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Failure to Restore the V γ 2-J γ 1.2 Repertoire in HIV-infected Men Receiving Highly Active Antiretroviral Therapy (HAART)

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Abstract

Gammadelta ($\gamma\delta$) T cells expressing the V γ 2-J γ 1.2V δ 2 (V γ 9-JPV δ 2, alternate nomenclature) T cell receptor (TCR) constitute the major peripheral blood population of $\gamma\delta$ T cells in adult humans and are specifically depleted during human immunodeficiency virus (HIV) disease. V γ 2-J γ 1.2V δ 2 T cells provide a convenient model for assessing the impact of antiretroviral therapy on cell populations that are not susceptible to direct infection because they do not express CD4 and depletion occurs by indirect mechanisms. We obtained longitudinal PBMC samples from 16 HIV-infected individuals who were enrolled in the Multicenter AIDS Cohort Study (MACS) and starting highly active antiretroviral therapy (HAART). V γ 2-J γ 1.2V δ 2 T cells were depleted in these individuals as a result of HIV infection. Despite evidence for clinical benefits of HAART, the V γ 2-J γ 1.2V δ 2 T cell repertoire did not recover after HAART initiation irrespective of treatment duration. These studies highlight important defects among cell subsets lost due to indirect effects of HIV.

Keywords

gamma/delta; HIV/AIDS; antiretroviral therapy; immune reconstitution; repertoire; T cell receptor

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Introduction

In the absence of therapy, human immunodeficiency virus (HIV) causes a chronic, debilitating infection characterized by the decline of CD4⁺ αβ T cells, ultimately resulting in immunodeficiency (AIDS) and death [1]. HIV infection promotes immunological dysregulation through both direct virus infection of susceptible target cells and indirect effects on uninfected cells [2]. Indirect mechanisms lead to CD8 αβ T cell effector function inhibition [3], chronic B cell hyperactivation [4–6], impairment of natural killer (NK) cell activity [7], and loss of functional responses by gammadelta (γδ) T cells [8]. Among γδ T cells, functional defects were attributed to loss of the Vγ2Vδ2 subset, with specific depletion of cells expressing the Vγ2-Jγ1.2 rearrangement [8].

Cells expressing the Vγ2-Jγ1.2Vδ2 T cell receptor (TCR) recognize tumor cells [9–11], mostly of hematopoietic origin [12] [13], and phosphoantigen metabolites derived from eukaryotic and prokaryotic sterol biosynthesis pathways [14–16]. Selection for this population is a common feature of human development [9,17,18]. The fetal gammadelta repertoire expresses the full potential of functional gamma or delta gene products, with substantial Vγ-Jγ or Vδ-Dδ-Jδ diversity [9,18]. Beginning soon after birth, the repertoire matures by positive selection in response to self or ubiquitous antigens, that amplifies the Vγ2Vδ2 subset by positive selection of cells that express the Vγ2-Jγ1.2Vδ2 T cell receptor; ultimately, this subset becomes the dominant, circulating γδ T cell population in healthy adults [9] [18–20].

HIV infection selectively depletes the Vγ2-Jγ1.2Vδ2 subset [8], thereby impairing responses to conventional γδ T cell stimulators including phosphoantigens or tumor cells [21]. The loss of Vγ2-Jγ1.2 T cells following HIV infection [8] is sufficient to account for the inverted Vδ2/Vδ1 cell ratio that is characteristic of HIV disease [8,22]. Vγ2Vδ2 T cells are highly stimulated during HIV infection and depletion likely occurs by activation-induced cell death [23,24]. In a previous cross-sectional study of HIV-infected adults receiving HAART [25], there was evidence for partial Vγ2-Jγ1.2 repertoire restoration, but circulating Vγ2-Jγ1.2 frequencies rarely returned to normal levels [25].

HAART promotes recovery of CD4 T cells with significant reductions in morbidity and mortality [26]. However, the effects of HAART are not well characterized for cell subsets that are altered during HIV disease by indirect mechanisms [27]. In this study, we analyzed viable frozen PBMC from 16 HIV-infected participants enrolled in the Multicenter AIDS Cohort Study (MACS) [28], looking at serial specimens during and after 2 years of HAART. We also studied uninfected adults over a similar two year period (with some extending to a seven year interval) and compared Vγ2 repertoire stability for both groups. Our goals were to determine whether HAART slows or stops the mechanisms for Vγ2-Jγ1.2Vδ2 depletion, and to understand the potential for γδ T cell reconstitution during prolonged therapy.

Materials and Methods

PBMC from the Multicenter AIDS Cohort Study

We requested PBMC specimens from HIV-infected donors who had five sequential visits (spaced every 6 months for a 2 year interval) and were beginning HAART, but had no prior history of HAART (see HAART definition below). We received PBMC specimens from 28 men who persistently suppressed virus to below 50 copies/ml in the 2 years following HAART initiation and 26 men who had at least 2 visits with > 5000 RNA copies/ml in the same time frame. Presumably, due to very low numbers of γδ T cells in PBMC from HIV-infected donors, we were only able to produce data sets for 16 of these individuals, that included 6 persons with viral suppression after HAART, 7 persons that did not fully suppress after HAART and 3 persons who had viral suppression but for which we did not have a specimen from the visit

immediately preceding HAART.. At the conclusion of these analyses, we obtained clinical and treatment data for each patient.

The definition of HAART was guided by the DHHS/Kaiser Panel guidelines. Treatment regimens that included antiretroviral drugs but did not meet the specified definition for HAART were designated ART (Table 1).

PBMC from healthy control donors

Heparinized blood was collected from nine healthy volunteers with approval from the Institutional Review Board at the University of Maryland Baltimore and informed consent of the donors. Peripheral blood mononuclear cells were isolated by centrifugation over Ficoll-Paque density gradients as described by the manufacturer (Pharmacia, Uppsala, Sweden).

Flow Cytometry

3×10^5 cells were washed twice in RPMI-1640 and stained at 4°C with fluorescein isothiocyanate (FITC)-conjugated CD3 (Clone UCHT1; BD Biosciences, San Diego, CA), phycoerythrin (PE)-conjugated anti-V δ 2 (clone B6; BD Biosciences, San Diego, CA), peridinin chlorophyll protein-conjugated anti-CD4 (clone L200; BD Biosciences, San Diego, CA) or the appropriate isotype controls. After 20 minutes, cells were washed once with RPMI-1640 and resuspended in PBS containing 2% paraformaldehyde. At least 10^4 lymphocytes (gated on the basis of forward and side scatter profiles) were acquired for each sample on a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Flow cytometry data were analyzed using FlowJo software (Tree Star, San Carlos, CA).

RNA Extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from at least 10^6 cells using the RNeasy Mini Kit as described by the manufacturer (Qiagen, Valencia, CA). One microgram of total RNA was then converted into cDNA using the Reverse Transcription System (Promega, Madison, WI) in a reaction containing: 500 ng of oligonucleotide A (T₁₅V), 1 mM deoxynucleotriphosphates, 5 mM MgCl₂, 10mM Tris-HCL pH 8.8, 50 mM KCL, 0.1% Triton X-100, 18 units of avian myeloblastosis virus reverse transcriptase and 10 units of RNasin ribonuclease inhibitor. Each reaction was incubated at 42°C for 2 hours, then cDNA were diluted to 100 μ L by adding to the reaction 80 μ L deionized water. PCR reactions were performed using 5 μ L cDNA as template and 500 nM each of forward and reverse primers, 0.2 mM dNTPs, 2 mM MgCl₂, 10 mM Tris-HCL pH 8.8, 50 mM KCl, 0.1% Triton X-100 and 1 unit of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The following primers were used: oligo-V γ 2 (5'-ATCAACGCTGGCAGTCC-3'), oligo-C γ 1 (5'-GTTGCTCTTCTTTTCTTGCC-3'), 5' β -actin (5'-GTGGGGCGCCCCAGGCACCA-3') and 3' β -actin (5'-CTCCTTAATGTCACGCACGATTTTC-3'). PCR was run with the following profile: denaturation for 1 minute at 94°C; 5 minutes at 68°C; 45 cycles (45 seconds at 94°C, 1 minute at 60°C, 1 minute at 72°C); extension for 10 minutes at 72°C. PCR products were separated on a 2% agarose/Tris-acetate-ethylenediaminetetraacetic acid buffer (TAE) gels containing 0.5 μ g/mL ethidium bromide.

Spectratype Analysis

Primer extension reactions were performed as described previously [18]. Each reaction contained 1 μ L PCR product, 3 mM MgCl₂, 0.2 mM dNTP, 0.2 units *Taq* DNA polymerase (Promega, Madison, WI), 10 mM Tris-HCL pH 8.8, 50 mM KCl, and 0.1 μ M – carboxyfluorescein (6-FAM)-labeled primer (C γ 6-FAM for V γ 2 chains: 5'-AATAGTGGGCTTGGGGGAAAC-3'; C δ 16-FAM for V δ 2 chains: 5'-ACGGATGGTTTGGTATGAGG-3'). Four microliters of run-off products were diluted in

deionized formamide and 1 μ L of N,N,N',N'-trimethyl-6-rhodamine-labelled molecular size standard was added to each sample. After a denaturation step (5 minutes at 95°C followed by immediate quenching on ice), products were loaded on an Applied Biosystem microcapillary genetic analyzer (Perkin-Elmer, Foster City, CA) and run for either 27 (V γ 2 chains) or 24 (V δ 2 chains) minutes on a performance optimized polymer (POP-4). Molecular size and relative frequency of extension products were determined using Genescan software (Perkin-Elmer, Foster City, CA). To standardize the data irrespective of the run-off primer position, CDR3 length variation was expressed in terms of the total V γ 2 or V δ 2 coding region lengths. Run-off product lengths were corrected by adding the lengths of known V γ 2 or V δ 2 mRNA coding regions that lay outside the run-off product. According to this calculation, the major peak for V γ 2 chains is 993 nucleotides with a corresponding run-off product length of 444 nucleotides.

Cloning and Sequencing

PCR products were purified by gel extraction using a Qiaquick Gel Extraction kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Purified products were denatured (1 minute at 94°C), incubated for 30 minutes at 72°C with 2 mM MgCl₂, 0.2 mM dATP and 2.5 units Amplitaq Gold (AB, Foster City, CA), then ligated into the pCR2.1 TOPO TA cloning vector (Invitrogen, Carlsbad, CA). Ligated vector was transfected into TOP10F' competent cells using the TA Cloning kit (Invitrogen) and bacterial colonies were grown overnight on agar plates containing 50 μ g/mL ampicillin, 500 μ M isopropylthiogalactoside (IPTG) and 80 μ g/mL bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal). Colonies containing recombinant plasmids were cultured overnight and plasmid DNA were purified using the Wizard Plus Minipreps DNA purification kit (Qiagen, Valencia, CA). Sequencing reactions were done with the Bigdye v3.1 fluorescent sequencing kit (AB, Foster City, CA) using both M13F and M13R oligonucleotide primers for each sample. Sequences were analyzed on an automated sequencer ABI3700 and aligned using Sequencher (GeneCodes Corp, Ann Arbor, MI) and MacClade software.

V γ 2, J γ 1.1, J γ 1.2, J γ 1.3/2.3, J γ 2.1, and C γ 1 were identified by comparison to GenBank accession numbers X07205, X08084, M12950, M12960/M12961, M16016, M14996, respectively and TCR γ locus reference sequences NG_001336 and AF159056.

Repertoire Stability Definition

In an effort to define repertoire stability, we calculated the proportion of V γ 2 chains present in two or more longitudinal specimens from each donor. V γ 2 chain sequences present repeatedly were considered to be stable and their proportion was calculated by dividing the number of V γ 2 sequences encoding stable V γ 2 nucleotides by the total number of sequences. Then, the conserved proportion of the V γ 2 repertoire in each specimen was plotted for all HIV-positive and HIV-negative donors.

Results

No increase in V γ 2V δ 2 T cell levels for HIV-infected MACS donors receiving HAART

We analyzed changes in the circulating CD4⁺ and V γ 2V δ 2⁺ T cell populations over a 2 year interval for PBMC specimens obtained from 16 HIV+ men participating in the Multicenter AIDS Cohort Study (MACS) and 9 age-matched HIV- controls. CD4⁺ T cell frequencies among HIV-infected donors were $4.6 \pm 3.3\%$ of lymphocytes (range 1.6–12.4%) at the visit prior to HAART initiation (baseline time point), compared to $44.9 \pm 5.7\%$ (range 36.3–53.3%) for HIV-negative donors (Figure 1). The absolute CD4 counts ranged from 41 to 609 cells per μ L of whole blood.

Of the HIV-infected donors analyzed, 6/13 were characterized as treatment responders after HAART initiation and viral suppression to <50 copies vRNA per mL. We observed that 7/13 patients did not have complete viral suppression. The remaining 3/16 patients did not have data for the visit immediately prior to HAART. The non-responder and responder groups had significantly different CD4 cell frequencies ($p=0.008$) and circulating HIV RNA levels at the final time points compared to the last visit before starting HAART. In contrast, there was no overall increase in the peripheral blood V γ 2V δ 2 T cell frequency or the proportion of V γ 2 T cells expressing the V γ 2-J γ 1.2 rearrangement (Figure 1).

HAART did not restore V γ 2₉₉₀₋₉₉₆ populations

To test whether HAART was associated with restoration of specific V γ 2 chains that were 990, 993 or 996 nucleotides in length (V γ 2₉₉₀₋₉₉₆), we compared the average V γ 2 chain length distribution for the 16 MACS donors at the initial and final time points of the 2 year study (Figure 2). We concentrated on the V γ 2 chain because several studies have linked the V γ 2 repertoire with functional responses to antigen [8] [18] [29] [30]. Further, we focus on V γ 2₉₉₀₋₉₉₆ because mRNA of this length mostly encode the V γ 2-J γ 1.2 rearrangement that is associated with functional responses to phosphoantigens and tumors [9]. We amplified V γ 2 cDNA and performed spectratype analyses. As expected, we observed low levels of V γ 2₉₉₀₋₉₉₆ at the pre-HAART visit (Figure 2), confirming that HIV infection preferentially impacts cells expressing V γ 2-J γ 1.2. For MACS donors, there were no significant differences between the pre-HAART and last post-HAART values for V γ 2 mRNA lengths (Figure 2). Many donors (9/15) successfully suppressed viral replication to below detectable levels at all visits in the 2 years following HAART initiation but effective viral suppression was not associated with V γ 2₉₉₀₋₉₉₆ restoration (Figure 1 and data not shown). Donors with CD4 T cell counts >350 cells/ μ L at HAART initiation (6/14) exhibited higher V γ 2V δ 2 T cell levels and V γ 2₉₉₀₋₉₉₆ values than donors with CD4 counts <350 cells/ μ L, but these differences were not statistically significant (data not shown). In conclusion, HAART did not promote V γ 2₉₉₀₋₉₉₆ restoration during the two year study.

V γ 2-J γ 1.2 Repertoire Instability for HIV-infected Donors Receiving HAART

Although spectratype data provide information about length diversity in a cell population, they do not show changes among V γ 2 chains from individual T cell clones. To characterize changes at the clonal level, we amplified and molecularly cloned V γ 2 cDNA from each specimen. V γ 2 sequence diversity was assessed at various time points for seven HIV-infected and nine HIV-negative donors (Figure 3 and Figure 4), by sequencing a representative number of V γ 2 cDNA clones. On average, 40 V γ 2 CDR3 nucleotide sequences (referred to as “V γ 2 nucleotypes”) were analyzed for each specimen (range 19–114); each V γ 2 nucleotype was aligned and CDR3 regions were translated into amino acid sequences (referred to as “V γ 2 clonotypes”). For each V γ 2 sequence, we identified J γ segment usage, chain length, and its proportion within the entire sample.

HIV-infected donors were severely depleted for V γ 2-J γ 1.2 chains (Figure 3) but still had cells expressing V γ 2 rearranged to other J segments and we observed a sustained depletion of the V γ 2-J γ 1.2 repertoire (Figure 3 and data not shown). Sequencing the V γ 2 repertoire from uninfected and infected donors allowed us to determine V γ 2-J γ 1.2 frequencies that had been estimated before on the basis of V γ 2 chain lengths (from spectratyping). For healthy, HIV-negative donors, there was a high correlation between V γ 2₉₉₀₋₉₉₆ frequency and V γ 2-J γ 1.2 frequency ($r^2=0.77$, data not shown). For HIV-infected donors with V γ 2 repertoire depletion, correlations between the proportion of V γ 2₉₉₀₋₉₉₆ chains and V γ 2-J γ 1.2 frequency were lower ($r^2=0.42$, data not shown). Extensive sampling of the V γ 2 repertoire for HIV-infected individuals also revealed unusual V γ 2 sequences that were 990–996 nucleotides in length but expressed rearrangements other than V γ 2-J γ 1.2. These atypical V γ 2 chains included major

CDR3 modifications (nucleotide addition/deletion) or use of J γ segments that are rare in healthy controls (data not shown) but are not expected to encode functional recognition of phosphoantigens.

In contrast, V γ 2 chain sequences from healthy, HIV-negative donors sampled 2 to 7 years apart showed a highly stable repertoire (Figure 4). For 5/9 seronegative donors, more than 50% of the V γ 2 repertoire was stably present for periods of up to 7 years. We had 7 HIV+ donors where sequence data was accumulated for every data point. Out of these 7, 4 persons had highly unstable repertoire, with less than 5% of the repertoire encoding stable V γ 2 nucleotypes.

CD4 Lymphocyte Reconstitution Not Correlated with Circulating V δ 2 Subset Restoration

To understand whether the modest increases in CD4 lymphocytes during therapy were linked with V δ 2 cell increases, we plotted the change in V δ 2+ cell percentage versus the change in CD4 lymphocyte count for 10 donors (5 suppressors and 5 non-suppressors) where we were able to perform flow cytometry on both specimens (Fig. 5, panel A). The R^2 value was 0.26, showing there was only a minor correlation between changes in CD4 cell count and the frequency of V δ 2+ cells after treatment. Similarly (Fig. 5, [panel B]), we plotted the change in V γ 2 chains with lengths between 990–996 nucleotides (covering the subset that would contain the V γ 2-J γ 1.2 rearrangements) versus the change in CD4 cell count for 13 HIV+ donors (6 suppressors, 7 non-suppressors). Again, there was no significant correlation between changes in the V γ 2 repertoire or CD4 cell count in this group. Thus, the effects of HAART on CD4 lymphocyte count were not obviously linked with V δ 2 lymphocyte frequency or V γ 2 repertoire recovery.

Discussion

In a study conducted with specimens from the Multicenter AIDS Cohort Study [28], up to 2 years of HAART did not increase the levels of V γ 2V δ 2 T cells in blood or increase the proportion of cells expressing V γ 2-J γ 1.2 chains that are important for functional responses to phosphoantigens or tumors. There were specific examples for increased frequencies of longer V γ 2 chains (with mRNA coding regions in the range of 990–996 nucleotides) but sequence analyses revealed this was due to unusual V γ 2 clones encoding other V γ 2-J γ rearrangements and not to recovery of the V γ 2-J γ 1.2 subset. HAART appeared beneficial in these patients, with 10/14 showing increased CD4 cell count, decreased viral load or both. However, these benefits were not extended to V γ 2V δ 2 T cells and this subset was not restored during two years of receiving HAART.

This effort extended our previous observation that HIV infection preferentially impacts the subset of $\gamma\delta$ T cells expressing the V γ 2-J γ 1.2 rearrangement. We concentrated on V γ 2 because previous studies linked the V γ 2-J γ 1.2 chain with functional responses to phosphoantigens or tumors [9] [30] [29], two common stimulators of $\gamma\delta$ T cell function. Cells expressing V γ 2 rearranged with other J γ segments (not J γ 1.2) are preserved during HIV disease. Thus, depletion mechanisms that remove V γ 2-J γ 1.2V δ 2 T cells are specific for this TCR subset. We believe that V γ 2V δ 2 depletion occurs early and in parallel with changes to NKT [31] and NK [7] subsets. Mechanistically, the depletion is an indirect effect, because V γ 2V δ 2 T cells are CD4-negative and not susceptible to HIV infection. However, these cell losses are likely linked to pathogenesis. A combined $\gamma\delta$ T cell, NK and NKT cell depletion will cripple important tumor immunity mechanisms, and will certainly alter fundamental immune regulatory mechanisms.

Our previous cross-sectional study predicted that HAART would partially restore the V γ 2-J γ 1.2 subset [25]. That hypothesis was not supported by the longitudinal study described here. Differences between the cross-sectional and longitudinal studies may have been due to

treatment initiation criteria, prior treatment history or treatment duration. HAART was initiated earlier in HIV disease for MACS donors (324 ± 191 CD4 cells/ μ L) than for infected individuals in our cross-sectional study (145 ± 159 CD4 cells/ μ L) [25]. There was a more severe depletion of V γ 2₉₉₀₋₉₉₆ in HAART-naïve individuals within the cross-sectional study ($17 \pm 8\%$ V γ 2₉₉₀₋₉₉₆ chains) compared to the MACS donors ($30 \pm 25\%$ V γ 2₉₉₀₋₉₉₆).

These differences may reflect a prior history of antiretroviral therapy before HAART for many MACS donors (Table 1) compared to patients in the cross-sectional study who were mostly treatment-naïve before receiving HAART. Prior therapy may have affected the V γ 2V δ 2 subset by arresting $\gamma\delta$ repertoire damage or providing some improvement that was not furthered by HAART. Perhaps V γ 2₉₉₀₋₉₉₆ restoration would be seen after longer treatment intervals with more extended viral suppression. It is also possible that small changes in the V γ 2 may have been more significant for individuals who started with lower V γ 2₉₉₀₋₉₉₆ [25] compared to the higher starting levels found within the MACS donor population.

The occasional appearance of novel V γ 2-J γ 1.2 clones in HIV-infected donors suggests that new cells continually enter the population and can be detected when the V γ 2 repertoire is repeatedly sequenced. However, these new cells fail to expand and become established as major elements in the V γ 2V δ 2 T cell population as would be expected for uninfected donors. Possibly, an underlying disease process continues to destabilize cells expressing the V γ 2-J γ 1.2V δ 2 TCR even when viremia is undetectable (<50 copies/mL). It is reasonable to speculate that the failure to reconstitute a normally complex repertoire of CD4⁺ T cells after HAART [32] might be due to similar mechanisms.

A small fraction of HIV-infected donors (2/16; MPD04, MPD05) had blood V γ 2V δ 2 frequencies in the normal range (5–10% of T cells). The reason for this observation is unknown although both of these donors still had severe V γ 2-J γ 1.2 depletion ($<5\%$ of V γ 2). However, a bulk cell increase without recovery of repertoire complexity is familiar from studies on CD4⁺ T cells during HAART where cell counts increase but damage to the repertoire persists [32,33].

The V γ 2-J γ 1.2 subset provides a convenient model for assessing the impact of HAART on cells that were depleted by indirect mechanisms. Our studies suggest that at least one type of immune damage persists even during effective antiretroviral therapy. Studies on V γ 2V δ 2 T cells may provide a means for measuring ongoing damage to the immune system and allow for comparisons of therapies that facilitate effective viral suppression but differentially affect subpopulations of immune cells.

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Figure 1. HAART effects on CD4+ or Vδ2+ cells and on the Vγ2 repertoire

For 13 HIV-infected donors (6 suppressors and 7 non-suppressors), we assessed the blood CD4 (upper row) and Vδ2 (middle row) frequency by flow cytometry and estimated the Vγ2-Jγ1.2 frequency (lower row) based on Vγ2 spectratype analysis (Vγ2₉₉₀₋₉₉₆). Vγ2₉₉₀₋₉₉₆ represents the sum relative frequency of Vγ2 chains 990, 993 and 996 nucleotides in length based on spectratyping. Data is plotted for each time point within the 2 year study interval (left panels), or by comparing (right panels) averaged values at initial and final time points for the 13 HIV-infected donors or 9 HIV-negative controls (over a comparable two year interval). Open bars in the right panels show data for HIV-negative or for the last visit before HAART initiation (Pre-HAART) for HIV+ individuals. Suppressor indicates patients with complete viral suppression after HAART. Non-Suppressor means there was viremia despite treatment. Solid bars show data for late time points that are 2–2.5 years after the early value. HIV-positive indicates the sum of 13 patients, whereas suppressors (n=6) and non-suppressors (n=7) are also shown separately. Error bars indicate standard error of the mean. A significant difference was

noted for CD4 lymphocyte frequency in the Suppressor population (upper right panel) with a value of $p = 0.008$. All other differences were not significant.

Figure 2. Selective Depletion of Longer Vγ2 Chains in HIV-positive Donors Despite HAART

We compared the Vγ2 chain length distribution for a group of HIV-negative adults (white) with Vγ2 spectratypes obtained from HAART-treated HIV-positive adults determined for an early time point (gray) and at an interval approximately 2 years later (black). The averaged relative frequency for each chain length among HIV-negative or HIV-positive donors is plotted ($\pm$ standard error of the mean). Asterisks (*) indicate statistical significance when comparing seronegative controls with both early and late time points from seropositive donors (Student's t-test, $p < 0.05$). No individual Vγ2 chain lengths reach statistical significance when comparing only early and late time point relative frequencies for HIV-positive donors.

Figure 3. Sustained Vγ2-Jγ1.2 depletion in HIV-infected individuals receiving HAART

The Vγ2-Jγ1.2 frequency was determined at two or three time points (minimum 6 months between intervals for HIV-positive donors and up to 7 years for uninfected controls) by analysis of Vγ2 sequences from 7 representative HIV-positive donors from the HIV-positive cohort (upper) or 9 healthy, HIV-negative donors (lower). The HIV+ donors MPD01 and MPD11 are separated by a dashed line to indicate that we did not have pre-HAART samples for these individuals and instead substituted data from the first visit after HAART initiation. Each data point represents the analysis of on average 40 Vγ2 CDR3 sequences (range: 19–114) at one of the time points (range: 0.5–2 years for HIV-positive donors, or 0.5–7 years for uninfected controls). The Vγ2 chain sequences with in-frame stop codons were excluded.

Figure 4. Targeted Instability within the V γ 2-J γ 1.2 Repertoire of HIV-infected donors

V γ 2 sequences were obtained from 7 HIV-infected donors at 6 month intervals for 2 years (study visits 1–5, left column) and 9 HIV-negative volunteers in 1999, 2004 or 2006 (right column). The HIV+ donors MPD01 and MPD11 are separated by a dashed line to indicate that we did not have pre-HAART samples for these individuals and instead substituted data from the first visit after HAART initiation. PBMC were purified from the blood of HIV-positive or negative donors and V γ 2 chains were amplified by RT-PCR. For each donor, V γ 2 (upper row) or V γ 2-J γ 1.2 (lower row) nucleotypes were compared between/among time points and those present at more than one time point were considered conserved. The number of sequences that encoded conserved V γ 2 or V γ 2-J γ 1.2 nucleotypes was divided by the total number of sequences encoding V γ 2 or V γ 2-J γ 1.2 nucleotypes, respectively. The conserved repertoire proportion was calculated for each time point and plotted for all donors. Each time point represents an average of 40 individual sequences (range: 19–114) but is a single determination and does not have a standard error. Truncated V γ 2 chains encoding in-frame stop codons were excluded from the analysis. Dotted lines indicate the approximate limit of detection for specific V γ 2 sequences (~10%). Part of these data for HIV-negative donors were reported earlier (Hebbeler et al. 2006).

Figure 5. Peripheral Blood Vδ2 Frequencies are not Related to Changes in CD4 cell Count

Flow cytometry was performed on specimens taken from 10 donors (5 suppressors and 5 non-suppressors) at the pre-HAART visit and at the peak of CD4 cell count post-HAART. We compared the Vδ2+ cell frequencies with the CD4 cell counts at these time points (panel A). There was no significant relationship between these values. Similarly, we compared the change in Vγ2 repertoire (on the basis of spectratyping to define the proportion of chains with lengths between 990–996 nucleotides) for 15 donors (6 suppressors, 7 non-suppressors and 2 persons where we used the initial visit after HAART as the baseline). Again, we did not see a significant relationship between Vγ2 chain repertoire and CD4 cell count.

Table 1

MACS Donors and Treatment Experience

Donor ID	Suppressors ⁺	Age [*]	Sex	TREATMENT HISTORY		NADIR BEFORE STUDY		INITIAL TIMEPOINT		PEAK DURING STUDY	
				Treatment Prior to the Study Interval	During Study Interval	CD4 Count		CD4 Count	Viral Load ^{**}	CD4 Count	Viral Load ^{**}
MPD03		40	M	NO	ART, HAART	20		51	271438	364	<50
MPD09		31	M	NO	HAART	335		470	5755	1029	<50
MPD15		34	M	NO	HAART	529		609	62067	1085	<50
MPD12		36	M	ART	HAART	400		462	519	665	<50
MPD07		41	M	ART	ART, HAART	13		41	13244	864	<50
MPD02		40	M	NO	HAART	690		164	189,796	538	<50
Non-suppressors ⁺											
MPD16		47	M	NO	ART, HAART	186		108	396618	479	69780
MPD10		39	M	ART	HAART	242		380	44341	477	65338
MPD14		41	M	ART	ART, HAART	259		253	98403	461	94329
MPD04		43	M	ART	ART, HAART	75		302	243165	296	91988
MPD06		37	M	ART	ART, HAART	142		130	12250	392	39268
MPD13		44	M	ART	ART, HAART	192		256	50473	340	46524
MPD05		37	M	NO	ART, HAART	335		370	4891	387	7891
Other ⁺											
MPD08		44	M	ART	ART, HAART	253		633	<50	815	<50
MPD11		33	M	NO	HAART	921		1481	<50	1481	<50
MPD01		47	M	ART	ART, HAART	88		143	<50	207	<50

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^{*} Age in years at the initial study visit.^{**} Viral load measurements listed as <50 (copies/mL) are below the detection limit for the viral RNA assay.⁺ *Suppressors* describes patients with complete (<50 vRNA copies per ml of plasma) suppression of HIV after HAART. *Non-suppressors* describes individuals with detectable viremia even during HAART. *Other* describes patients with viral suppression during HAART but we did not have specimens from the visit immediately preceding HAART initiation.