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The γδ T-cell receptor repertoire is reconstituted in HIV patients after prolonged antiretroviral therapy

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Abstract

Objective—Determine whether reconstitution of Vγ2Vδ2 T cells in patients with HIV is due to new cell synthesis with recovery of the T-cell receptor repertoire or proliferative expansion of residual cells from the time of treatment initiation.

Design—Perform a cross-sectional analysis of the T-cell receptor complexity of Vγ2 chain in patients treated for HIV, natural virus suppressors who control viremia to undetectable levels, patients with chronic low-level viremia in the absence of therapy, and uninfected controls. Apply quantitative methods for repertoire analysis to assess the degree of Vδ2 repertoire loss or reconstitution.

Methods—T-cell receptor Vγ2 chain DNA clones (up to 300 per patient sample) were sequenced and aligned to enumerate the antigen-reactive subset with Vγ2-Jγ1.2 rearrangements. Predominant shared (public) sequences in each patient were compared to a reference library of public sequences from uninfected controls to assess the extent of similarity. Repertoire comparisons were quantified through bioinformatics testing.

Results—Patients with prolonged virus suppression due to antiretroviral therapy reconstituted the Vγ2 T-cell repertoire to near-normal levels. Natural virus suppressors were similar to the treatment group. Severe defects in the Vγ2 T-cell receptor repertoire were observed in patients with chronic viremia despite the absence of overt disease.

Conclusion—Prolonged HIV suppression with antiretroviral therapy leads to reconstitution of the Vγ2Vδ2 T-cell subset deleted in HIV disease. Direct evidence for repair of the T-cell receptor repertoire supports a view that treatment-associated immune reconstitution is due to new cell synthesis and not to expansion of residual cell populations.

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Conflicts of interest
The authors have no conflict of interests related to these studies.

Keywords

antiretroviral therapy; HIV; immune reconstitution; repertoire; T-cell receptor; thymic function; Vgamma9; Vgamma2

Introduction

HIV infection causes specific depletion of CD4-negative Vγ2Vδ2 T cells (a subset of γδ T cells) in patients with HIV disease [1,2]. Vγ2Vδ2 T-cell depletion is remarkably consistent among individuals with HIV disease and may occur when Envelope glycoprotein binds to elevated levels of CCR5, which activates caspase and causes cell death; CCR5 levels are very high in these cells [3]. Prolonged antiretroviral therapy (ART) appears to reconstitute Vγ2Vδ2 T cells [4]. Untreated natural virus suppressors (viral loads <400 RNA copies/ml of blood in the absence of ART [5]) also called elite controllers, had Vγ2Vδ2 T-cell counts similar to uninfected controls, but the Vγ2 repertoire was damaged [6], and higher cell counts reflected expansion of the residual population [7]. Untreated HIV-positive patients with persistent low-level viremia had low Vγ2Vδ2 T cells [8] and significant damage to the T-cell receptor (TCR) repertoire [2].

Here, we compared the Vγ2 repertoires (also designated Vγ9 in an alternate nomenclature) among three groups of HIV patients to determine the mechanism for Vγ2Vδ2 T-cell reconstitution after long-term virus suppression. We focus on public Vγ2 chains found in many unrelated individuals due to the lack of major histocompatibility complex (MHC) restriction for γδ T-cell antigen responses [9]; at least eight public clonotypes were found in more than 50% of all healthy volunteers [10]. Comparing the presence or absence of these public Vγ2 chains among HIV-positive groups defines the extent of depletion or reconstitution of the TCR repertoire.

Results

Peripheral blood mononuclear cells from four groups: patients receiving ARTand having CD4 counts above 300 cells/μl (ART), natural virus suppressors who control HIV to undetectable levels in the absence of therapy (NVS), patients with chronic viremia not receiving therapy (VIR), and HIV-negative controls. Specimens were used for cloning cDNA for individual Vγ2 chains, followed by analysis of the T-cell receptor repertoire (Supplemental digital content Table 1 and Methods, http://links.lww.com/QAD/A342). Approximately 250 Vγ2 chain sequences were analyzed for each patient or control specimen. In control donors, more than 60% have the Vγ2-Jγ1.2 rearrangement. All HIV-infected patients had profound depletion of phosphoantigen-reactive Vγ2-Jγ1.2 chains (Fig. 1b). This value was as low as 10% for the VIR group. For the ARTand NVS groups, Vγ2-Jγ1.2 comprised approximately 30% of all Vγ2 chains.

The normal Vγ2 repertoire is dominated by public clonotypes [11], each of which is encoded by multiple nucleotypes. We matched Vγ2 clonotype sequences from our HIV-positive groups to a table of public clonotypes from HIV-negative African Americans (Supplemental Digital Content Table 2, http://links.lww.com/QAD/A342) [10]. A significant decrease

among public clonotypes was apparent for the ART, NVS, and VIR groups. In healthy controls, 52% of all Vγ2-Jγ1.2 rearrangements were public clonotypes (Fig. 1c). The NVS group had approximately 20%, a result similar to the VIR group. The ART group had approximately 30% public Vγ2 clonotypes, a value lower than controls but higher than other HIV-infected groups. Our earlier studies implied higher levels of Vγ2-Jγ1.2 cells in ART compared to other groups, but did not enumerate public clonotypes [4,12].

Next, we examined the distribution of nucleotypes for several of the most common public clonotypes including (PubSeq1) (Fig. 1c). PubSeq1 was encoded by five different nucleotypes in controls and ART patients, but was less frequent in NVS or VIR groups (Supplemental digital content Table 3, http://links.lww.com/QAD/A342). Controls had an average of 2.4 nucleotypes for PubSeq1; values were lower in all HIV-positive groups. However, we found examples where nucleotype abundance was similar to controls even though clonotype expression was lower (e.g. PubSeq2 for ART and NVS groups). For PubSeq6 and PubSeq7 there were substantial defects in both clonotype and nucleotype expression for all HIV-positive groups. PubSeq8 had low clonotype abundance for all HIV-positive groups, but nucleotype levels were similar to controls for ART and NVS groups.

For the VIR group, five of the seven patients had PubSeq1 and only one patient had more than one nucleotype (Fig. 1c). For PubSeq2, two of the seven VIR and six of the eight controls had this clonotype. Patients in the NVS group were likely to express PubSeq1 (five of nine) or PubSeq2 (four of nine). Most surprising was the group of ART patients where all the eight patients expressed PubSeq1 with an average of 1.9 nucleotypes per person (Fig. 1c). Thus, every control or ART patient had PubSeq1 and had similar nucleotype abundance. When PubSeq1 was present in NVS or VIR groups, it was encoded by nine nucleotypes in five of the NVS patients (average 1.8) and seven nucleotypes in five of the VIR Vγ2 patients (average 1.4). Patient 5 in the ART group showed strong selection for PubSeq1 (three nucleotypes), PubSeq2 (three nucleotypes), and the less frequent PubSeq8. A similar pattern was seen for patient #2 of the NVS group who had three nucleotypes encoding PubSeq1 and six nucleotypes encoding PubSeq2. Surprisingly, nucleotype diversity for ART or NVS patients was sometimes greater than for uninfected controls and we found several nucleotypes that were not present in control groups. Whether these Vγ2 sequences are selected for their response to HIV, another infectious agent, malignancy, or unknown pathology could not be determined.

T-cell receptor-Vγ2 repertoires are similar for antiretroviral therapy and uninfected control groups

Individual TCR repertoires are complex because of clonal diversity (increasing the number of unique TCR sequences) and selection, which causes individual clones to be present above or below the population averages. In ‘shallow’ sequencing studies like ours, the Vγ2 sequence sample size (about 250 sequences per sample) is below the number required to cover an entire repertoire. Most Vγ2 sequences are present once within each sample set and a few are present at higher, and sometimes much higher frequencies. Specialized statistical methods are required to make group-wise comparisons of repertoire complexity (see online

Methods). One approach is to compare, between groups, the level of similarity between pairs of individual repertoires within each group [13,14].

We compared the appearance of common clonotypes between pairs of individual repertoires. Differences in the median number of common clonotypes per group between control and ART patients were not statistically significant (Fig. 2a). There were significantly more common clonotypes between the control and ART groups compared with NVS, or between the control and VIR groups. We calculated the Morisita-Horn similarity index between pairs of clonotype repertoires in each group, which measures the abundance of individual clonotypes (or nucleotypes) and total number of clonotypes (or nucleotypes) in pair-wise comparisons; significant differences were observed between control and all other groups and there was a trend to lower similarity indices for NVS and VIR groups (Fig. 2b). Statistically significant differences in Morisita-Horn similarity indices between control and ART groups reflect differences in the abundance of common clonotypes, showing the ART group is actually more heterogeneous than the control group. Similar results were obtained when comparing the numbers of common nucleotypes (Fig. 2c). The Morisita-Horn similarity analysis for nucleotype repertoires showed significantly greater similarity in the control group compared with either NVS or VIR repertoires and in ART compared with NVS groups (Fig. 2d). Again, control and ART groups were distinguishable from the NVS and VIR groups in terms of nucleotype complexity.

Discussion

Profound depletion of CD4-negative Vγ2Vδ2 T cells in HIV disease is specific for cells expressing a Vγ2-Jγ1.2Vδ2 TCR [1], the same receptor that recognizes stimulatory phosphoantigens [15–17] and tumor cells [18–20]. Now, nearly 10 years after the introduction of combination therapy, we can assess the long-term effects of virus suppression on reconstitution of the Vγ2Vδ2 TCR repertoire.

Focusing on γδ T cells is a new approach for studying treatment effects. Immune reconstitution in HIV disease is usually judged by treatment-associated increases in the $CD4^+$T-cell count [21] which are related to fewer opportunistic infections and improved responses to vaccination [22]. The extent of $CD4^+$T-cell reconstitution has been related to nadir CD4 cell count [23] and the duration of viremia prior to treatment [24].

Proliferative expansion of surviving $CD4^+$T cells is one model for immune reconstitution [25]. This mechanism would increase CD4 cell counts without affecting TCR complexity. Alternately, reconstitution of $CD4^+$T cells by de-novo thymic output would increase both counts and repertoire complexity. Studies on TCR recombination excisional circles (TRECs), a byproduct of TCR rearrangement [26], and phenotypically defined recent thymic emigrants [27] supported thymic output as the driver of immune reconstitution. Using spectratyping methods, the TCR Vβ repertoire complexity was increasing during reconstitution [28] although spectra-type data are not corrected for sample size effects which can be especially important for low $CD4^+$ cell counts, and expansion of individual clones can skew the spectratype and confuse interpretation of data. Sequencing studies are needed to confirm whether reconstitution is associated with increasing TCR repertoire complexity

due to new cell synthesis. However, the large size of the TCRVβ repertoire with more than 10^5 rearranged sequences in healthy adults, and MHC restriction differences reduce the frequency of public clonotypes. These technical issues are obstacles to proving whether or not reconstitution is associated with increased TCR repertoire complexity.

Knowing that the thymus produces γδ, CD4αβ and CD8αβ T cells from the same precursor cell pool [29], reconstitution of the $CD4^+$ TCR repertoire must be matched by similar changes in the γδ TCR repertoire. Our finding that ART reconstitutes Vγ2 repertoire complexity supports the thymic output model for immune reconstitution of both γδ and $CD4^+$ T cells. Although these are cross-sectional studies, our earlier work documented near-extinction of Vγ2-Jγ1.2 sequences in viremic patients with $CD4^+$T-cell counts below 200 cells/μl [2]. All patients in the ART group had nadir $CD4^+$ cell counts below 200 cells/μl two of them with nadir $CD4^+$ below 70 cells/μl. In our experience, it is unlikely that these individuals with such low nadir $CD4^+$ cell counts were harboring substantial Vγ2-Jγ1.2 cells in blood. Surviving γδ T cells sequestered in tissues are unlikely to account for repertoire changes because longitudinal studies on patients initiating combination ART did not detect a rapid rebound in Vγ2Vδ2 T cells [2]. The increased complexity of reconstituted γδ TCR repertoire should be matched by similar changes in the αβ TCR repertoire in $CD4^+$ cells, a result consistent with some recent studies [30].

Even though treatment reconstituted the Vγ2 chain receptor repertoire, functional responses to phosphoantigen were not restored fully [12]. We did find that bisphosphonate (zoledronate) induced Vγ2Vδ2 cell proliferation and antibody-dependent cellular cytotoxicity (ADCC) in HIV-positive patients who had received ART [31]. Bisphosphonate treatment has been developed as an immunotherapy for cancer, by increasing tumor cytolysis or elevating ADCC activity [32,33], and was well tolerated by HIV patients [34]. Knowing that treated HIV patients can recover a functional Vγ2 chain repertoire and their effector function can be stimulated by bisphosphonates, we are encouraged to initiate bisphosphonate therapy studies in patients with HIV disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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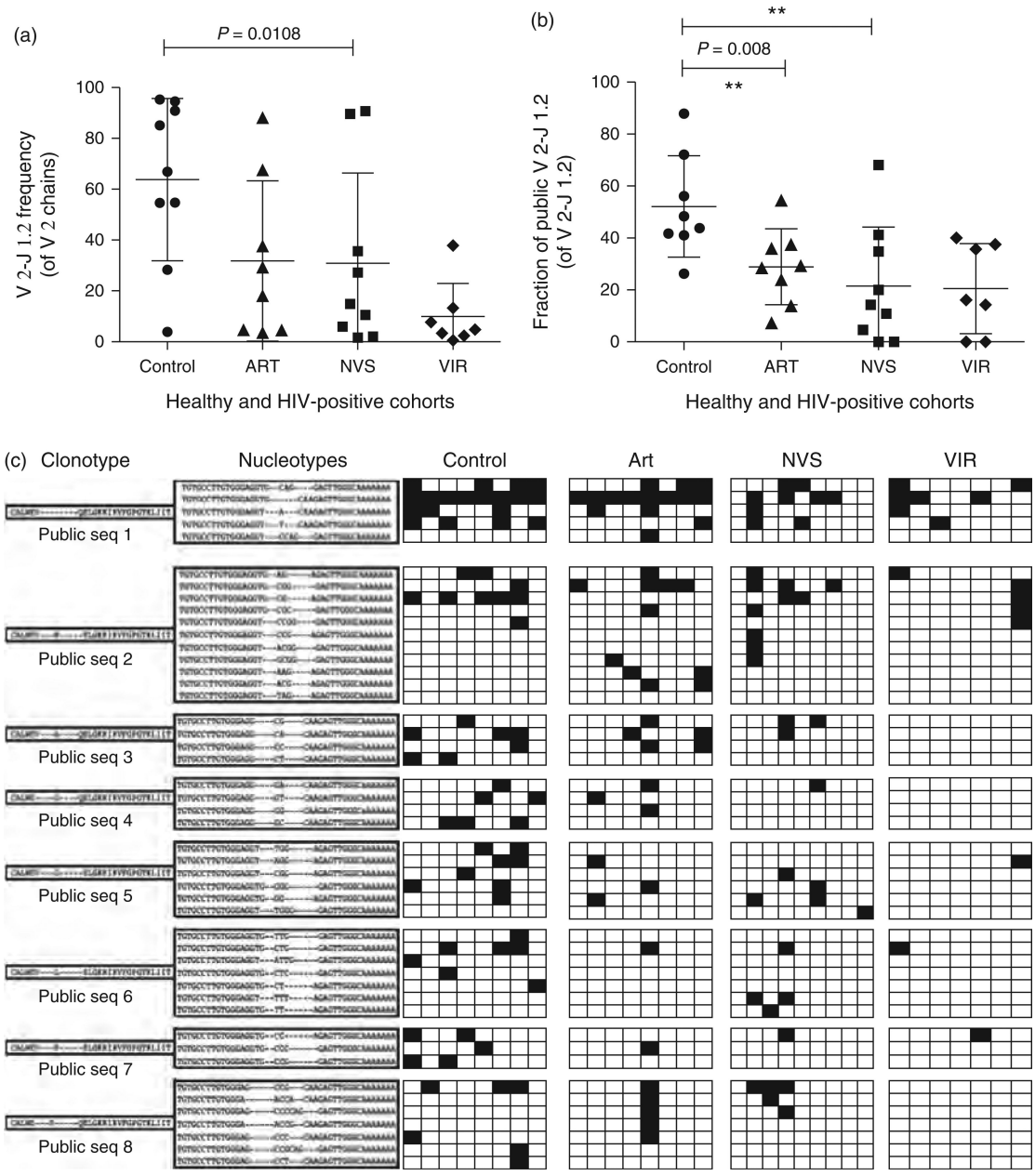

Fig. 1. HIV infection depletes circulating Vγ2-Jγ1.2Vδ2 T cells

(a) The fraction of Vγ2 chains expressing Jγ1.2 segment is lowest for the VIR group. The fraction of chains expressing Jγ1.2 was calculated and plotted for the four groups (mean + SD). On average, approximately 60% of Jγ2 chains expressed the Jγ1.2 segment in healthy controls, whereas only approximately 10% Jγ2 chains were Jγ1.2 in VIR. For NVS and ART groups this number was approximately 30%. (b) The fraction of Vγ2-Jγ1.2 chains expressing public Jγ1.2 clonotype is lowest for the VIR group. Public clonotypes were identified as aa sequences present in more than one patient in the control group, and these sequences were scored for their presence in HIV-positive groups. For control group, approximately 52% of Vγ2-Jγ1.2 chains were found in two or more donors, whereas approximately 20% of Vγ2-Jγ1.2 chains were public for VIR and NVS groups. ART group

had approximately 30% public Vγ2-Jγ1.2 chain. For each panel horizontal lines represent mean values. Statistical comparisons used Kruskal–Wallis test; P values less than 0.05 were considered significant. (c) Clonotype and nucleotype abundance for the most common public Vγ2-Jγ1.2 chains in control, ART, NVS, and VIR groups. The eight most common public clonotypes (PubSeq 1–8) are listed along with all their nucleotypes found in this study. Vertical columns are grouped into control, ART, NVS, and VIR groups as described in the text. Each individual column represents sequencing data for an individual patient. A shaded box indicates that nucleotype was present in the set of TCR-Vγ2 sequences from the individual patient or control. ART, antiretroviral therapy; NVS, natural virus suppressor; TCR, T-cell receptor; VIR, viremic and not receiving therapy.

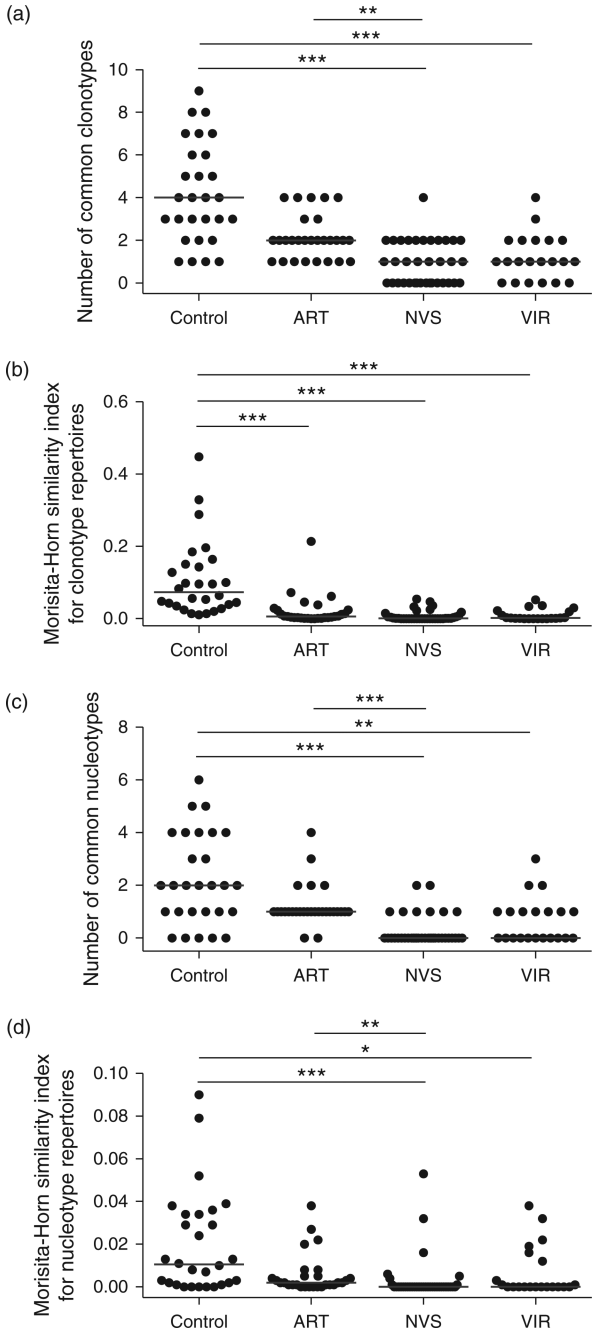

Fig. 2. Similarity analysis between the TCR-Vγ2 repertoires of pairs of individuals within each group

The number of clonotypes (a) or nucleotypes (c) common between individual repertoires and the Morisita-Horn similarity indices for clonotype (b) and nucleotype (d) repertoires were estimated for a sample size of 98 TCR-Vγ2 sequences per individual repertoire. Horizontal lines represent the median similarity values per group and asterisks show $P <$ 0.0005 (***), ($P < 0.005$ (**), or $P < 0.05$ (*). Statistical comparisons were made using a Kruskal–Wallis test and Dunn's multiple comparison post-tests. In terms of common

nucleotypes (c) or similarity index for nucleotype repertoire (d), control and ART groups were not different at $P < 0.05$. ART, antiretroviral therapy; TCR, T-cell receptor.