The Potential Role of CD16⁺ V γ 2V δ 2 T Cell-Mediated Antibody-Dependent Cell-Mediated Cytotoxicity in Control of HIV Type 1 Disease

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Abstract

Increasing evidence has suggested that HIV infection severely damages the V γ 2V δ 2 (V δ 2) T cells that play an important role in the first-line host response to infectious disease. However, little is known about V δ 2 T cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) in HIV disease. We found that although the CD16⁺ V δ 2 T cell subset hardly participated in phosphoantigen responses dominated by the CD16⁻ V δ 2 T cell subset, the potency of the ADCC function of V δ 2 T cells was correlated with the frequency of the CD16⁺ subset. Thus, two distinct and complementary V δ 2 T cell subsets discriminated by CD16 were characterized to explore the respective impacts of HIV-1 infection on them. HIV-1 disease progression was not only associated with the phosphoantigen responsiveness of the CD16⁻ V δ 2 subset, but also with the ability of the CD16⁺ V δ 2 subset to kill antibody-coated target cells. Furthermore, both of the two V δ 2 functional subsets could be partially restored in HIV-infected patients with antiretroviral therapy. Notably, in the context of an overall HIV-mediated V δ 2 T cell depletion, despite the decline of phosphoantigen-responsive CD16⁻ V δ 2 cells, CD16⁺ V δ 2 cell-mediated ADCC was not compromised but exhibited a functional switch with dramatic promotion of degranulation in the early phase of HIV infection and chronic infection with slower disease progression. Our study reveals functional characterizations of the two V δ 2 T cell subsets with different activation pathways during HIV-1 infection and provides a rational direction for activating the CD16⁺ V δ 2 T cells capable of mediating ADCC as a means to control HIV-1 disease.

Introduction

HUMAN V γ 2V δ 2 T CELLS (V δ 2 T CELLS) are believed to play a vital role in both innate and adaptive immunity.^{1,2} Unlike conventional T cells bearing $\alpha\beta$ T cell receptors (TCR), V δ 2 T cells function in an MHC-independent manner, which do not require antigen processing and presentation by antigen-presenting cells.³⁻⁶ Preprogramming allows V δ 2 T cells to rapidly initiate a “lymphoid stress-surveillance response” without any delay by obligatory clonal expansions or differentiations.⁷ They recognize phosphorylated non-peptidic antigens, which are produced by stressed or infected cells. Phosphoantigen stimulation, such as by isopentenyl pyrophosphate (IPP), has been considered as a model for the normal response of V δ 2 T cells to infection.⁸⁻¹⁰ Several groups have demonstrated that the capacity of V δ 2 T cells to

respond to IPP inversely correlates with HIV-1 disease progression.¹¹⁻¹⁴ The impaired function of V δ 2 T cells in HIV-1 disease could be explained by the specific depletion of the V γ 2J γ 1.2 V δ 2 T cell subpopulation, which is normally most responsive to phosphoantigen stimulation.¹⁵

Antibody-dependent cell-mediated cytotoxicity (ADCC), which relies on specific antibodies and Fc receptor-bearing effector cells for a proper antiviral response, plays an important role in controlling HIV infection. Previous studies have documented compromised ADCC responses in progressive HIV-1 infection from the perspective of HIV-specific antibodies.¹⁶⁻¹⁸ Furthermore, the RV144 Thai trial demonstrated that nonneutralizing antibodies elicited by the vaccination might protect against HIV acquisition, potentially preventing infection through the ADCC mechanism.¹⁹ Effector cells, including natural killer (NK) cells, V δ 2 T cells, and monocytes,

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are able to recognize the antibodies bound to infected cells through a low-affinity Fc receptor for IgG, called Fc γ RIIIa (CD16). Recently, impaired ADCC function of NK cells was observed in HIV-infected individuals,²⁰ which indicates that in addition to antibodies, the capacity of effector cells to respond to target cells should also be studied when evaluating ADCC activity. Similar to NK cells, V δ 2 T cells also express CD16 that can be used for ADCC, but little is known about the V δ 2 T cells with respect to their activity as ADCC effectors during HIV-1 disease progression.

It has been reported that memory V δ 2 T cells can be divided into two subsets with unique effector functions based on the expression of CD16 and these subsets represent different pathways of maturation for circulating V δ 2 T cells.²¹ Thus, V δ 2 T cells comprise a number of distinct effector subsets and likely have complex activities during HIV infection. We propose that one of these activities is ADCC, which is mediated by a unique V δ 2 T cell subset with CD16 expression. Given the divergent activation pathways, we wanted to study in detail the two V δ 2 T cell subsets discriminated by CD16 from uninfected controls, HIV-1-positive subjects without treatment at different stages of infection and HIV-1-positive subjects receiving highly active antiretroviral therapy (HAART). We examined the magnitudes of IPP-induced activation in the two subsets across different cohorts. We also focused on the V δ 2 T cell-mediated ADCC in terms of degranulation and cytokine production, and measured CD16 expression on V δ 2 T cells to see if the baseline frequency of the Fc receptor correlated with their ADCC function. These studies offered us a comprehensive view of V δ 2 T cell activity during HIV infection with an in-depth analysis of these two complementary effector subsets.

Materials and Methods

Subjects

A total of 91 subjects were recruited for the study, including 21 healthy HIV-1-negative controls, 18 treatment-naïve early HIV-1-infected subjects, 42 treatment-naïve chronic HIV-1-infected subjects, and 10 HIV-1-infected subjects receiving HAART (Table 1). All the treatment-naïve HIV-1-infected subjects were from a cohort of HIV-1-positive men who have sex with men (MSM) infected for different lengths of time. The MSM cohort was recruited at Chaoyang CDC in China. The estimated time of HIV infection was interpolated as the midpoint between the negative and HIV-1 seroconversion. Early HIV-1 infection was defined as infection within 6

months (2–6 months). Chronic HIV-1-infected subjects had been infected for at least 1 year (12–36 months); they were further divided into three subgroups based on CD4 T cell counts. The cohort of treated HIV-1-infected subjects was recruited at Dehui CDC in China and had received HAART for at least 1 year (12–72 months) with prolonged limited viral loads <100 copies/ml and with a CD4 T cell count >300 cells/ μ l. Ethical approval was granted by the Institutional Review Board of the National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention. All patients gave informed consent for participation in the study.

Cell processing

Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-treated venous blood by Ficoll Hypaque centrifugation (Sigma) within 6 h of sample collection. After cell counts and a viability check, PBMCs were resuspended at a density of 1×10^6 cells/ml in R-10 medium [RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin] for subsequent experiments.

Phenotypic analysis of V δ 2 T cells

Cell surface staining was performed using the following monoclonal antibodies: CD3 (PerCP conjugate, Clone SK7, BD Biosciences), V δ 2 (PE conjugate, Clone B6, BD Biosciences), and CD16 (PB conjugate, Clone CB16, eBioscience). PBMCs (1×10^6) were used for each staining. After staining, cells were washed with phosphate-buffered saline (PBS), fixed with 2% paraformaldehyde (PFA) solution, and collected on BD FACS Aria. Data were analyzed by FlowJo software (TreeStar Inc.).

V δ 2 T cell stimulation by IPP

PBMCs were cultured at 1.5×10^6 cells/well in R-10 medium in 96-well round-bottomed plates and stimulated with medium alone or isopentenyl pyrophosphate (Sigma), respectively. CD107a (APC conjugate, Clone H4A3, BD Biosciences), Golgi-Stop (BD Biosciences), and Brefeldin A (Sigma) were added to each well. Following an 11-h incubation at 37°C with 5% CO₂, PBMCs were collected, washed with PBS, and stained with monoclonal antibodies CD3, V δ 2, and CD16. Cells were then permeabilized using Caltag Fix & Perm (Invitrogen) and intracellular cytokine staining was carried out for interferon (IFN)- γ (Alexa700 conjugate, Clone B27, BD Biosciences). After staining, cells were washed with PBS and

TABLE 1. CHARACTERISTICS OF SUBJECT GROUPS

Group	Number (males/females)	Age (years)	Viral load (RNA copies/ml)	CD4 count (cells/ μ l)	V δ 2 T count (cells/ μ l)	V δ 2 T % (of CD3 ⁺ T cells)
Early untreated	18 (18/0)	29	42,100 (49–213,000)	349.9 (211.3–662.7)	40.4 (5.3–138.4)	2.6 (0.3–9.3)
Chronic untreated	42 (42/0)					
CD4 < 300	16 (16/0)	33	74,000 (6,100–180,000)	219.3 (21.0–280.2)	17.4 (5.8–118.1)	1.5 (0.6–6.0)
300 $\leq$ CD4 $\leq$ 500	15 (15/0)	31	8,700 (6,100–23,000)	389.1 (316.9–482.9)	28.8 (6.6–135.4)	1.5 (0.5–6.6)
CD4 > 500	11 (11/0)	34	2,630 (790–14,700)	616.0 (509.9–928.6)	43.47 (15.1–80.3)	2.4 (0.9–4.7)
Chronic treated	10 (6/4)	56	50 (50–81)	487.4 (322.7–715.1)	58.4 (9.6–149.8)	4.4 (0.9–10.2)
HIV-negative control	21 (12/9)	30	NA	757.5 (435.8–1044.9)	82.2 (20.8–444.4)	6.6 (1.4–31.9)

Data are presented as median (range); NA, not applicable.

fixed in 2% PFA solution. Labeled cells were acquired on BD FACS Aria and analyzed by FlowJo software.

V δ 2 T cell-mediated ADCC assay

A single cell line p815 (mouse leukemic cell line) coated with rabbit antibodies specific to p815 cells (Accurate Chemical & Scientific Corporation) was used as target cells. Target cells were prepared by culturing p815 cells with p815 antibodies in R-10 medium for 1 h at 37°C with 5% CO₂ prior to ADCC assay. PBMCs were used as effector cells and stimulated with R-10 medium, uncoated p815 cells, or antibody-coated (Ab-coated) p815 cells, respectively. CD107a, Golgi-Stop, and Brefeldin A were added to each well and samples were incubated for 11 h at 37°C with 5% CO₂. After incubation, cells underwent surface staining with monoclonal antibodies CD3, V δ 2, and CD16 and intracellular staining for IFN- γ . After staining, cells were washed with PBS and fixed in 2% PFA solution. Labeled cells were acquired on BD FACS Aria and analyzed by FlowJo software.

Cell counts of CD4⁺ T cells and V δ 2 T cells

The cell counts of CD4⁺ T cells and CD3⁺ T cells were measured in EDTA-treated venous blood with FACS Calibur TruCount tube (Becton Dickinson), staining by multicolor antibodies that included CD3-FITC, CD4-PE, CD8-APC, and CD45-PerCP (Becton Dickinson). Labeled cells were acquired on FACS Calibur. Data were analyzed by MultiSETTM software (BD Bioscience). Cell counts of V δ 2 T cells or functional V δ 2 T cells were determined based on their percentage among CD3⁺ T cells and CD3⁺ T cell counts.¹¹

Viral load quantification

Plasma viral RNA was quantified in EDTA-treated venous blood by fluorescent real-time polymerase chain reaction (PCR). Plasma viral loads were analyzed by the Amplicor ultrasensitive assay (Hoffmann-La Roche) with a detection limit of 50 viral RNA copies/ml of plasma.

Statistical analysis

All the statistical and graphic analyses were performed using GraphPad Prism software (GraphPad Software Inc.) and Sigma Plot software (SPSS Inc.). Statistical comparisons between different groups were made using one-way ANOVA. *p* values of pairwise comparisons were adjusted for multiple comparisons using Dunn's method. Spearman rank order correlation was used to analyze the correlation between variables. *p* values less than 0.05 were considered to be significant.

Results

Two different patterns of activation of V δ 2 T cells discriminated by CD16 expression

V δ 2 T cells can be specifically and efficiently activated by phosphoantigens, such as IPP, in a TCR-dependent manner.⁸ Fc γ RIIIa (CD16), a low-affinity receptor that binds only to clustered IgG bound to cell surface, is found on V δ 2 T cells and can mediate ADCC activity.²² In this study, we measured both the TCR-dependent IPP response and CD16-dependent ADCC function of V δ 2 T cells. The method for measuring NK

cell-mediated ADCC²⁰ was applied to V δ 2 T cells in this study. We used a single target cell line p815 coated with p815-specific antibodies as Fc target cells (Fc targets) to induce the ADCC activity of V δ 2 T cells. Similar to NK cells, we found an obvious decline of CD16 expression on V δ 2 T cells following stimulation with Ab-coated p815 cells, but not in the presence of medium, IPP stimuli, or p815 cells alone (Fig. 1a).

CD107a, a degranulation marker, is a necessary indicator for cellular cytotoxicity assays.²³ In this study, CD107a and IFN- γ were both used to evaluate the cytotoxicity and cytokine-producing capacity of V δ 2 T cells, respectively. After treating with IPP or Ab-coated p815 cells, a significant expression of CD107a and IFN- γ was detected on V δ 2 T cells, while medium alone and uncoated p815 cells did not induce any response (Fig. 1a). In particular, IPP induced degranulation and IFN- γ production mostly by the CD16⁺ V δ 2 T cell subset (% median for CD16⁺ subset in IPP-responsive V δ 2 T cells, 98.82% of CD107a⁺ V δ 2 T cells and 99.40% of IFN- γ ⁺ V δ 2 T cells), and rarely by CD16⁺ V δ 2 T cells (Fig. 1a), and the IPP response dominated by CD16⁺ V δ 2 T cells was observed across cohorts independent of disease progression (Fig. 2b and c).

Meanwhile, stimulation with Fc targets resulted in a profound activation of V δ 2 T cells as seen by increased degranulation and IFN- γ production, implicating ADCC as a key effector function of V δ 2 T cells. Given that CD16 is the only Fc gamma receptor reported on V δ 2 T cells, we expected that CD16⁺ V δ 2 T cells, and not CD16⁺ V δ 2 T cells, were the ones exerting ADCC. Subsequent regression analysis of the cohorts revealed a strong correlation between the CD16⁺ V δ 2 T cells and Fc target responders, CD107a⁺ V δ 2 T cells and IFN- γ ⁺ V δ 2 T cells, in terms of either frequencies or cell counts (Fig. 1b). These data indicated that an increased baseline frequency of CD16⁺ V δ 2 T cells allowed them to better respond to Fc targets. This is consistent with the report showing that CD16 expression closely correlates with ADCC function in NK cells.²⁰ Thus, we defined and characterized V δ 2 T cells on the basis of CD16 expression to study the respective impact of HIV-1 infection on the immune response of two functional V δ 2 T cell subsets with distinct antigen recognition pathways.

Compromised IPP-induced response of V δ 2 T cells dominated by the CD16⁺ subset in HIV-1 infection

Although a decreased cell number and impaired phosphoantigen response of V δ 2 T cells in HIV disease have been reported,^{11,13} the detailed impact of HIV infection on CD16⁺ and CD16⁺ V δ 2 T cell subsets is not yet clear. In our cohorts, untreated subjects at different stages of chronic HIV-1 infection were divided into three subgroups based on CD4 T cell counts (cells/ μ l): CD4 < 300, 300 $\leq$ CD4 $\leq$ 500, and CD4 > 500. During early HIV-1 infection, when a rapid decline of CD4 T cells was observed in comparison with healthy controls (*p* < 0.001), V δ 2 T cell counts had been reduced significantly and the loss of V δ 2 T cells was also observed in untreated chronic HIV-1 infection (*p* < 0.05; Fig. 2a). In terms of IPP responders, dramatic declines of CD107a⁺ CD16⁺ V δ 2 T cells and IFN- γ ⁺ CD16⁺ V δ 2 T cells (*p* < 0.05 for both) were observed in early HIV infection compared to healthy controls (Fig. 2c). In addition, there was an overall loss of IPP-responsive CD107a⁺ and IFN- γ ⁺ CD16⁺ V δ 2 T cells in

FIG. 1. Response of V δ 2 T cells to isopentenyl pyrophosphate (IPP) and Fc targets stimulation. Levels of CD16 expression in V δ 2 T cells (upper panel) as well as CD107a and IFN- γ (lower panel) were measured in the assay (a). The flow plots represent the V δ 2 T cells from a single individual in response to medium alone, IPP, the cell line p815, and p815 cells coated with p815-specific Abs (Fc targets), respectively (from left to right). Expression of CD107a and interferon (IFN)- γ was specifically analyzed for CD16⁺ and CD16⁻ V δ 2 T cell subsets after IPP stimulation, respectively. Correlations between CD16 expression and V δ 2 T cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) response (b) are illustrated as percentages or cell counts. Spearman rank order correlation coefficients r and corresponding p values are indicated.

chronic untreated HIV-infected individuals when compared to healthy subjects ($p < 0.05$ for all). The chronic untreated patients with CD4 < 300 had a decreased frequency of IFN- γ ⁺ CD16⁻ V δ 2 T cells in response to IPP compared to patients with higher CD4 T cell counts ($p < 0.05$; Fig. 2b). Furthermore, we observed an elevated percentage and cell count of IPP-responsive IFN- γ ⁺ CD16⁻ V δ 2 T cells from treated individuals in comparison to untreated individuals with CD4 < 300 ($p < 0.05$ for both).

Consistent with one previous study¹¹, IPP-responsive V δ 2 T cells were positively correlated with CD4 T cell counts ($r = 0.369$, $p = 0.027$ for CD107a⁺ V δ 2 T cells and $r = 0.431$, $p = 0.009$ for IFN- γ ⁺ V δ 2 T cells) and inversely correlated with viral loads ($r = -0.401$, $p = 0.035$ for CD107a⁺ V δ 2 T cells and $r = -0.460$, $p = 0.014$ for IFN- γ ⁺ V δ 2 T cells) in chronic HIV infection (Supplementary Fig. S1a; Supplementary Data are available online at www.liebertpub.com/aid). Interestingly, we found that some early-infected individuals who progressed rapidly after HIV infection with CD4 < 300 had a significantly higher frequency of IPP-responsive V δ 2 T cells than the chronically infected individuals with CD4 < 300 ($p < 0.05$; Supplementary Fig. S2a). In contrast to chronic HIV infection, the IPP-responsive V δ 2 T cells in early HIV infection had no correlation with CD4 T cell counts or viral load (Supplementary Fig. S1a).

Defective ADCC function of V δ 2 T cells during chronic HIV-1 infection

CD16, which may serve as a predictor for FcR-mediated activation of V δ 2 T cells, was examined during the course of HIV-1 infection. As shown in Fig. 3a, there was no difference in the overall proportions of CD16⁺ V δ 2 T cells. However, there were notable differences in the CD16⁺ V δ 2 T cell counts between the two chronic subgroups with CD4 ≤ 500 and healthy controls ($p < 0.05$ for both), while the CD4 > 500 subgroup maintained the CD16⁺ V δ 2 T cell level (quantity median, 11.78 cells/ μ l; range, 3.92–13.43 cells/ μ l) compared to healthy controls (quantity median, 11.94 cells/ μ l; range, 4.09–39.51 cells/ μ l). Given the strong correlation between CD16 expression and V δ 2 T cell-mediated ADCC, the decline of CD16⁺ V δ 2 T cells could result in the V δ 2 T cells being incapable of exerting ADCC in chronic HIV-1 infection with fast disease progression.

In the V δ 2 T cell-mediated ADCC assay, although there was a sharp reduction of V δ 2 T cells after HIV-1 infection, a modestly higher percentage as well as nearly equal number of antibody-dependent cytotoxic V δ 2 T cells were found in early-infected subjects (frequency median, 7.83%; range, 3.26–15.36%) (quantity median, 2.09 cells/ μ l; range, 0.76–7.49 cells/ μ l) as compared to healthy controls (frequency median,

FIG. 2. IPP-induced responses of V δ 2 T cells based on CD16 expression during different stages of HIV-1 infection. Dot plots show the differences in V δ 2 T cell counts (**a**) at different stages of HIV-1 infection. Frequencies (**b**) and cell counts (**c**) of V δ 2 T cells discriminated by CD16 that are able to degranulate (expression of CD107a) and to produce IFN- γ after IPP stimulation were analyzed in all patient populations. Horizontal bars indicate medians. * p < 0.05; ** p < 0.01; *** p < 0.001.

3.57%; range, 0.28–10.17%) (quantity median, 1.41 cells/ μ l; range, 0.10–7.34 cells/ μ l) (Fig. 3b and c). In chronic HIV-1 infection, V δ 2 T cell-mediated ADCC was compromised most severely in the chronic subgroup with CD4 < 300 in all respects when compared to both the chronic subgroup with CD4 > 500 and healthy controls (p < 0.05). Notably, CD107a⁺ V δ 2 T cells responding to Fc targets were not compromised in the CD4 > 500 subgroup. The median percentage of CD107a⁺ V δ 2 T cells in the CD4 > 500 subgroup (frequency median, 11.39%; range, 4.56–15.42%) was over 3-fold higher than in healthy controls, and the median quantity of CD107a⁺ V δ 2 T cells in the CD4 > 500 subgroup (quantity median, 3.39 cells/ μ l; range, 1.66–7.18 cells/ μ l) was slightly higher than in healthy controls. Higher cell counts of degranulated and cytokine-producing V δ 2 T cells responding to Fc targets were observed in chronic treated individuals when compared with chronic untreated individuals with CD4 < 300 (p < 0.05).

The ADCC functional V δ 2 T cells exhibited a positive correlation with CD4 T cell counts (r = 0.518, p < 0.001 for CD107a⁺ V δ 2 T cells and r = 0.624, p < 0.001 for IFN- γ ⁺ V δ 2 T cells) and an inverse correlation with viral loads (r = -0.378, p = 0.043 for CD107a⁺ V δ 2 T cells and r = -0.461, p = 0.012 for IFN- γ ⁺ V δ 2 T cells) in chronic subjects (Supplementary Fig. S1b), which was consistent with the varying levels of ADCC activities at different stages of chronic HIV-1 infection. In accordance with the IPP-activated V δ 2 T cells, the individuals with CD4 < 300 during early HIV-1 infection had much higher frequencies and cell counts, both with respect to degranulated and cytokine-producing V δ 2 T cells, than the chronic subgroup with CD4 < 300 (p < 0.05 for all; Supplementary Fig. S2b). No relationships were observed between the ADCC functional V δ 2 T cells and CD4 T cell counts or viral load during early HIV-1 infection (Supplementary Fig. S1b).

FIG. 3. $CD16^+$ $V\delta 2$ T cell-mediated ADCC responses during different stages of HIV-1 infection. The dot plots depict changes in frequencies and cell counts of $CD16^+$ $V\delta 2$ T cells (**a**) among individuals at different stages of HIV-1 disease. The capacity of $V\delta 2$ T cells from the individuals in different populations to respond to Fc targets is shown in terms of frequencies (**b**) and cell counts (**c**) of degranulation as well as cytokine production. Horizontal bars indicate medians. * $p < 0.05$; ** $p < 0.01$.

Functional profile of $V\delta 2$ T cells in response to IPP and Fc targets

We next analyzed the functional repertoires of $V\delta 2$ T cells activated by IPP or Fc targets in both HIV-infected and healthy subjects (Fig. 4). Following stimulation with IPP, the dual-functional cells with expression of CD107a and IFN- γ dominated the functional proportion independent of HIV-1 infection. Significant expansion of the monoexpression of CD107a was found in $V\delta 2$ T cells responding to IPP in early HIV-infected subjects compared to healthy controls ($p < 0.001$).

Different from the profile of the IPP-responsive $V\delta 2$ T cells, the functional division between the degranulated and cytokine-secreting subset was much clearer in ADCC activity. Strikingly, the $V\delta 2$ T cells from the HIV-infected and HIV-uninfected groups displayed an almost reverse ratio of the two monofunctional subgroups in response to Fc targets. Significant differences in the frequencies of monoexpression of CD107a on $V\delta 2$ T cells were found between healthy controls and the two untreated HIV-infected subgroups, includ-

ing the early HIV-infected subgroup ($p < 0.001$) and the chronic subgroup with $CD4 > 500$ ($p < 0.05$). The great degranulation capacity of $V\delta 2$ T cells after HIV infection indicates that CD16-dependent ADCC activity in $V\delta 2$ T cells might contribute to the killing of HIV-infected cells.

Discussion

Here we have demonstrated that irrespective of HIV-1 infection status, $CD16^-$ and $CD16^+$ $V\delta 2$ T cell subsets performed different functions in response to various stimuli, suggesting the existence of distinct activation pathways. This result is consistent with the functions of $V\delta 2$ T effector memory subsets discriminated by CD16 expression, including $V\delta 2$ T_{EMh} cells, which lack CD16 expression and respond to TCR-dependent phosphoantigen stimulation, and $V\delta 2$ T_{EMRA} cells, which mediate ADCC function via CD16.²¹ Previous studies have investigated $V\delta 2$ T cell activation in response to phosphoantigen and revealed a close relationship between cell-surface TCR and phosphoantigen recognition.⁸⁻¹⁰

FIG. 4. Different functional profiles of IPP-responsive and ADCC-mediating Vδ2 T cells in HIV infection. The pies depict the two functional profiles of Vδ2 T cells in response to IPP and Fc targets, respectively, among individuals at different stages of HIV-1 disease. Vδ2 T cells were divided into three subpopulations based on their production of CD107a and IFN-γ individually or in combination (nonresponsive cells were excluded for the analyses). Median values for individuals from different populations are shown.

Of importance, our data show that the baseline frequency of CD16⁺ Vδ2 T cells correlated with the capacity of the Vδ2 T cell pool to respond to Fc targets. Moreover, when comparing the different patient cohorts, a significant decline in CD16⁺ Vδ2 T cells was observed specifically in subjects with faster disease progression compared to healthy controls, while this decline was not observed in patients with better controlled HIV infection. In addition, this preserved frequency of CD16⁺ Vδ2 T cells in better controlled HIV⁺ patients was associated with a higher capacity of the Vδ2 T cell population to exert ADCC. This suggests that CD16⁺ Vδ2 cells (possibly of the T_{EMRA} phenotype) could be significantly contributing to the control of HIV infection via their strong capacity to exert ADCC. However, the observed decline of CD16⁺ Vδ2 T cells in HIV infection begs the question of what is the mechanism of this depletion.

One possibility includes CD16 downregulation of otherwise CD16⁺ Vδ2 T cells by cell surface proteolytic cleavage following activation, as has been described in NK cells,^{20,24,25} and is inferred from our *in vitro* Vδ2 T cell-mediated ADCC assay (although the likelihood that Fc targets might block the detection of CD16 could not be excluded); another possibility is the progressive destruction of CD16⁺ Vδ2 T cells by HIV virus-mediated cytopathic effects, analogous to the pronounced damages to the TCR γ-2 chain repertoire seen in HIV viremic patients.²⁶ The mechanism of the CD16⁺ Vδ2 T cell decline needs further study, and may help to guide research aimed at improving the efficacy of Vδ2 T cell-mediated ADCC activity.

Consistent with previous studies,^{13,27} we found the Vδ2 T cells declined at least as rapidly as CD4 T cells in early HIV infection, accompanied by the decreased IPP-responsive cells in the CD16⁺ Vδ2 T cell subset. However, with a strong capacity for degranulation, the CD16⁺ Vδ2 T cells from the early-infected group maintained cytotoxic Vδ2 T cells as the potentially efficient ADCC effectors. The shift in ADCC functional repertoire with dominant degranulation, which irreversibly continued throughout HIV-1 infection, might be the result of CD16⁺ Vδ2 T cell effectors in response to HIV through ADCC.

Recently, the HIV envelope-mediated, CCR5/α₄β₇-dependent killing mechanism has been revealed by Pauza's group to explain the depletion of Vδ2 T cells. They also observed the highest level of HIV envelope-induced cell death in Vδ2 T cells when exposed to phosphoantigen, which suggests that phosphoantigen activation might be the reason for the specific loss of the Vγ2Jγ1.2 Vδ2 T cell subpopulation.²⁷ This finding may partially explain the different status of the two functional Vδ2 T cells in early HIV-1 infection: the preservation of the functional Vδ2 T cells responding to Fc targets, but not to IPP, in early HIV-1 infection was probably due to the barely phosphoantigen-activated CD16⁺ Vδ2 T cell subset.

A strong and consistent relationship between viral load and the level of either CD4⁺ or CD8⁺ T cell activation has been found during early HIV infection.²⁸ As opposed to conventional T cells bearing αβ TCR, neither the IPP-induced nor the Fc target-induced activation of Vδ2 T cells from the early-infected group was related to the viral load or the CD4 T cell counts. Individuals who progressed rapidly after HIV-1 infection with CD4 < 300 in the early phase still maintained a functional Vδ2 T cell pool, in contrast to chronically infected subjects with CD4 < 300, who had a severe impairment of IPP responses and ADCC function. This indirectly suggests that the prolonged exposure to HIV-1 in the chronic phase may have a great impact on the overall defects of Vδ2 T cells, which is consistent with the study examining the ongoing damage to the Vδ2 T cell population caused by persistent chronic HIV-1 infection, in terms of both cell count and function.²⁶

Contrary to individuals in the early stage of HIV-1 infection, the capacity of Vδ2 T cells to respond to both IPP and to Fc targets had a close relationship with disease progression in chronic infection. Vδ2 T cells from chronically HIV-infected subjects with fast disease progression—as seen by low CD4 T cell counts—were compromised in their ability to respond to IPP and exert ADCC. Of importance, in the subjects with slower disease progression, despite the ongoing depletion of IPP-responsive Vδ2 T cells, a high quality of Vδ2 T cell cytotoxic response to Ab-coated targets was observed, strongly suggesting that CD16⁺ Vδ2 T cells might

contribute to the control of HIV-1 disease progression as potent ADCC effector cells.

Studies involving HIV-infected subjects receiving HAART have shown that suppression of viral load is closely related to partial recovery of the V δ 2 T cell receptor repertoire.^{29,30} However, there is scarce evidence for the V δ 2 T cell-mediated ADCC response in treated HIV-1 patients. Our study shows that treatment not only partially restored IPP-responsive V δ 2 T cells, but also significantly increased ADCC functional V δ 2 T cells in a treated cohort versus untreated HIV-infected subjects with a low CD4 T cell count. Recently, zoledronate, together with interleukin-2, has been found to amplify the CD16⁺ V δ 2 T cell subset and could expand V δ 2 T cells from HIV-infected individuals to become cytotoxic effectors for ADCC *in vitro*.³¹ Here we demonstrate a restoration of V δ 2 T cell-mediated ADCC in treated patients with low viral load and immune recovery of CD4 T cells, which supports the new direction of research aimed at bolstering ADCC to improve HIV-1 treatment.

Altogether, we identified two unique V δ 2 T cell subsets discriminated by CD16 and demonstrate that HIV-1 disease progression is not only related to the magnitude of the IPP-activated response dominated by the CD16⁻ V δ 2 T cell subset, but is also related to the capacity of CD16⁺ V δ 2 T cells to mediate ADCC. Although the p815 assay does not directly evaluate the capacity of HIV-specific antibodies to mediate ADCC, it provides an indirect measure of the potential capacity of the V δ 2 T cells as the ADCC effectors to clear the HIV-infected cells. Notably, HIV-1 infection has different impacts on the functional activity of the two V δ 2 T cell subsets: HIV-induced depletion of V δ 2 T cells eliminates phosphoantigen-stimulated CD16⁻ V δ 2 T cells but not the cytotoxic, Fc target-responsive CD16⁺ V δ 2 T cells, a phenomenon that is seen in patients with early and slow-progressed chronic HIV-1 infection but not in individuals with fast HIV-1 disease progression. As a member of the ADCC effectors, the V δ 2 T cell pool has alterations in its overall functional profile with significantly increased cytotoxicity in HIV-1 infection. These data highlight the CD16⁺ V δ 2 T cell subset as one with preserved ADCC activity throughout HIV-1 disease progression despite the generally damaged V δ 2 T cell pool, and warrants the selective generation of CD16⁺ V δ 2 T cells capable of mediating ADCC as a promising strategy for HIV vaccine and therapy research.

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Author Disclosure Statement

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