Combination Antiretroviral Therapy Results in a Rapid Increase in T Cell Receptor Variable Region β Repertoire Diversity within CD45RA CD8 T Cells in Human Immunodeficiency Virus–Infected Children

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Human immunodeficiency virus (HIV) type 1 disrupts the T cell receptor (TCR) variable region (V) β repertoire in CD8 T cells by impairing thymic capacity and skewing postthymic cellular maturation. The TCR repertoire was examined using spectratyping of CDR3 length diversity within CD45RA and CD45RO CD8 T cells in HIV-infected and healthy children. In healthy children, CDR3 lengths displayed Gaussian distribution in both CD45RA and CD45RO subsets. Vβ families in HIV-infected children displayed a large proportion of perturbations in both subsets. High virus load and advanced immunosuppression correlated with increased perturbations within CD45RA but not CD45RO CD8 T cells. After therapy and virus suppression, there was rapid reestablishment of Gaussian distributions in CD45RA cells. HIV-1–induced disruption of TCR diversity within CD45RA CD8 T cells correlates with disease progression. Suppression of viral replication by treatment results in the rapid correction of TCR diversity in this CD8 subset because of emergence of new T cells from the thymus.

Human immunodeficiency virus (HIV) type 1 infection results in perturbations of T cell receptor (TCR) variable region (V) β repertoire within the CD8 T cells of infected children and adults [1–4]. Although CD8 T cells play an important role in the suppression of viral replication, virus-induced anergy leads to defective T cell responses and contributes to the failure of immunity to control infection [5, 6]. The prognostic significance of alterations within the TCR repertoire remains uncertain, particularly in HIV-infected children whose T cell dynamics are affected by their high thymic capacity and immaturity of the cellular immune response [7–9]. HIV-1 infection skews the maturation of antigen-specific CD8 T cells and alters their homing and effector cell functions [10]. This is one mechanism that enables HIV-1 to evade the T cell immune response [11]. CD8 T lymphocytes are composed of different subpopulations on the basis of their stage of differentiation, activation state, cellular phenotype, and functional capacity [12, 13]. There is a dynamic steady state among CD8 T cells as they emigrate from the thymus,
encounter antigens, become activated to proliferate as memory T cells, or differentiate into cytotoxic effector T cells [5, 11]. CD8 T cells can be subdivided into different maturation lineages on the basis of the expression of the different isoforms of the leukocyte common antigen CD45 [14, 15]. The high-molecular-weight isoform, CD45RA, is expressed on both naive and antigen-primed effector CD8 T cells [12, 16]. Activated CD8 T cells predominantly express the low-molecular-weight isoform, CD45RO [17]. The isoforms of CD45 can be used to separate CD8 T cells into distinct populations, which allows for the accurate assessment TCR diversity within different CD8 T cell subsets [18, 19].

Recombination of germ line–encoded V, diversity, and joining (J) region gene elements that make up the β chain of the TCR establishes antigen specificity and diversity of the cellular immune response [20–22]. Recombination forms the hyper-variable fragment CDR3 region, which varies in both amino acid sequence and length [23]. Variation in CDR3 length can be exploited to monitor changes within the TCR repertoire as CD8 subpopulations differentiate during antigen-induced T cell activation [19, 24, 25]. Patterns of clonal dominance within CD8 T cells are associated with slower disease progression in HIV-infected children [4]. However, limited oligoclonal expansion of HIV-1–specific CD8 T cells during the acute phase of infection is associated with the emergence of viral immune escape, high levels of viral replication, and disease progression [26]. This apparent paradox forms the basis of the present study, which examines TCR diversity within different CD8 T cell subpopulations before and after antiretroviral therapy. Effective control of viral replication should lead to the reestablishment of a normal, Gaussian distribution of CDR3 lengths in HIV-infected children, similar to that in adults [2, 25]. However, TCR diversity within different CD8 T cell subsets has not been performed. Our study examines a relationship among perturbations in TCR Vβ repertoire, virus load, and the extent of CD4 T cell attrition. In addition, changes in TCR diversity within CD45RA and CD45RO CD8 T cells after effective control of viral replication were evaluated.

SUBJECTS, MATERIALS, AND METHODS

Subjects. The study consisted of a cross-sectional analysis of blood samples obtained from 16 HIV-infected and 10 healthy age-matched children. Samples were obtained from HIV-infected children who were at different Centers for Disease Control and Prevention clinical and immune stages of HIV disease [27] and who were receiving different antiretroviral regimens. Samples were obtained from 6 subjects before and after the initiation of combination antiretroviral therapy that included 2 different nucleotide reverse-transcriptase inhibitors plus a protease inhibitor. The median age of the study cohort was 6 years (range, 2–16 years) for the healthy children and 7.5 years (range, 4–16 years) for the HIV-infected children (P = .38). Healthy children selected for study had no underlying medical condition, recent illnesses, or immunizations. Plasma HIV-1 RNA copy number was determined using the Roche Amplicor 1.0 assay, according to the manufacturer’s protocol (Roche Molecular Systems). The sensitivity of the assay has 50 copies/mL as the lower limit of detection. T cell subsets were determined by use of flow cytometry [28].

Isolation and purification of T lymphocyte subsets. Peripheral blood mononuclear cells (PBMC) were isolated by ficoll-hypaque centrifugation. Separation of T cell subpopulations from PBMC was done as described elsewhere [18, 19, 29]. CD8 T cells were selected using magnetic multisort microbeads coated with an anti-CD8 antibody (Miltenyi Biotech) and a high-gradient magnetic separation column (Miltenyi Biotech). Microbeads coated with monoclonal antibody (Mab) and mononuclear cells were mixed and incubated at 4°C for 15 min. Labeled CD8 T cells, which adhered to the column, were washed 3 times with wash buffer and collected. Purified CD8 cells were released from the beads by using release reagent at 4°C for 15 min. The CD8 T cell subpopulations were initially separated into CD45RA, and, subsequently, the CD45RO subpopulation was isolated using microbeads coated with the appropriate MAb (Miltenyi Biotech). The purity of each T cell subpopulation was >95%, as determined by flow cytometry and molecular analysis, with <1% contamination with the reciprocal subpopulation [18, 30].

Extraction of mRNA and synthesis of cDNA. Lymphocyte mRNA was isolated and purified using procedures described elsewhere [31, 32]. In brief, mRNA was extracted from 1–5 × 10^6 purified T lymphocytes by using guanidinium isothiocyanate and a spin column with oligo (dT)–cellulose (mRNA purification kit; Pharmacia). First-strand cDNA was synthesized from 0.2 to 1 μg of mRNA by mixing with 1 μg of random hexanucleotides (Promega) and 5 μL of supertranscriptase II (Gibco BRL) in a total volume of 20 μL at 37°C for 1 h. The reaction was terminated by heating to 70°C for 10 min.

Analysis of CDR3 length using spectratyping. CDR3 size analysis within TCR–β chain was performed using 2-step polymerase chain reaction (PCR) [19, 33]. Separate amplification reactions for 22/24 human Vβ gene families were carried out in 25-μL reactions containing 0.5 μL (20 μM) of each forward Vβ and reverse Cβ primers, 0.5 μL (10 mM) of dNTPs, 2.5 μL of 10X PCR buffer, 20.5 μL of sterile H2O, 0.25 μL of cDNA, and 1.25 U of Taq polymerase. Amplifications were started with an initial denaturing step of 3 min at 95°C followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension step of 7 min. Primer sequences have been published elsewhere [19]. Second-round PCR was done with first-round products as...
Therefore, the perturbation of CDR3 profile in CD8 CD45RA distributions. For uninfected subjects, their TCR profile in CD8 vector PCR 2.1 (Invitrogen), sequenced by fluorescent dye terminators, and analyzed on an Applied Biosystems model 337A automated sequencer. Sequence of the CDR3 amino acid was analyzed using DNAMAN software (Lynnon).

**PCR amplification of T cell–receptor excisional circles (TRECs) in CD8 CD45RA T cells.** Purified CD8 CD45RA T cells were lysed in 100 μL of K buffer (containing 100 μg/mL proteinase K) at a concentration of 5000 cells/μL for 1 h at 56°C and then for 10 min at 95°C to inactivate the protease. A pair of coding joint primers within the δRec-ψα region, 5′-CAT ATG GAA GAA TGA ATC TAA TG-3′ and 5′-GAA TCT CTC ACT GAT AAT TTT CAG-3′, were used to amplify TRECs. Primers for β-actin consisted of 5′-CTA GGA GCA GTT TCC GTG GAC-3′ and 5′-GAA ACT ACC TTC AAC TCC ATC GTG GAC-3′. PCRs were prepared in final volume of 50 μL, including 1 μL of coding joint primers (10 pmol each) or 1 μL of β-actin primers (5 pmol each), 1 μL of dNTPs (10 mM), 5 μL of 10× PCR buffer, 37.5 μL of sterile H2O, 4 μL of the cell lysates, and 0.5 μL of Taq DNA polymerase. The PCRs were performed in a cycle sequencer (Model 9600; Perkin-Elmer) with an initial 3 cycles: 97°C for 1 min, 55°C for 2 min, and 72°C for 1 min, followed by 37 cycles: 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min. Approximately 20 μL of the PCR products was separated on 2% agarose gel in Tris-acetate/EDTA electrophoresis buffer. Gels were stained with ethidium bromide for visualization under UV light.

**Immunofluorescence staining and flow cytometric analysis.** Lymphocyte subset enumeration was performed by standard 3-color flow cytometry analysis of PBMC using a FACSscan (Becton Dickinson). Enumeration of CD3-, CD4-, and CD8-expressing T cells was carried out as described elsewhere [28]. Further analysis of CD8 T cell subpopulations to enumerate CD8 CD45RA CD11a T cells was assessed using MAbS directly conjugated with peridininchlorophyll protein, fluorescein isothiocyanate (FITC), or phycoerythrin. These MAbS were anti-CD8 (Leu2a; Becton Dickinson), anti-CD45RA FITC (Leu18; Becton Dickinson), and anti-CD11a (Becton Dickinson). T lymphocytes were gated, and a minimum of 15,000 cells/sample were acquired. The data were analyzed using CELLQUEST software (Becton Dickinson).

**Statistical analyses.** Statistical differences between study groups and changes in CDR3 length after therapy were determined using Sigma Stat (Jandel Scientific). A paired Student’s t test was used to compare difference in CDR3 length distribution between healthy and HIV-infected children. Student’s t test was also used to compare therapy-induced changes in CD4 T cell counts, virus load, and changes in CDR3 lengths. In addition, linear-regression analyses were performed to determine the relationship between virus load or CD4 T cell percentages with the
degree of CDR3 length perturbations in CD45RA and CD45RO CD8 T cells from HIV-infected children.

RESULTS

Differences in CDR3 length distribution in CD45RA and CD45RO CD8 T cells from healthy and HIV-infected children. TCR CDR3 length distributions for each of 22 Vβ families were determined among 16 HIV-infected children and 10 age-matched healthy children. An average $D$ for all CDR3 profiles in CD8 CD45RA T cells from HIV-infected children was $26.5\% \pm 6.5\%$ Vβ family, compared with $14.3\% \pm 3\%$ from uninfected healthy control subjects ($P<.0001$). The average $D$ in CD8 CD45RO T cells in HIV-infected children was $35\% \pm 9\%$ Vβ family, compared with $17.5\% \pm 2.5\%$ in the healthy control subjects ($P<.0001$).

A summary of perturbations in all Vβ families in healthy and HIV-1–infected children is shown in figure 1. A perturbation for any particular Vβ family, in either CD45RA or CD45RO CD8 T cells, is defined as an average deviation, compared with the average of CDR3 length distributions of that individual Vβ family from healthy children. If all the CDR3 lengths are within 3 SD of the average $D$, then the family is considered to have a Gaussian distribution of CDR3 lengths. The TCR Vβ families within the CD8 T cell subsets from healthy children had fewer perturbations than did subsets from HIV-infected children. The median number of Vβ families with perturbations in either CD45RA or CD45RO T cells was 5.5/healthy child, compared with 18 Vβ families/HIV-infected child ($P<.001$). Among the healthy children, the frequency of Vβ families with perturbations was similar in the CD45RA (median, 2.0; quartile range, 1.0–5.0) and CD45RO cells (median, 3.0; quartile range, 2.0–5.0), respectively ($P=.938$). In comparison to healthy children, HIV-infected children had significantly greater numbers of Vβ families with perturbations in both CD8 subsets (CD45RA cells median, 10.0; quartile range, 5.5–14.0 and CD45RO cells median, 14.0; quartile range, 8.5–19.5) ($P<.01$ for each subset, HIV-infected vs. healthy chil-

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Figure 1. Frequency of CDR3 length perturbation among 22 variable region (V) β subfamilies within CD45RA and CD45RO subsets of CD8 T cells in human immunodeficiency virus (HIV)–infected children vs. control subjects. Subjects P1–P6 have not received combination antiretroviral therapy; subjects P7–P16 are receiving treatment. Gray boxes with “A” represent a perturbation within CD45RA T cells; hatched boxes with “O” represent perturbation within CD45RO T cells; “A/O” represents perturbations within both CD45RA and CD45RO T cells. Ctr, control; Pt, patient; TCR, T cell receptor.
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Figure 2. CDR3 length distributions of selected variable region (V)β subfamilies within CD45RA and CD45RO CD8 T cell subsets in a human immunodeficiency virus (HIV)–infected and a healthy child. A, CDR3 amino acid length distributions (X-axis) compared in relative fluorescent intensity (FI) (Y-axis) for 12 representative Vβ families from CD45RA and CD45RO CD8 T cells from a healthy child. B, CDR3 length distribution in the same Vβ families from an HIV-infected child of the same age. *, Predominant CDR3 lengths (>25% of average distance in CD8 CD45RA T cells or >35% of average distance in CD8 CD45RO T cells).

dren). Perturbations were more frequent in the CD45RO than the CD45RA CD8 T cells from HIV-infected children \((P = .004)\).

Perturbations within both CD45RA and CD45RO subsets of the same Vβ family occurred in only 3 (1.4%) of 220 Vβ families evaluated among healthy children. In contrast, 116 (33%) of 352 separate analyses of CDR3 length from the HIV-infected children showed simultaneous perturbations in both CD45RA and CD45RO CD8 T cells in the same Vβ family \((P < .001, \text{Student's } t \text{ test})\). Figure 2 shows an example of CDR3 length distributions for 12 representative Vβ families from a healthy child, CD8 T cell subsets display predominantly Gaussian distributions, although perturbations within the CD45RO subsets can be observed, as is shown in Vβ16 and Vβ24 (figure 2A, asterisks). In HIV-infected children, patterns of CDR3 perturbations occurred in CD45RA or CD45RO CD8 T cell subpopulations. The patterns of CDR3 lengths within the subsets were variable (figure 2B). In some families, such as Vβ3, Vβ13, Vβ16, or Vβ21, perturbations occurred within CD45RO cells, whereas a Gaussian pattern for the same families appeared in the CD45RA cells. The reciprocal pattern was also observed for Vβ1, Vβ2, Vβ9, and Vβ14. A pattern observed almost exclusively in HIV-infected children was perturbations within both CD45RA and CD45RO CD8 T cell subsets as in Vβ7, Vβ15, and Vβ18.

Oligoclonal expansions of TCR Vβ families within CD45RA and CD45RO CD8 T cell subsets. Most Vβ families that displayed perturbations (figure 1) had single predominant CDR3 lengths that accounted for the deviation from Gaussian distribution. To evaluate the composition of perturbations in Vβ families, CD3 regions expressed by an individual Vβ family were sequenced after amplification and cloning \([34]\). Representative families that were sequenced are shown in figure 3. CDR3 clones from the CD8 CD45RA Vβ18 family had a Gaussian distribution of 8–14 aa. Each CDR3 region was composed of a different sequence, which indicates a polyclonal profile (figure 3A, upper panel). In contrast, Vβ18 TCR within the
Figure 3. Patterns of CDR3 length distribution and amino acid sequences of cloned variable region (V) \(\beta\) polymerase chain reaction products from CD45RA and CD45RO CD8 T cells from human immunodeficiency virus (HIV)-infected children. CDR3 amino acid lengths (X-axis), compared with relative fluorescence intensity (FI) (Y-axis). The V\(\beta\), CDR3, and joining region (J) \(\beta\) amino acid sequences and length with relative clonal frequency are shown on the right columns.

A, V\(\beta\)18 CDR3 length distribution and amino acid sequences from an HIV-infected child in which CD45RA cells are Gaussian and CD45RO T cells have a predominant CDR3 lengths of 10 aa.

B, V\(\beta\)22 for the same child in which CD45RA cells contain a predominant peak and CD45RO cells are Gaussian.

C, V\(\beta\)16 CDR3 lengths in CD45RA and CD45RO cells that have predominant peaks of the same length and amino acid sequence.

D, V\(\beta\)7 CDR3 lengths in which the predominant peaks in CD45RA and CD45RO CD8 T cells has different lengths. Bold type denotes predominant sequences.

CD45RO cells from the same HIV-infected subject expressed a 10-aa predominant CDR3 length that was identical among 15 of 22 clones, which indicates an oligoclonal profile (figure 3A, lower panel). A similar result was found in the V\(\beta\)22 TCR family (figure 3B). In this example, 18 (72%) of 25 V\(\beta\)22 clones in the CD45RA subset expressed a identical 12-aa predominant CDR3 length (figure 3B, upper panel), whereas all 11 V\(\beta\)22 clones from the CD45RO subset, which displayed a Gaussian distribution of CDR3 lengths, had unique amino acid sequences (figure 3B, lower panel). Among both HIV-infected and healthy children, sequencing revealed that the predominant CDR3 length was always oligoclonal (data not shown).

Predominant CDR3 peaks of 8 aa occurred within both CD45RA and CD45RO T cells of the V\(\beta\)16 family from an HIV-infected child (figure 3C). Five of 10 clones from the CD45RA subset were identical in amino acid sequence to 9 of 10 clones from the CD45RO subset. In contrast, CD45RA and CD45RO T cells in the same V\(\beta\)7 family contained discordant CDR3 lengths with distinct amino acid sequences (figure 3D).

In CD45RA cells, the predominant 11-aa V\(\beta\)7 sequence made up approximately half (11/20) of the CDR3 clones (figure 3D, upper panel) but was undetectable among the V\(\beta\)7 CDR3 clones from the CD45RO subset (figure 3D, lower panel). Yet predominant 9-aa CDR3 length in the CD45RO T cells was detected as a minor sequence (2/20) in the CD45RA T cells. These findings indicate that, during the course of HIV-1 infection, the same expanded T cell clone can be detected simultaneously within different CD8 T cell subsets.

Relationship between V\(\beta\) perturbations and immune or viral status among HIV-infected children. A cross-sectional analysis that evaluated a relationship between the percentage of CD4 T cells and the extent of V\(\beta\) families with perturbations within CD8 subsets was performed in 16 HIV-infected children who had varying CD4 T cell counts and HIV loads. The extent of V\(\beta\) families with perturbations was inversely correlated with CD4 percentage among the CD45RA subset (figure 4A) but failed to show a significant relationship among the CD45RO subset (figure 4B).
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Figure 4. Relationships between the frequency of variable region (V)ß CDR3 length perturbations in CD45RA and CD45RO CD8 T cell subsets to the CD4 T cell percentage and plasma virus load in a cohort of human immunodeficiency virus (HIV)–infected children. A and B, Relationship between the percentage of average distance (D) of CDR3 length profile per subject (X-axis) in CD8 CD45RA T cells (A) and CD8 CD45RO T cells (B) to the CD4 T cell percentage (Y-axis). C and D, Relationship between HIV-1 load, calculated in log_{10} RNA copies/mL, and perturbations in CD45RA and CD45RO T cells. Correlation coefficient and P values were determined using linear regression.

There was a positive correlation between increased average D, with perturbations and plasma virus burden among the CD45RA subset (r = 0.53; P = .03) (figure 4C) but not in the CD45RO subset (r = −0.04; P = .88) (figure 4D).

**Antiretroviral therapy restores CDR3 length diversity in CD8 CD45RA T cells.** Changes in CDR3 length diversity over time was evaluated among 5 therapy-naive children who were treated successfully with combination antiretroviral therapy (figure 5). At the start of therapy, the mean virus burden was 3.9 ± 3.6 log_{10} copies/mL, and the mean CD4 T cell percentage was 31.4% ± 4.6%. Treatment produced a decline in virus burden to <50 copies/mL within 8–16 weeks in all 5 children. A sixth child with advanced disease and severe immunosuppression prior to treatment, who did not respond to therapy, was included as a comparison. In this child, virus burden declined ~2 logs (from 5.8 log_{10} copies/mL before therapy to 3.9 log_{10} copies/mL) by 60 weeks of treatment, whereas the percentage of CD4 T cell increased from pretherapy levels of 7% to only 8% after 60 weeks.

In the 5 successfully treated children, the pretherapy Vß average D within CD45RO T cells did not change significantly after treatment (P > .15). In contrast, the average D improved significantly in the CD45RA T cells, from 36.9% ± 2.3% before therapy to 17.4% ± 1.7% after therapy (P < .001). As shown in figure 5, the average number of the perturbed Vß families among CD8 CD45RA T cells per subject prior to treatment was 11.0 ± 4.0, which declined to 2.0 ± 1.0 after 8–16 weeks of successful therapy (P = .003). Among the children who experienced suppression of viral replication, this improvement in CDR3 distribution was not observed in CD8 CD45RO T cells (P = .69).

As shown in the representative subject in figure 6A, effective control of viral replication resulted in the emergence of new CDR3 lengths among Vß1, Vß9, Vß11, and Vß22 families in CD45RA cells. In each Vß family, the predominant CDR3 length (10, 11, 9, or 12 aa, respectively) persisted over the course of therapy (figure 6A, asterisks). Yet the average D of the predominant peaks decreased with the emergence of new CDR3 lengths (figure 6B). The predominant peaks represented 65% ± 0.16% of the average distances prior to therapy but only 19% ± 6% of the average D as virus load declined to <50 copies/mL (P < .006). Gaussian distributions of CDR3 lengths reappeared within 4–8 weeks after the initiation of therapy.

To evaluate changes in TCR clonality after therapy, CDR3 products from amplification of Vß22 within CD8 CD45RA cells prior to and after 4 and 8 weeks of treatment were analyzed. In the representative subject shown in figure 7, prior to therapy, 80% of Vß22 CDR3 sequences were composed of an identical 12-aa length, which represented oligoclonality of a Vß22 to Jß2.5 recombination (figure 7, upper panel). The predominant
Figure 5. Changes of T cell receptor (TCR) CDR3 length profile within CD8 CD45RA and CD8 CD45RO T cells after highly active antiretroviral therapy with different time points in human immunodeficiency virus (HIV)-infected cells. HIV-infected children (as shown in figure 1) prior to (week 0) and after treatment. Gray boxes with “A” represent a perturbation within CD45RA T cells; hatched boxes with “O” represent perturbation within CD45RO T cells; “A/O” represents perturbations within both CD45RA and CD45RO T cells. Pt, patient; Vb, variable region β.

12-aa peak declined to 20% of the sequenced CDR3 lengths after only 4 weeks of therapy (figure 7, middle panel) and to 10% of the CDR3 sequences after 8 weeks of treatment (figure 7, lower panel). The new sequences identified within the emerging CDR3 lengths contained different amino acids from those detected at 4 weeks after therapy. The shift to a Gaussian distribution of CDR3 lengths resulted from emergence of new and more diverse CDR3 lengths.

Change in thymic output in HIV-infected children after antiretroviral therapy. CD8 CD45RA T cells are a heterogeneous population consisting of both new thymic emigrants and terminally differentiated effector T cells [11]. To determine whether the changes in TCR diversity within this population were due to increased thymic output, the proportion of naive CD8 CD45RA CD11a(dim) and relative number of CD45RA cells containing TREC were evaluated before and after treatment. Three subjects who had adequate sample availability were assessed. The relative proportions of CD8 CD45RA T cells expressing CD11a(dim) (naive) or CD11a(bright) (effector) was determined using flow cytometry analysis. Prior to therapy, the number and percentage of CD8 CD45RA expressing CD11a(dim) in the 2 successfully treated subjects was 43% (220 cells/μL) and 41% (210 cells/μL), respectively (figure 8C). By 8–16 weeks after therapy, there was nearly a doubling in the number of CD45RA CD11a(dim) CD8 T cells, as well as an increase in the percentage of cells to 68% (445 cells/μL) and 62% (388 cells/μL), respectively. The subject who failed to respond to antiretroviral treatment continued to display low numbers and percentages of CD11a(dim) cells, with no change after treatment (31%–27%).

To further assess changes in thymic output before and after therapy, an analysis of TREC was performed using DNA from purified CD8 CD45RA T cells from the same 3 subjects. Therapy-induced virus suppression in subjects P1 and P3 resulted in increased detection of TREC at 8–12 weeks after the initiation of treatment (figure 8A, lanes 1–5). Increases in detectable TREC were not seen in the subject (P2) for whom therapy failed (figure 8A, lanes 6 and 7). Results of β-actin amplification (figure 8B) indicated that similar amounts of input cellular DNA were amplified in all subjects. Together, these findings suggest that the reestablishment of TCR diversity in CD45RA cells is due to increases in thymic output of naive CD8 T cells.

DISCUSSION

HIV-1 infection affects CD8 T cell development and function in several ways. Virus impairs thymic output, thus decreasing the number of thymic emigrants in both CD4 and CD8 T cell lineages [35]. In addition, ongoing viral replication induces a chronic state of T cell activation that leads to clonal expansion and, ultimately, clonal exhaustion due to persistent antigenemia [36]. In this way, HIV-1 skews CD8 T cell maturation and leads to an accumulation of activated CD8 T cells that cannot effectively control viremia [10]. Virus-induced aberrations within...
Figure 6. Changes in CDR3 length distribution in CD45RA CD8 T cells after therapy. CDR3 lengths distributions prior to (0 weeks) and at 4 and 8 weeks after the initiation of combination antiretroviral therapy for a representative subject. Therapy resulted in a decline in virus load to undetectable levels (<50 copies/mL) by 4 weeks of treatment.

A, Changes in CDR3 length distributions in 4 representative variable region (V) β families from CD45RA CD8 T cells. B, Therapy induced changes over time in the percentage of average distance (D) for the predominant peak (*) in each Vβ family. FI, fluorescent intensity.

CD8 T cells are also evident when examining their TCR Vβ repertoire [1, 2]. However, the impact of viral replication on the TCR Vβ repertoire within CD8 T cells of different maturational stages and activation states has not been evaluated. The present study provides the first detailed examination of virus-induced alterations of the TCR diversity within different CD8 T cell subsets.

We examined CDR3 length distribution within CD45RA and CD45RO CD8 T cells, because these CD45 isoforms provide the best initial dissection of the CD8 T cell subpopulations on the basis of activation state and stage of differentiation [12, 14, 37, 38]. Both CD8 and CD4 T cells undergo a postthymic differentiation that is driven by antigenic activation [39]. CD45RA CD8 T cells are composed of both naive T cells that have recently left the thymus and terminally differentiated effector T cells [12]. CD45RO CD8 T cells are functionally and phenotypically more homogenous and are composed primarily of activated and proliferating T cells [12]. Further subdivision of CD8 CD45RA CD8 T cells into naive and effector phenotypes can be made on the basis of the expression of CCR7, CD27, CD103, and CD11a [12, 14, 40]. Antigen activation results in the down-regulation of CCR7, CD107, and CD45RA and the up-regulation of CD11a and CD45RO [10, 12, 13, 38, 41, 42]. Differentiation into effector CD8 cells is associated with expression of CD11a<sup>high</sup>, reexpression of CD45RA, and loss of expression of CD27 and CD45RO [12, 37, 42, 43]. These cell-surface markers can be applied to further track CD8 T cell activation and differentiate naive T cells from activated effector cells.

A comprehensive assessment of the differences in TCR CDR3 length diversity in different CD8 T cell subsets from both HIV-infected and healthy children has not been carried out; to our knowledge, our study is the first. Previous examinations of CDR3 length perturbations in CD8 T cells from healthy adults have found a limited degree of clonal expansions [2, 44, 45]. Furthermore, on the basis of comparisons between young and elderly adults, there appears to be an age-related increase in clonal T cell expansions in CD8 T cells [46]. Separation into CD45RA and CD45RO subsets enables a more sensitive eval-
Figure 7. Analysis of the changes in clonotypes and CDR3 length-restricted patterns within CD45RA CD8 T cells after combination therapy. Polymerase chain reaction products for CDR3 lengths amplified, cloned, and sequenced from variable region (V) β22 CD45RA CD8 T cells from a human immunodeficiency virus–infected child receiving combination antiretroviral therapy. CDR3 lengths distribution are shown in the left side of the figure, and the Vβ, CDR3, and joining region (J) sequences with amino acid length and relative clonal frequency are shown on the right side. The changes CDR3 length and frequency of different clonotypes prior to therapy (0 weeks) and at 4 and 8 weeks after therapy are shown as the designated time points. FI, fluorescent intensity.

ulation of the cellular phenotypes undergoing clonal expansion [19]. In healthy children, CDR3 length distributions are predominantly Gaussian in both CD45RA and CD45RO CD8 T cells, although perturbations occasionally occur in either subset, an indication that clonal expansion can be detected in healthy children, perhaps in response to previous infection or antigenic stimulation.

In comparison to the age-matched, healthy children, there is a 3–4-fold higher incidence of CDR3 length perturbations in both the CD45RA and CD45RO CD8 T cells, although perturbations occasionally occur in either subset, an indication that clonal expansion can be detected in healthy children, perhaps in response to previous infection or antigenic stimulation.

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After combination antiretroviral therapy with subsequent control of viral replication, there is a rapid reestablishment of TCR diversity within the CD45RA but not the CD45RO CD8 T cells. Early increases in CD8 T cells, B cells, and CD4 T cells after the initiation of antiretroviral therapy are thought to be due primarily to lymphocyte recirculation after treatment-induced declines in viral burden [8, 9, 49]. However, our previous studies showed that children display an early increase in naive CD4 T cells, as well as increases in total CD8 T cell numbers after therapy [28]. We were able to carry out a limited evaluation using a small number of subjects to determine the cellular origin of the CD8 CD45RA T cells that contributed to the reestablishment of TCR diversity. The assessment of the changes in TREC and CD11αdim expression within the CD45RA CD8 T cell subset demonstrated a dramatic increase in naive CD8 T cells. This finding supports a thymic origin of the new CDR3 lengths. The rapid restoration of the Gaussian distribution indicates that HIV-infected children, unlike adults, can rapidly restore their TCR repertoire after therapy [50]. However, sequence analysis of the CDR3 lengths within the CD45RA cells indicates that the oligoclonal expanded cells can persist for many weeks after therapy, even as TCR diversity within the CD45RA cells increases.

Recent analysis of the HIV-specific cytotoxic T lymphocyte response has indicated that a large proportion of activated CD8 T cells are directed toward HIV-1 antigenic epitopes [5, 51, 52]. Our study shows that effective control of viral replication by antiretroviral therapy leads to a significant decrease in the levels of oligoclonal expansion within the Vβ repertoire of the CD45RA but not CD45RO T cells. Early reestablishment of TCR diversity in CD45RA cells after optimal therapy may have prognostic significance in predicting whether an HIV-infected individual will be able to sustain control viral replication after the initiation of treatment. The persistent perturbations in the CD45RO subset may reflect delayed reestablishment of the Vβ repertoire in this subpopulation. Effective antiretroviral therapy that leads to a rapid decline and optimal control of viral replication results in increases in both CD4 and CD8 T cell populations [28, 49]. Therapy-induced increases in T cell numbers are also associated with an increase in TCR diversity and improved T cell function [53]. The present study expands these earlier observations by showing that HIV infection disrupts the TCR repertoire within both CD45RA and CD45RO CD8 T cell subpopulations. The degree of TCR perturbations within the CD45RA CD8 T cells correlates significantly with the degree of immune suppression and viral burden. In HIV-infected children, control of viral replication through combination antiretroviral therapy resulted in a rapid increase in TCR diversity within the CD45RA CD8 T cells, most likely because of early posttherapy increases in thymic output.

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References


