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Measuring the contribution of γδ T cells to the persistent HIV reservoir

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Abstract

Objective: To study the contribution of γδ T cells to the persistent HIV reservoir.

Design: Fifteen HIV-seropositive individuals on suppressive ART were included. We performed parallel quantitative viral outgrowth assays (QVOA) of resting CD4+ T (rCD4) cells in the presence or absence of γδ T cells.

Methods: Resting αβ+CD4$^+$ T cells were magnetically isolated from PBMCs using two different custom cocktails, only one kit contained antibodies to deplete γδ T cells, resulting in two populations: rCD4 cells and rCD4 cells depleted of γδ cells. Frequency of infection was analyzed by QVOA and DNA measurements.

Results: Recovery of replication competent HIV from cultures of rCD4 cells was similar in 11 individuals despite the presence of γδ T cells. In four donors, HIV recovery was lower when γδ T cells were present. Expression of the cytotoxic marker CD16 on Vδ2 cells was the only variable associated with the lower HIV recovery. Our results highlight the potency of those responses since a mean of 10.000 γδ T cells were present within 2.5million rCD4 cells. However, despite the low frequency of γδ T cells, the presence of cytotoxic Vδ2 cells correlated with lower HIV recovery from cultures of rCD4 cells.

Conclusions: Results of this study show that quantification of the contribution of γδ T cells to the reservoir is challenging due to their low numbers compared to conventional rCD4 cells and

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highlights the potent antiviral function of γδ T cells and the impact of their presence on the frequency of latent HIV infection.

Keywords

gammadelta T cells; HIV latency; resting CD4 cells; HIV reservoir; CD16; antiviral function

INTRODUCTION

The major obstacle in achieving a cure for HIV infection is the existence of a persistent reservoir within latently infected cells that can produce replication-competent virus if antiretroviral therapy (ART) is stopped [1, 2]. The HIV reservoir concentrates mainly in resting CD4+ T (rCD4) cells [3], including CD4 T cell subsets such as T memory stem cells (TSCM) and T follicular helper (Tfh) [4, 5]. In addition, we previously reported that γδ T cells can harbor replication-competent HIV [6, 7] suggesting that these cells can contribute to the recovery of HIV upon latency reversal.

γδ T cells represent between 2–10% of circulating $CD3^+$ T cells [8] and express T -cell receptors (TCR) composed of γ and δ chains. They are classified according to their TCRδ chain usage into Vδ1 and Vδ2 cells [9]. Vδ2 cells are the major circulating subpopulation of γδ T cells in healthy individuals and constitute around 0.5–5% of total CD3+ T cells [8]. However, Vδ2 cells are depleted during HIV infection via direct, through transient upregulation of the CD4 receptor [8], and indirect mechanisms [6, 10]. Concomitantly with this depletion, the number of circulating Vδ1 cells which are mainly found in tissues increases and the Vδ2/Vδ1 ratio relative to that in HIV-seronegative individuals is inversed [11]. Vδ2 cells can be productively infected, harbor high levels of HIV DNA [6, 12] and constitute a novel reservoir of HIV infection [6]. However, in our previous study due to the low γδ T cell numbers, we could not measure the extent of the contribution of this cell population to the total HIV reservoir. To overcome this difficulty, in the present study, we performed QVOA on parallel cultures of rCD4 cells in the presence (rCD4+γδ) or absence (rCD4--$\gamma\delta_{dep}$) of γδ T cells to analyze the contribution of γδ T cells to the inducible replication-competent HIV reservoir. We could not detect differences between QVOA performed in the presence or absence of γδ T cells highlighting the difficulty of measuring the contribution of γδ T cells to the cellular reservoir. However, we found that despite their scarce number, γδ T cells exerted a potent anti-HIV function that translated into a lower recovery of HIV in 27% of the donors.

METHODS

HIV-seropositive and HIV-seronegative donors

Characteristics of the ART-suppressed cohort of HIV participants included in this study have been previously reported [13, 14]. Briefly, all HIV-seropositive participants were recruited through the University of North Carolina (UNC) Aids Clinical Trials Unit and the Women Interagency HIV Study (WIHS) (UNC and University of California, San Francisco sites). Participants were on stable ART with plasma HIV-1 RNA <50 copies/mL for ≥10 months before enrollment and no blips (i.e., >50copies/mL) were detected for at least two years prior to enrollment, when applicable. We included HIV-seropositive donors treated in chronic and acute HIV infection. HIV-seropositive donors treated in the acute phase of HIV infection started therapy within 45 days of the estimated date of infection. In addition, HIV-seronegative donors were screened for high CCR5 expression and following leukapheresis, isolated cells were frozen. All HIV-seropositive and seronegative participants provided written informed consent. Studies were approved by the Institutional Review Board of the University of North Carolina and the University of San Francisco. Buffy coats from HIV-seronegative donors were obtained from the New York Blood Center (Long Island City, NY, USA).

Isolation of resting CD4 cells

Leukapheresis was performed and peripheral blood mononuclear cells (PBMCs) were isolated via Ficoll gradient. Total resting $\alpha\beta$+CD4$^+$ T cells (rCD4) were then magnetically isolated by negative selection from ART-suppressed HIV-seropositive participants using two different custom antibody cocktails from Stemcell Technologies in parallel. One cocktail contained anti-$\gamma\delta$TCR antibodies (pan-$\gamma\delta$TCR) to deplete $\gamma\delta$ T cells and therefore rCD4 cells that did not contain $\gamma\delta$ T cells (rCD4--$\gamma\delta_{dep}$) were recovered, while the other kit did not have anti-$\gamma\delta$TCR antibodies and rCD4 containing $\gamma\delta$ T cells (rCD4+$\gamma\delta$) were isolated. Both cocktails contained the following antibodies: CD8, CD14, CD16, CD19, CD20, CD36, CD56, CD123, Glycophorin A, CD66b, CD25, HLADR and CD69. Isolations were performed in parallel following the manufacturer's instructions. An aliquot of the isolated fractions was analyzed by flow cytometry for purity, as described below.

Quantitative viral outgrowth assays (QVOA)

After rCD4 cell isolation, QVOA was performed as previously reported [15–19]. Briefly, an average of 42.6×10^6 rCD4 cells from each isolation method (rCD4--$\gamma\delta_{dep}$ and rCD4+$\gamma\delta$) were cultured in parallel in limiting dilution. The cells were activated with 2¼g/mL purified PHA (Thermofisher) and 60U/mL IL-2, in the presence of allogeneic irradiated PBMCs from an HIV-seronegative donor. After 24 hours of activation, the cells were washed to remove the PHA and feeder cells (PHA-activated PBMCs depleted of CD8+ T cells from an HIV-negative donor displaying high CCR5 expression) were added to amplify outgrowth of the virus. Each participant is matched to a specific HIV-negative donor and feeder cells from the same donor are used throughout the experiment. Media was refreshed every three to four days and supernatants were harvested at day 15 to perform HIV p24 ELISA quantification (ABL, Rockville, MA, USA). Positivity of the HIV p24 ELISA was confirmed at day 19 of culture. Infectious units per million (IUPM) cells was calculated for each QVOA using the HIV p24 ELISA data, as described in the statistical methods.

Quantification of total HIV DNA levels

On the day of the HIV-seropositive participant's leukapheresis, if enough cells were available, aliquots of 5 million purified rCD4+$\gamma\delta$ cells, and 5 million purified rCD4--$\gamma\delta_{dep}$ cells were pelleted, snap frozen, and stored at −80°C. DNA was extracted from the two isolated cell populations using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. The extracted DNA concentration and purity was determined using the Nanodrop 2000 spectrophotometer (Thermo Scientific). Total HIV-1 DNA copies were assessed by quantitative PCR (qPCR) using primers and probes to amplify HIV-1 *gag*, as previously described. Primers and probes used targeted conserved regions of HIV-1 *gag* [20]. Briefly, 500ng of total cellular DNA were run per PCR reaction in triplicate and for each qPCR run, an HIV-1 *gag* standard curve was generated as described elsewhere [21]. Results were expressed as HIV-1 *gag* copies per million cells.

3' Half genome sequencing

We used phylogenetic analysis to explore whether there is evidence that γδ T cells reduce virus production in QVOA by eliminating a genetically distinct subset of inducible, replication competent viruses. The following protocol was used to sequence and perform phylogenetic analyses of outgrowth viruses from three HIV-seropositive participants (participants 354, 357 and 363). First, viral RNA was isolated from p24 positive QVOA wells and converted to cDNA using Superscript III Reverse Transcriptase and an oligo(dT) primer. 3' half genomes (4924 to 9604 on HXB2) were amplified by PCR using barcoded primers, and the PCR products were gel purified. The SMARTbell Template Prep Kit (PacBio) was used to add adaptors to amplicons and amplicons were sequenced using the PacBio Sequel platform (movie time of 10 hours). Sequences were grouped by barcode and high-quality sequences were analyzed using the PacBio Long Amplicon Analysis (LAA) package. Sequences were visually screened to confirm that reading frames were intact. Sequences from each participant were aligned using MUSCLE (v3.8.1) and a neighbor-joining phylogenetic tree (XXCITE Capoferri) was constructed for each individual.

Flow Cytometry

To estimate the frequency of γδ T cells in each population (rCD4+γδ and rCD4--$\gamma\delta_{dep}$), an aliquot from both cell populations was stained with monoclonal antibodies (mAbs) against CD3 (clone SK7), CD4 (clone OKT4), Vδ1 (clone TS8.2) and Vδ2 (clone M-T271) (all antibodies from Biolegend, San Diego, CA USA, except Vδ1 from Thermofisher). To analyze the expression of markers associated with cytotoxic functions in γδ T cells, PBMCs from the participants were stained with mAbs against CD16 (clone 3G8) and CD56 (clone HCD56). Cells were incubated in staining buffer (i.e. 10% FBS in PBS) for 20 minutes on ice in the dark, washed and then fixed in 2% paraformaldehyde solution. Standard controls including compensation and fluorescence minus one controls (FMO), were used and data were acquired on an Attune Nxt instrument. Analysis was performed using Flow Jo version 10.1.

HIV inhibition assays

Viral inhibition assays were performed as previously described [22]. Briefly, HIV-infected CD4 T cells were cultured alone as a control of HIV production, or co-cultured with Vδ2 cells in triplicate at a 1:10 effector:target ratio. Supernatants were harvested at day 7 and stored at −20°C until HIVp24 ELISA quantification (ABL_{inc}, Rockville, MA, USA) was performed. Results of 1:10 effector:target cell ratio cocultures are shown. Results are expressed as percent of viral inhibition normalized to HIV p24 production when target CD4 T cells were cultured alone.

Statistical Analysis

Infectious units per million (IUPM) for rCD4+γδ and rCD4--$γδ_{dep}$ samples were estimated using the SLDAssay R software package [23]. The bias-corrected (BC) maximum likelihood estimate, corresponding exact confidence intervals, and goodness of fit p-values (PGOF) were computed. For each individual, the log of the ratio of the IUPM estimates between rCD4+γδ and rCD4--$γδ_{dep}$ cells was calculated. In addition, the IUPM difference between rCD4+γδ and rCD4--$γδ_{dep}$ and a corresponding exact 95% CI was calculated for each individual by inverting a likelihood ratio test. In order to assess associations between covariates of interest and the change in IUPM between rCD4+γδ and rCD4--$γδ_{dep}$ cells within an individual, the log of the ratio of IUPM estimates was compared to covariates. To assess the association between the log of the ratio of IUPM estimates and categorical covariates with two levels (gender and acute classification), the Wilcoxon rank sum test was used. For categorical covariates with more than two levels (race), the Kruskal-Wallis test was used. For continuous covariates, the Spearman rank correlation p-value with permutation and the mid-ranks tie handling method was used. All statistical analyses were conducted using R version 3.4.3. Following recommendations from the American Statistical Association [24], instead of presenting results according to the dichotomy of $p<0.05$ ("statistically significant") or $p>=0.05$ ("no statistical difference"), p-values are presented as continuous statistics as well as other numerical results, such as confidence intervals, which provide additional context.

RESULTS

Participants' characteristics

The 15 HIV-seropositive participants had been suppressed for a median period of 3.6 years [range 0.87, 9.35 years]. The median time on ART was 4.8 years [range 0.9, 19.9 years]. Eleven (73.3%) participants were male and four (26.6%) were female. Six (40%) were Caucasian, six African American (40%), two (13.3%) belonged to other races, and one was of unknown race (6.7%). Four (26.7%) participants were treated in the acute phase of infection, and eleven (73.3%) were treated in the chronic phase of infection.

Efficacy of γδ T cell depletion

rCD4 cells isolated from PBMCs using two different cocktails in parallel resulted in two different cell populations: rCD4 cells with γδ T cells (rCD4+γδ cells), and rCD4 cells depleted of γδ T cells (rCD4--$γδ_{dep}$). Frequency of Vδ1 and Vδ2 cells after rCD4 cell isolation was compared by flow cytometry (Supplemental Fig. 1), and the efficacy of the depletion was calculated as the difference between the frequency of γδ T cells present within rCD4+γδ cells minus the frequency of γδ T cells within rCD4--$γδ_{dep}$ cells (Table 2). Within rCD4+γδ cells, mean frequency of CD3+ Vδ2+ cells was 0.2% [range 0.03, 0.70], and mean frequency of CD3+ Vδ1+ cells was 1.06% [range 0.6, 1.9]. However, in cultures of rCD4--$γδ_{dep}$ cells, the mean frequency of Vδ2 cells decreased to 0.02% [range 0, 0.1] and the mean frequency of Vδ1 cells decreased to 0.2% [range 0, 0.75].

Impact of the presence of γδ T cells on the recovery of replication-competent HIV

A mean of 42.6 million rCD4 cells were assayed in parallel cultures and bias corrected (BC)-IUPM values were calculated (Table 1). The mean IUPM was 0.583 (95% CI 0.312, 1.311) for rCD4+γδ cells compared to 1.044 (95% CI 0.557, 2.484) for rCD4--$γδ_{dep}$ cells (p=0.07, Fig. 1A). Interestingly, the pattern of HIV recovery was heterogeneous and some donors had a higher recovery in cultures when γδ T cells were present and vice versa. IUPM estimates and corresponding CIs for rCD4+γδ and rCD4--$γδ_{dep}$ cells for each individual are displayed in Fig. 1B. For most participants, IUPM was similar in wells with or without γδ cells with observed differences likely to be due solely to assay variability. However, in 27% of the participants (313, 363, 354 and 357), the difference in IUPM between wells with and without γδ T cells was greater than expected from only random assay variation; this is reflected by the 95% confidence intervals in Table 2 having lower bounds greater than or equal to 0. In these four participants, the recovery of replication-competent HIV was lower when γδ T cells were present in cultures of rCD4 cells. Finally, in the seven participants with available cells, total *gag* HIV DNA levels were similar between rCD4+γδ and rCD4--$γδ_{dep}$ cells (p=0.15, Fig. 1C). Since the depletion of γδ T cells was not 100% effective (Table 2), we performed correlations between the differences in IUPM in rCD4+γδ and rCD4--$γδ_{dep}$ cells and the efficacy of the depletion showing no correlation (Table 3).

Expression of CD16 on Vδ2 cells is associated with a lower recovery of replication-competent HIV

We hypothesized that the lower recovery of HIV from cultures of rCD4+γδ cells in some donors, was due to a more potent antiviral function from γδ T cells. In order to test this hypothesis, we analyzed the expression of the cytotoxic markers CD16 on Vδ2 cells, and CD56 on both Vδ1 and Vδ2 T cell populations (Fig. 2A) in the HIV-seropositive donors. Since the original flow cytometry panel did not include CD16 to be measured on Vδ1 cells, only the frequency of Vδ2 cells expressing CD16 was accounted for in the analysis.

We calculated the log ratio of the two IUPMs defined as the log(IUPMγδ+/IUPMγδ-) to evaluate what covariates were involved in the difference in IUPMs of rCD4+γδ and rCD4--$γδ_{dep}$ (Table 3). Covariates included; i) demographic characteristics (age, sex and race); ii) immune status (nadir CD4, and CD4 and CD8 counts at the time of leukapheresis); iii) culture-related data (number of cells and total wells cultured); iv) frequency of Vδ1 and Vδ2 cells in the cultures; v) expression of cytotoxic markers (CD16 and CD56) on Vδ1 and Vδ2 cell T cells; and vi) ART-related data (including time on ART, treatment in acute/chronic infection and time participants had been suppressed). Interestingly, expression of the cytotoxic marker CD16 on Vδ2 cells was associated with a lower recovery of replication-competent HIV (R= 0.598, p=0.03, Table 2).

Strengthening our hypothesis, seven of the donors analyzed in this study, had been included in a previous study where the antiviral capacity of their Vδ2 cells had been directly measured in HIV inhibition assays [22]. In that study, isolated Vδ2 cells showed a mean inhibition capacity of 84% when cocultured with isolated autologous CD4+ T cells. Interestingly, in the present study, donors with a more potent antiviral capacity (313, 363, 354 and 357) showed a lower HIV recovery from cultures of rCD4 cells when γδ T cells were present (Fig. 2B).

Similar viral sequences between cultures of rCD4 cells containing or lacking γδ T cells

In order to explore whether viral sequences generated from cultures with or without γδ T cells may be identifiable as a genetically distinct portion of the inducible HIV-1 reservoir, we sequenced and phylogenetically compared outgrowth viruses from three donors (354, 357 and 363) that had lower IUPM estimates in rCD4+γδ compared to rCD4--$\gamma\delta_{dep}$. We did not observe evidence of sequence differences between rCD4+γδ and rCD4--$\gamma\delta_{dep}$ cells (Supplemental Fig. 2). Similar numbers of sequences from the rCD4 cells with γδ T cells (Participant 354 = 29; Participant 357= 52, and Participant 363 = 21) and those depleted of γδ T cells (Participant 354 =28; Participant 357=22, and Participant 363 = 21) were generated. Sequences from both populations were interspersed in the phylogenetic tree and therefore, there was no evidence of a genetically distinct viral lineages found in cultures of rCD4--$\gamma\delta_{dep}$ and rCD4+γδ cells (Supplemental Fig. 2). While we did not detect evidence that specific viral lineages are different from rCD4 cell cultures containing versus lacking γδ T cells, we did not sequence a sufficient number of viruses to detect changes in rare cell populations.

DISCUSSION

In this study, we aimed to assess γδ T cell contribution to the persistent reservoir. We previously demonstrated the capacity of these cells to harbor replication-competent HIV in long-term ART-suppressed individuals [6]. We show that since γδ T cell frequency in peripheral blood is very low and conventional CD4 cells outnumber γδ T cells, measuring their contribution to the total latent reservoir is challenging. However, despite the low frequency of γδ T cells, they exert a potent antiviral function that can directly have an impact on the recovery of replication competent HIV from cultures of rCD4 cells, as supported by the results of this study.

We performed QVOA on cultures of isolated rCD4 cells that had been depleted of γδ T cell populations and compared them to cultures of isolated rCD4 cells with γδ T cells. When possible, experiments were performed in parallel using the same numbers of cells and limiting dilution conditions to avoid assay fluctuations. Unfortunately, we could not measure the extent of the contribution of γδ T cells to the replication-competent virus most likely due to the scarce number of γδ T cells within cultures of rCD4 cells, highlighting the difficulty of working with rare populations. However, for some of the participants included herein the frequency of infection within isolated γδ T cells was quantified in a previous work [6] and in ongoing studies. In participants 263, 321, 354 and 357 HIV was previously recovered from isolated Vδ2 cells [6]. However, when cultured within rCD4 cells, the specific contribution to the viral reservoir from γδ T cells was not quantifiable, possibly because classical rCD4 cells vastly outnumber γδ T cells. Analysis of half 3’ half-genome sequences from HIV p24 positive wells from QVOA showed no evidence of genetic differences between the cultures with versus without γδ T cells, suggesting that virus contained within classical CD4+ T cells and γδ T cells are not different or if different, we were not able to detect them possibly due to the low frequency. Ongoing work will help elucidate the nature of viral isolates from γδ T cells.

Interestingly, 27% of the donors showed increased frequency of infection when γδ T cells were depleted from cultures of rCD4 cell demonstrating the potent antiviral capacity of these cells given their low frequency (less than 10,000 γδ T cells in 2.5×10^6 rCD4 cells). This finding is consistent with our prior work showing that antiviral function of γδ T cells reduced the recovery of replication-competent virus from isolated Vδ2 cells [6]. Although γδ T cells are recognized for their antiviral functions, their potency to reduce the HIV production in cultures of rCD4 cells has not been previously shown. We hypothesized that the potent antiviral function from γδ T cells was responsible for the lower recovery of virus upon reactivation. To test this hypothesis we interrogated the implication of different covariates, including markers of cytotoxicity in γδ T cells [25, 26], in the outcome of HIV recovery. Similar to NK cells, CD16 expression on γδ T cells is associated with cytotoxic function. CD16 expression on Vδ2 cells identifies two different subsets with different responses. Specifically, Vδ2 cells expression CD16 are more phenotypically similar to NK cells, express NK receptors, and high levels of perforin [27]. In addition, a functional linkage has been reported between CD16 and CD3 expression that points to a mechanism to be explored [26]. Our correlation analyses showed that the frequency of Vδ2+ CD16+ cells was associated with a lower recovery of HIV in cultures of rCD4 cells, although we cannot exclude the involvement of other factors, from either γδ T cell population. Strengthening our hypothesis, seven of the donors analyzed in this study, had been included in a previous study where their antiviral capacity had been directly measured in HIV inhibition assays [22]. In the four donors where HIV recovery was lower when γδ T cells were present, we saw a more potent γδ antiviral activity when compared to the other participants in that study. Other covariates, including demographics, immune status at the time of leukapheresis, time on ART or ART initiation in the acute or chronic phase of the infection, frequency of remaining γδ T cells in the cultures after rCD4 cell isolation, were analyzed and did not seem to have a significant role on the recovery of replication competent HIV.

In summary, results of this study show that quantification of the contribution of γδ T cells to the reservoir is challenging due to their low frequency compared to rCD4 cells. However, despite their low frequency, our results show that γδ T cells from some participants, have the ability to reduce the production of replication-competent HIV recovered in cultures of rCD4 cells and highlights the importance of depleting γδ T cells from cultures of rCD4 cells for accurate QVOA measurements and when testing the efficacy of LRAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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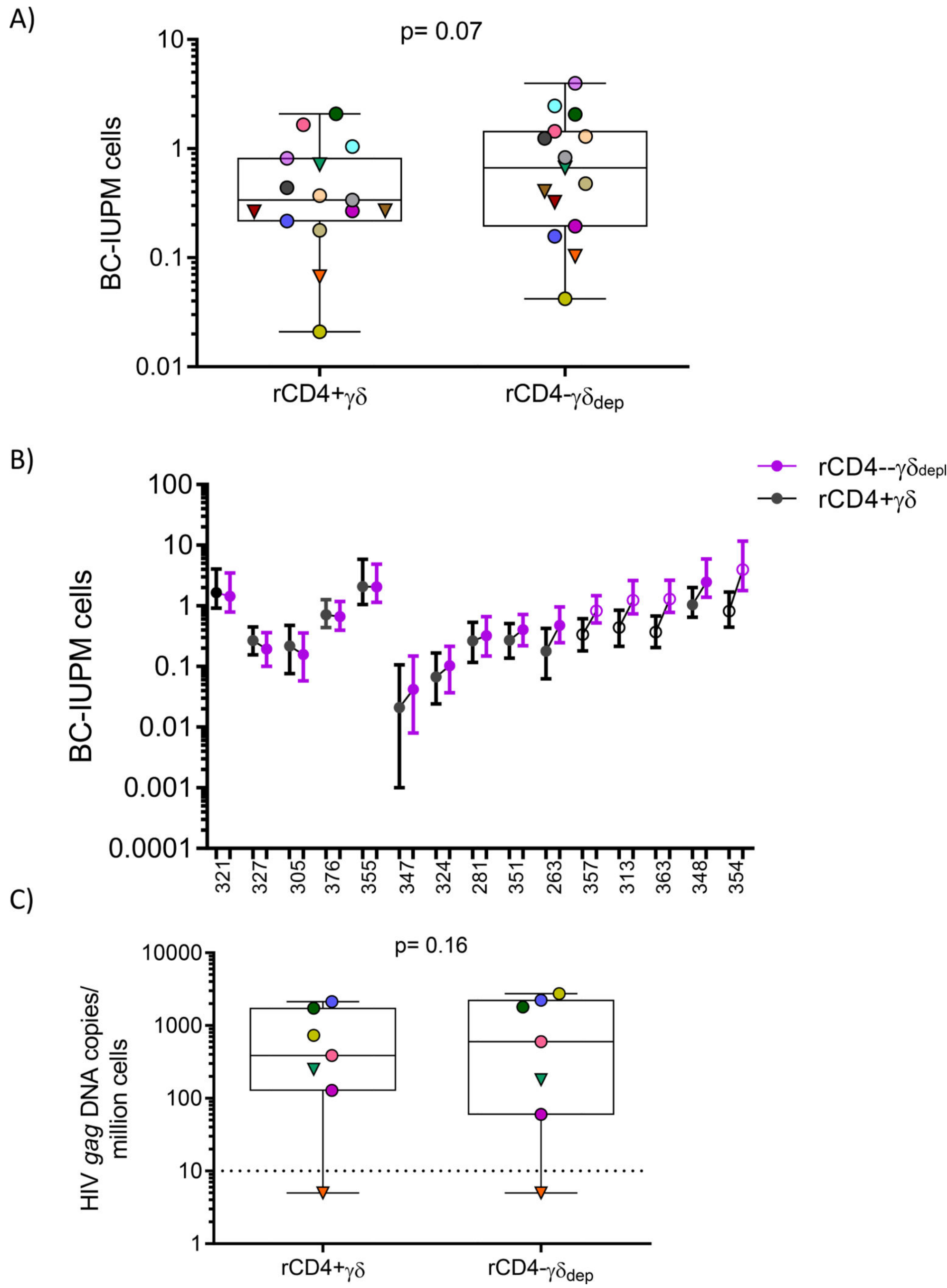

Figure 1. Frequency of persistent HIV infection.

A) Recovery of replication-competent HIV by QVOA. BC-IUPM from 15 HIV-seropositive participants was calculated in parallel cultures of rCD4 cells in the presence (rCD4+γδ cells) or absence of γδ T cells (rCD4--$γδ_{dep}$). Cultures depleted of γδ T cells tended to have a higher BC-IUPM (p=0.07, Wilcoxon rank sum test). **B) Infectious units per million (IUPM) rCD4+γδ cells and IUPM rCD4--$γδ_{dep}$.** Bias corrected (BC)-IUPM estimates and corresponding exact 95% CI are represented for the 15 HIV-seropositive participants included in the study. Open symbols represent donors where the recovery of replication competent HIV was greater in cultures of rCD4--$\gamma\delta_{dep}$ cells than in cultures of rCD4+$\gamma\delta$ cells. **C) Levels of total *gag* HIV DNA.** HIV DNA levels were analyzed in 7 of the 15 participants similar levels between cultures of rCD4+$\gamma\delta$ cells and rCD4--$\gamma\delta_{dep}$ (p=0.16, Wilcoxon rank sum test). In A) and B) circles represent HIV-seropositive participants treated in the chronic phase of the infection and triangles represent participants treated in the acute phase of the infection.

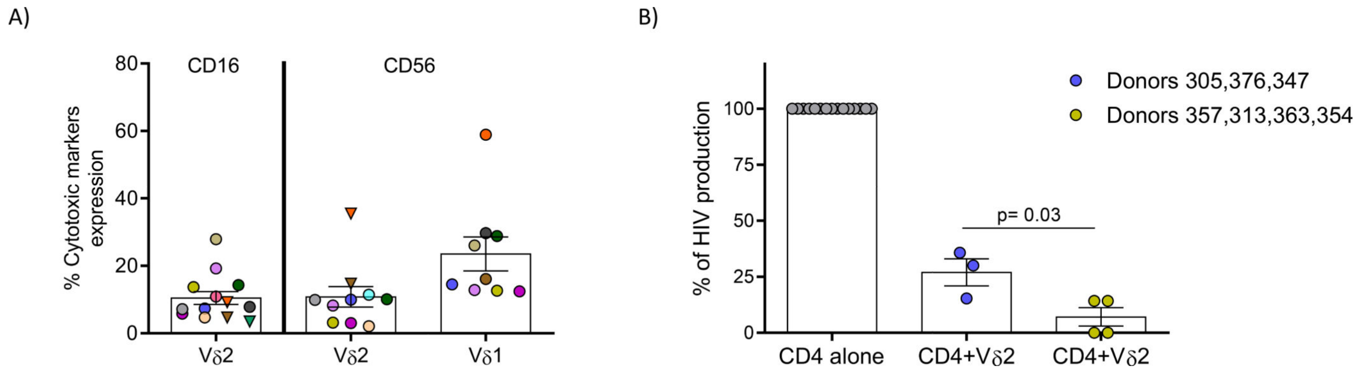

Figure 2. Expression of cytotoxic markers CD16 and CD56 in γδ T cell populations and association with HIV recovery from QVOA.

A) Expression of CD16 in Vδ2 cells and CD56 in Vδ2 and Vδ1 cells. Mean CD16 expression on Vδ2 cells was 10.3%, while CD56 was expressed by a mean of 10.8% in Vδ2 cells compared to a mean of 23.3% in Vδ1 cells. Circles represent HIV-seropositive participants treated in the chronic phase of the infection and triangles represent participants treated in the acute phase of the infection. **B) Enhanced viral inhibition capacity by Vδ2 cells from donor 357, 313, 363 and 354.** HIV inhibition assays from isolated Vδ2 cells cocultured with autologous isolated CD4 cells from ART-suppressed HIV-infected donors. HIV p24 production (measured by ELISA) normalized to the condition where only isolated CD4 cells were cultured is represented. Donors 305, 376 and 327, represented in blue, did not show differences in HIV recovery from cultures of rCD4+γδ and rCD4--γδ$_{dep}$. In Donors 357, 313, 363 and 354, HIV recovery was higher when γδ T cells had been depleted. The capacity to inhibit viral replication from autologous isolated CD4 cells, was higher in Donors 357, 313, 363 and 354 compared to 305, 376 and 327 (p=0.03).

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Table 1.

Culture conditions and estimated IUPM rCD4+γδ and rCD4--γδ_{dep} cells.

DONOR	Cells (x10^6)	Total wells	rCD4+γδ				rCD4--γδ_{dep}			
			Positive wells	BC-IUPM	Lower CI	Upper CI	Positive wells	BC-IUPM	Lower CI	Upper CI
263	33.6	24	5	0.178	0.063	0.424	10	0.476	0.247	0.959
281	33.75	30	6	0.264	0.117	0.532	8	0.323	0.148	0.664
305	33.6	24	6	0.217	0.076	0.477	5	0.157	0.058	0.355
313	33.75	30	10	0.438	0.214	0.843	16	1.239	0.736	2.610
321	33.75	30	17	1.656	0.919	4.043	16	1.440	0.788	3.498
324	63.6	36	4	0.067	0.024	0.167	6	0.103	0.037	0.215
327	63.75	42	14	0.268	0.156	0.451	10	0.194	0.100	0.359
347	48.6	30	1	0.021	0.001	0.107	2	0.042	0.008	0.148
348	33.6	24	17	1.042	0.648	2.011	19	2.468	1.381	5.946
351	48.6	30	10	0.270	0.137	0.507	13	0.406	0.221	0.720
354	33.6	24	13	0.813	0.446	1.682	16	3.966	1.793	11.617
355	33.6	24	17	2.087	1.052	5.870	18	2.057	1.136	4.862
357	48.6	30	12	0.338	0.180	0.612	20	0.827	0.519	1.464
363	48.6	30	12	0.370	0.205	0.679	17	1.292	0.780	2.654
376	48.6	30	18	0.716	0.439	1.261	17	0.667	0.394	1.185

BC, Bias corrected. CI, Confidence Interval.

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Table 2.

Difference in rCD4+γδ and rCD4--$γδ_{dep}$ BC-IUPM estimates per participant, with corresponding exact 95% Confidence Interval (CI).

	Participant ID	Difference in IUPM	Exact CI		Efficacy of γδ depletion	
					Vδ2	Vδ1
1	321	−0.22	−3.26	2.56	100	70
2	327	−0.07	−0.33	0.17	100	55
3	305	−0.06	−0.47	0.23	100	73
4	376	−0.05	−0.82	0.67	43	86
5	355	−0.03	−5.45	3.96	100	100
6	347	0.02	−0.01	0.16	70	74
7	324	0.03	−0.10	0.18	100	87
8	281	0.05	−0.33	0.51	98	82
9	351	0.14	−0.24	0.59	100	100
10	263	0.29	−0.11	0.92	63	100
11	348	1.43	−0.52	6.32	93	51
12	**357**	**0.49**	**−0.00**	**1.21**	**100**	**94**
13	**313**	**0.80**	**0.05**	**2.44**	**50**	**92**
14	**363**	**0.92**	**0.16**	**2.87**	**100**	**100**
15	**354**	**3.15**	**0.49**	**11.43**	**100**	**95**

Bolded text in rows indicate exact CIs with lower endpoint ≥ 0.0, which indicates higher recovery of replication-competent HIV when γδ T cells were depleted from cultures of rCD4 cells.

Table 3.

Tests for association between covariates of interest and log(IUPM rCD4+γδ / IUPM rCD4--$\gamma\delta_{dep}$).

Covariate	P-value	Spearman Rank Correlation
Age	0.77	0.08
Sex	0.85 *	N/A
Race	0.79 **	N/A
Nadir CD4	0.54	−0.16
CD4	0.93	0.03
CD8	0.65	0.13
Number of cells cultured	0.58	−0.15
Number of wells plated	0.44	−0.21
Presence of Vδ2 in rCD4+γδ	0.10	0.45
Presence of Vδ2 in rCD4--$\gamma\delta_{dep}$	0.41	0.22
Presence of Vδ1 in rCD4+γδ	0.48	0.19
Presence of Vδ1 in rCD4--$\gamma\delta_{dep}$	0.83	−0.06
Expression of CD16 in Vδ2 cells	**0.03**	**0.60**
Expression of CD56 in Vδ2 cells	0.41	−0.27
Time on ART	0.27	0.30
Treated in acute phase of infection	0.41 *	N/A
Years of suppression	0.46	0.21
HIV DNA levels in rCD4+γδ	0.49	−0.26
HIV DNA levels in rCD4--$\gamma\delta_{dep}$	0.80	−0.10
Ratio of HIV DNA levels in rCD4+γδ to rCD4--$\gamma\delta_{dep}$	0.38	−0.36

The Spearman rank correlation p-value was used for continuous covariates. The Spearman rank correlation estimate is also provided when applicable.

* The Wilcoxon rank sum test was used for categorical covariates with two levels.

** The Kruskal-Wallis was used for categorical covariates with more than two levels.