

Comparison of TCR Vβ CDR3 Length Distribution in Naïve and Memory CD4 and CD8 T Cells in Healthy and HIV-Infected Children Before and After Antiretroviral Therapy (ART)

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Introduction

HIV infection perturbs TCR Vβ CDR3 length distributions in CD8 CD45RA and CD45RO T cells that are restored in the CD45RA subset with ART. This study examines the extent of TCR Vβ perturbations in CD4 T cell subsets in HIV-infected children before and after antiretroviral therapy and compares with CD8 T cells.

Methods

• TCR Vβ CDR3 length diversity expressed by each of 21 Vβ families was examined by spectratyping of purified CD45RA and CD45RO CD4 and CD8 T cells (Kou et al., *CDLI*, 7:953-959, 2000).

• A total of 13 HIV-infected children (P1 to P13) were examined prior to ART, including 6 children (P3, P4, P5, P8, P12 and P13) in whom both CD4 and CD8 subsets were evaluated in the same subset. A cohort of healthy, age-matched children was used for comparisons. A longitudinal analysis of the changes in CDR3 length diversity during the first 12 weeks following initial treatment with combination antiretroviral therapy (ART) was performed in four children (P5, P9, P12 and P13).

• The number of Vβ families with perturbations per child (NP) defined the extent of TCR disruption (Kou et al., *JID* 187:385-97, 2003).

• Mann-Whitney Rank Sum test was used to test the statistical differences.

References

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• Kou ZC, Puh J, Wu SS, Goodenow MM, Sleasman JW. Combination antiretroviral therapy results in a rapid increase in T cell receptor variable region repertoire diversity within CD45RA CD8 T cells in human immunodeficiency virus-infected children. *JID* 187:385-397, 2003.

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Results

TCR CDR3 length profiles in T cell subsets of healthy children.

Figure 1. TCR CDR3 Vβ perturbations in healthy children. Individual Vβ families are shown across the top of the grid. Boxes marked with (A) represent a perturbation within CD45RA T cells; boxes marked with (O) represent perturbation within CD45RO T cells. If marked with (A/O) then both CD45RA and CD45RO subsets are perturbed.

A. CD4 CD45RA and CD45RO T cell subsets in 8 healthy children.

Healthy	TCR Vβ families																				
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	20	21	22	24
C1																					
C2																					
C3																					
C4																					
C5																					
C6																					
C7																					
C8																					

B. CD8 CD45RA and CD45RO T cell subsets in 10 healthy children.

Healthy	TCR Vβ families																				
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	20	21	22	24
C1																					
C2																					
C3																					
C4																					
C5																					
C6																					
C7																					
C8																					
C9																					
C10																					

• Vβ families within CD4 T cells from healthy children displayed few perturbations (Figure 1A). The median (range) of the NP in CD4 CD45RA T cell subset was 2.0 (0.0-2.0) and 0.0 (0.0-2.0) in the CD4 CD45RO T cell subset. The difference was not statistically significant ($P = 0.51$).

• In contrast to CD4 T cells, many Vβ families within CD8 subpopulations of healthy cohort displayed perturbations (Figure 1B). The median (range) of the NP was not significantly different in CD8 CD45RA T cells, 1.0 (1.0-4.0), compared to CD8 CD45RO T cells, 3.0 (1.0-4.0) ($P = 0.27$).

• When comparing perturbations among subsets between CD4 and CD8 T cell subpopulations, NP in CD4 CD45RA was similar to that in CD8 CD45RA T cells ($P = 0.86$). However, NP was higher within the CD8 CD45RO T cells than that in CD4 CD45RO T cells ($P = 0.03$).

Comparison of TCR CDR3 length distributions in CD4 T cell subpopulations from healthy and HIV-1-infected children.

Figure 2. TCR CDR3 Vβ perturbations in CD4 CD45RA and CD45RO T cell subsets from 13 therapy-naïve HIV-infected children.

HIV-1 infected	TCR Vβ families																				
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	20	21	22	24
P1																					
P2																					
P3																					
P4																					
P5																					
P6																					
P7																					
P8																					
P9																					
P10																					
P11																					
P12																					
P13																					

• HIV-infected children showed Vβ perturbations widely spreading among Vβ families.

• HIV-infected children demonstrated significantly higher numbers of perturbed Vβ families within CD4 CD45RA T cells when compared to CD4 CD45RA T cells in age-matched healthy individuals. Median NP was 5.0 (2.5-11.3) in HIV-infected children and 2.0 (0.0-2.0) in healthy controls ($P = 0.03$).

• In contrast, no significant difference in number of Vβ perturbations in CD45RO CD4 T cells between HIV-infected and healthy cohorts was detected. Median (range) of NP was 2.0 (1.0-4.0) among HIV-infected and 0.0 (0.0-2.0) in healthy individuals ($P = 0.09$).

Comparison of TCR CDR3 length distribution in CD4 and CD8 T cell subpopulations.

Figure 3. TCR CDR3 Vβ perturbations within CD4 and CD8 T cell subpopulations among 6 therapy-naïve HIV-infected patients. CD4 and CD8 T cell subpopulations were isolated from the same individuals (P3, P4, P5, P8, P12 and P13).

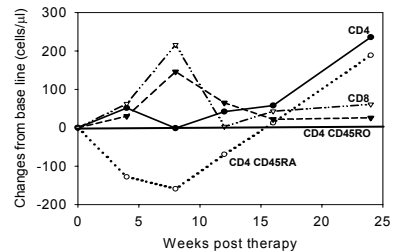
HIV-1 infected	TCR Vβ families																				
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	20	21	22	24
P3																					
P4																					
P5																					
P8																					
P12																					
P13																					

• The median (range) of NP within CD4 CD45RA T cells was 7.5 (3.0 - 11.0), which was similar to NP 11.5 (4.0-12.0) in CD8 CD45RA T cells ($P = 0.4$).

• The median (range) of NP in CD4 CD45RO T cells was 2.0 (1.0-3.0), which was significantly different from median NP of 15.5 (9.0-17.0) among CD8 CD45RO T cells ($P = 0.002$).

Impact of antiretroviral therapy on TCR CDR3 length diversity in CD45RA and CD45RO subpopulations.

Figure 4. Median changes from baseline in lymphocyte populations over 24 weeks of ART with protease inhibitor. The 4 children (P5, P9, P12 and P13) had optimal control of viral replication. Median (range) viral load fell from 3.8 (3.4-4.1) log₁₀ copies/ml at baseline to undetectable levels < 2.6 log₁₀ copies/ml by 4 to 8 weeks. Viral load remained undetectable throughout the 24 weeks of observation.



• A major increase of CD4 T cells appeared after 16 weeks post therapy. The median rise from baseline in CD4 T cell number was 177 cells/μl by 24 weeks on treatment.

• CD4 CD45RA T cells increased sharply after an initial 8-week decrease. The median rise from baseline in CD4 CD45RA T cell number was 197 cells/μl by 24 week post-therapy.

• There was a sharp median rise from baseline in CD4 CD45RO T cells (141 cells/μl) over the initial 4 to 8 weeks, and the increase became modest thereafter. The median rise from baseline was 26 cells/μl by 24 weeks on therapy.

• A transient increase in CD8 T cell number occurred over the initial 4 to 8 weeks on therapy, although the median rise from baseline was 26 cells/μl by 24 weeks on therapy.

Figure 5. Changes in TCR CDR3 Vβ profile within CD4 CD45RA and CD4 CD45RO T cells with ART.

Patient	Time Point (weeks)	TCR Vβ families																					NP	
		1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	20	21	22	24		
P5	0																							
	24																							
P9	0																							
	24																							
P12	0																							
	24																							
P13	0																							
	24																							

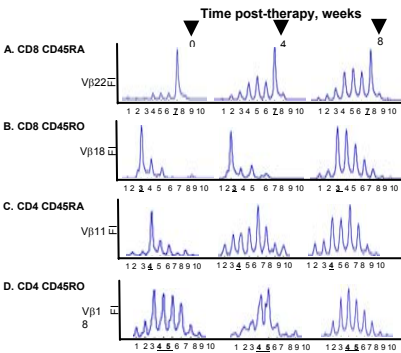
• The NP median (range) within CD4 CD45RA T cells fell from 13.5 (10.5-18.0) pre-therapy level to 5.5 (2.0-10.5) following 8 to 12 weeks of therapy ($P = 0.11$).

• In contrast, NP within CD4 CD45RO T cells fluctuated considerably among the treated children.

• Within 4 weeks following the initiation of treatment, three of the four children (P9, P12 and P13) demonstrated dramatic net increase in NP to 6, 12 and 7 compared with pre-therapy NP of 1, 7 and 3 respectively. These increases correlates with the sharp median rise in CD4 CD45RO T cells over the initial 4 to 8 weeks.

• The NP then declined to 0 in P12 and P13 by 8 weeks on therapy.

Figure 6. Representative distribution in TCR CDR3 length in CD45RA and CD45RO subsets of CD4 and CD8 T cells before and after ART. Relative amino acid lengths are shown on the x axis. Time points post-therapy are marked with arrows.



• Following control of viral replication, new CDR3 lengths emerged in majority (>90%) of perturbed Vβ families within both CD45RA and CD45RO CD8 T cells to restore a Gaussian distribution. However, the predominant pre-therapy CDR3 length persists and remains evident four or more weeks (Figure 6A, 6B).

• In CD4 CD45RA T cells less than 20% of the perturbed Vβ families that reverted to Gaussian distributions demonstrated persistence of the pre-therapy oligoclonal peak. In this subpopulation re-establishment of a Gaussian distribution generally involved the appearance of new CDR3 lengths (Figure 6C).

• Therapy induced a paradoxical shift in TCR diversity within CD4 CD45RO T cells. A high proportion of the Vβ families that showed Gaussian distributions pre-therapy became perturbed in the first few weeks of treatment but returned to Gaussian by 8 weeks (Figure 6D.). These increases correlate with the sharp median rise in CD4 CD45RO T cells over the initial 4 to 8 weeks following start of therapy.

Summary of Results

• TCR Vβ perturbations take place in healthy children. NP was higher in CD8 CD45RO T cells than in CD4 CD45RO ($P = 0.03$), but these differences were not seen in CD45RA subsets.

• HIV-infected children showed greater NP in CD4 CD45RA when compared to healthy children ($P = 0.03$), but there was no difference when comparing CD4 CD45RO subsets ($P = 0.09$).

• In CD4 and CD8 T cells in the same untreated HIV-infected individuals, NP was similar in CD45RA subsets ($P = 0.49$) but was significantly higher in CD8 CD45RO T cells compared to CD4 CD45RO T cells ($P = 0.002$).

• Four HIV-infected children were followed longitudinally after initiation of ART.

• All had optimal suppression of viral replication and median increases from base line in CD4 T cells to 177 cells/μl by 24 weeks.

• Within CD4 CD45RA T cells NP declined from a median of 13.5 pre-therapy to 5.5 by 12 weeks post-therapy.

• In contrast, NP in CD4 CD45RO T cells increased during the initial 4 to 8 weeks on ART, correlating with rising CD4 CD45RO T cells of 141 cells/μl (median rise from the baseline). NP returned to 0 to 1 by 8 to 12 weeks post therapy correlating with a rapid increase of CD4 CD45RA T cells in periphery.

• Analysis of individual CDR3 lengths demonstrated that, unlike what has been previously shown in CD8 T cells, individual CDR3 peaks did not persist in CD4 T cells.

Conclusions and Discussion

• TCR Vβ perturbations occur mainly in CD8 CD45RO T cell subset among healthy children. Perturbations may result from persistent oligoclonal expansion within cytokine-driven memory CD45RO CD8 T cells that can be maintained overtime without further antigen stimulation.

• In therapy-naïve HIV-infected children, disruption of Vβ families within CD4 CD45RA T cell subset, but not in CD4 CD45RO T cells, was observed. This may reflect circulation of oligoclonal CD4 CD45RA T cells and compartmentalization of CD4 CD45RO T cells within tissues.

• A greater number of perturbed Vβ families within CD8 CD45RO than in CD4 CD45RO T cells among therapy-naïve HIV-infected children. This finding may reflect the greater magnitude and longer duration of proliferation of CD8 compared to CD4 effector/memory T cells subsequent to antigen encounter, localization of CD4 CD45RO T cells within the tissue, and persistent circulation of CD8 CD45RO T cells in the blood.

• Optimal control of viral replication by antiretroviral therapy results in increases in CD4 CD45RA T cells, which contributes to the overall increase of total CD4 T cells after treatment.

• Increase of CD4 CD45RA T cells reflects enhanced thymic output and explains the rapid reconstitution of TCR Vβ CDR3 length diversity in CD4 CD45RA T cells.

• Increase in Vβ perturbations in CD4 CD45RO T cells during the initial 4 to 8 weeks on ART correlates with rising CD4 CD45RO T cells suggesting a re-circulation of CD4 CD45RO cells from lymph nodes to peripheral blood following the control of viral replication.