-1-specific CTL, and thymic CT scans: These assays were performed as previously described in detail(1-4).

Novel TCR Vβ spectratyping: This method is described in Figure 1. See also poster # 382.

Statistical analyses: Significance of differences in T cell counts, TREC numbers, thymic volume and total perturbations in TCR-Vβ repertoire between HIV-1-infected and HIV-1-uninfected subjects were determined using a 2-sample t-test with unequal variance. Linear regression was performed on log₁₀-transformed ELISpot SFC values with respect to the total perturbations in the TCR-Vβ repertoires of HIV-1-infected subjects, using Prism statistical analysis software.

QUANTITATIVE TCR Vβ SPECTRATYPE ANALYSIS

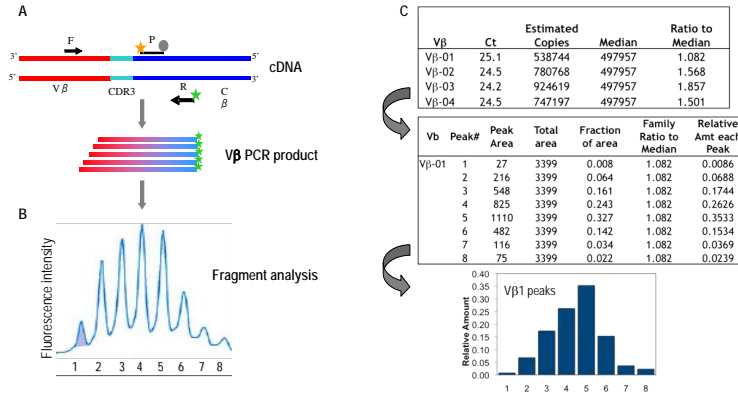


Figure 1. Schematic of quantitative spectratype analysis. (A) TCR-Vβ transcripts were reverse-transcribed as described (5), except using real-time PCR: 24 aliquots of the cDNA were amplified in 25μl reactions with a forward primer specific for each of 24 TCR-Vβ genes, a fluorescent labeled reverse primer, and a TaqMan probe specific for the TCR-Vβ chain constant region. (B) TCR-Vβ size distributions were analyzed using a 3130 Gene Analyzer and Peak Scanner software (ABI). (C) Top table - Ct values from (A) were converted to copy numbers compared to a real-time PCR standard. The relative amount of each TCR-Vβ family transcript was normalized to the median of total amount for all Vβ family genes combined. Example calculations are shown for the first 4 families. Middle table. The contribution of each size peak within a Vβ family was calculated from the histograms in (B), and the relative concentration of each individual peak was calculated by multiplying that fraction times the total relative concentration of the entire Vβ family derived above. Calculations are shown for the first family. Bottom graph. The final values calculated for the size distributions within the first Vβ family are plotted for the example. The y-axis units indicate the relative amount of transcripts within each peak in respect to all 24 Vβ families in total.

RESULTS

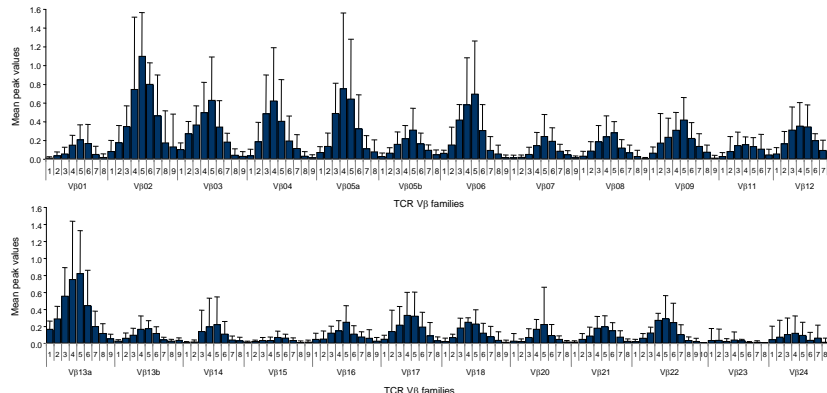


Figure 2. Mean relative peak values of TCR Vβ families in the CD31⁺ naive CD4 T cell subset from 6 HIV-1-uninfected controls. Error bars are 2 S.D.

RESULTS (CONTINUED)

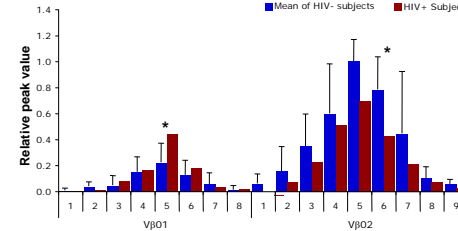


Figure 3. TCR repertoire perturbation analysis. Perturbation of the TCR repertoire in each naive T cell subset was defined as a change in a TCR population of either 2 standard deviations above or below the mean expression level seen in HIV-1-uninfected controls (* Denotes perturbation).

Variable	Control Group (n=6)	HIV Infected Youth (n=10)	P Value
% CD4 ⁺	39.2 ± 2.1	27.9 ± 6.4	<0.05
# CD4 ⁺ cells/μl	708.4 ± 116.2	656.8 ± 209	NS
% CD31 ⁺ Naive CD4 ⁺	42.8 ± 10.4	50 ± 11	NS
# CD31 ⁺ Naive CD4 ⁺ cells/μL	305.7 ± 98.6	339 ± 146	NS
Thymic volume (mL)	17.2 ± 7.3	18.68 ± 17	NS
TREC/million PBMC	11370 ± 9254	14332.5 ± 8968	NS

Table 1. Comparison of immunological parameters between HIV-1-uninfected and infected subjects.

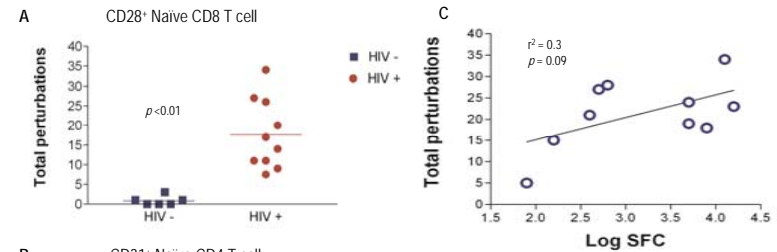


Figure 4. Total perturbations in TCR repertoire of control and HIV-1-infected subjects. For each individual, TCR Vβ perturbations were calculated as shown in Figure 3. (A) CD8⁺CD45RA⁻CD28⁺ and (B) CD4⁺CD45RA⁻CD31⁻ T cell subset. (C) A trend between HIV-specific CTL responses and the degrees of perturbation in CD31⁺ naive CD4 T cell subset in HIV-1-uninfected subjects (R²=0.3, p=0.09).

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CONCLUSIONS

While most gross thymic parameters (thymic tissue mass, T-cell counts, TREC) appear to be normal in perinatally HIV-1-infected subjects on suppressive ART, our more detailed examination of TCRs in naive T cells suggests defects in the repertoire of the reconstituting T-cells. Given that spectratyping is a rather gross measurement of TCR breadth, this suggests that there may be significantly altered immunologic reserve. The association of perturbation with HIV-1-specific CTL responses indicates that ongoing subclinical viral replication may have a key role. These data illustrate the complex relationship between viral suppression and thymopoiesis on immune reconstitution and suggest for intensification of therapy may be required to promote long-term preservation of T-cell diversity.