Association between Vγ2Vδ2 T Cells and Disease Progression after Infection with Closely Related Strains of HIV in China

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(See the editorial commentary by Nunnari on pages 1473–5)

Background. Human immunodeficiency virus (HIV) infection and disease are accompanied by decreases in the absolute number and function of Vγ2Vδ2 T cells, suggesting that this subset of cells may play an important role in controlling disease. We performed a cross-sectional study involving HIV-infected former blood donors and assessed the association between Vγ2Vδ2 T cells and markers of disease progression.

Methods. Changes in Vγ2Vδ2 T cell count and function were compared between HIV-infected individuals and healthy blood donors using the Mann-Whitney U test. The relationships between Vγ2Vδ2 T cell count, plasma viral load, and CD4 T cell count were analyzed using the Spearman correlation.

Results. We found significant positive correlations between CD4 T cell count and both total Vγ2Vδ2 T cell count (P < .001) and functional (isopentenyl pyrophosphate–responsive) Vγ2Vδ2 T cell count (P < .001). We found significant reverse correlations between viral load and both total Vγ2Vδ2 T cell count (P < .05) and functional Vγ2Vδ2 T cell count (P < .05).

Conclusions. The association of Vγ2Vδ2 T cells with disease progression in 146 HIV-infected participants supports the view that intact Vγ2Vδ2 T cell populations are important for controlling HIV disease.

Human γδ T cells comprise ~3% of the total peripheral blood T cell population. Of these γδ T cells, a majority express the Vγ2Vδ2 receptor, and ~75% have the Vγ2-Jγ1.2 rearrangement [1]. In contrast to αβ T cells, the γδ subset generally lacks CD4 or CD8 expression and recognizes nonpeptidic microbial antigens in a major histocompatibility complex–unrestricted manner, without antigen processing by professional antigen-presenting cells [2–5]. Similar to natural killer cells, Vγ2Vδ2 T cells express major histocompatibility complex I receptors, including the inhibitory CD94/NKG2 complexes, and also express killer immunoglobulin–like receptors [6, 7]. Vγ2Vδ2 T cells produce TNF-α, IFN-γ [8], and β-chemokines (macrophage inflammatory protein–α, macrophage inflammatory protein–β, and regulated on activation normal T cell expressed and secreted) [9, 10] in response to stimulatory ligands [11]. Because Vγ2Vδ2 T cells are broadly reactive against various intracellular pathogens, it is probable that Vγ2Vδ2 T cells do not respond to specific viral antigens but, instead, respond to host molecules, such as phosphoantigens and major histocompatibility complex products induced or modified by viral infections [12]. In vitro, Vγ2Vδ2 T cells display both proliferative and lytic responses to HIV-infected cells [13], and activated Vγ2Vδ2 T cells can suppress HIV replication by releasing soluble factors, including β-chemokines [9].

Alterations in γδ T cell distribution in the peripheral blood of HIV-infected persons, including a dramatic reduction in the absolute number of Vγ2Vδ2 T cells, have been previously reported [14]. In most HIV-infected persons, damage to the Vγ2Vδ2 T cells also eliminates the response to phosphoantigen stimulation [15, 16]. These changes occur before a significant decrease...
in the CD4 T cell count occurs [15] and are among the earliest defects in cellular immunity after infection with HIV. However, it is still not clear whether damage to Vγ2Vδ2 T cells is associated with disease progression in HIV infection. To address this question, we analyzed the Vγ2Vδ2 T cells from a cohort of 146 people with chronic, untreated HIV infection in Anhui Province, China.

**PATIENTS, MATERIALS, AND METHODS**

**Study participants.** A group of former blood and/or plasma donors were infected with HIV from 1992 through 1995 because of unregulated commercial blood and/or plasma collection; our volunteers were recruited from local clinics in Fuyang City (Anhui Province, China). A previous baseline investigation revealed that the ages of the participants ranged from 27 to 65 years and that approximately one-half of the participants were male. No injection drug use was identified and all participants were naive to antiretroviral therapy. Informed consent was obtained from all participants. The study protocol was sequentially approved by the National Institutes of Health, the institutional review board of the China Center for AIDS/STD Control and Prevention, and the institutional review board of the Anhui Provincial Center for Disease Control and Prevention. Details about HIV testing, sample collection, PBMC isolation, and other relevant information are described elsewhere [17].

**In vitro stimulation of Vγ2Vδ2 T cells and effector function assessment.** PBMCs were isolated from EDTA anticoagulated blood by Ficoll-Hyphaque (Pharmacia) gradient centrifugation. The PBMCs were cultured at 5 × 10⁵ cells/well in complete Roswell Park Memorial Institute 1640 medium (supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) in 96-well round-bottomed plates (Corning) and were stimulated in vitro for 10 h with either medium alone, 15 μmol/L isopentenyl pyrophosphate (IPP; Sigma), or 10 μg/mL phytohemagglutinin. Brefeldin A (Sigma) was added 3 h before staining for cytokine production. IPP selectively stimulates T cells expressing the Vγ2Vδ2 T cell receptor.

**Flow cytometry.** Unless noted otherwise, cells were stained with fluo ophore-conjugated monoclonal antibodies from BD Biosciences. CD3, CD4, and CD8 T cell counts were measured with a FACSCalibur TruCount tube (Becton Dickinson) with multicolor antibody (CD3, CD8, CD45, and CD4 T cells). Results were analyzed by Multiset software (BD Biosciences). To determine the frequency of circulating Vγ2Vδ2 T cells, 3 × 10⁵–5 × 10⁶ cells were washed, resuspended in 50–100 μL of RPMI 1640, and stained with mouse antihuman Vγ9–fluorescein isothiocyanate clone (FITC) 7A5 (Pierce Biotechnology; note that Vγ9 and Vγ2 are alternate names for the same chain of the T cell receptor), mouse antihuman CD3–allophycocyanin (antigen-presenting cells) clone UCHT1, and isotype controls (including rabbit antimouse IgG1–FITC clone X40 and IgG1 antigen–presenting cells clone X40). For detecting intracellular IFN-γ, IPP-stimulated cells were stained with Vγ9–FITC, fixed permeabilized, and incubated for 45 min at 4 °C with mouse antihuman IFN-γ–PE clone 4S.B3. Intracellular staining so-

**Figure 1.** Alteration of Vγ2Vδ2 T cells in the peripheral blood of HIV-infected persons. The percentage of Vγ2Vδ2 T cells among total CD3 T cells (A) and absolute Vγ2Vδ2 T cell counts (B) in 146 HIV-infected persons significantly decreased, compared with those in 42 healthy donors. Statistical comparisons were made using the Mann-Whitney U test.

**Figure 2.** Association of Vγ2Vδ2 T cell count and functional Vγ2Vδ2 T cell count. There was a positive relationship between Vγ2Vδ2 T cell count and functional (isopentenyl pyrophosphate–responsive) Vγ2Vδ2 T cell count. Correlation statistics were analyzed using the Spearman correlation.
Figure 3. Vγ2Vδ2 T cell count associated with HIV disease progression. A. Positive correlation between Vγ2Vδ2 T cell count and CD4 T cell count. B. Inverse correlation between Vγ2Vδ2 T cell count and viral load. C. Significant differences in Vγ2Vδ2 T cell counts between subgroups stratified by CD4 T cell count. Correlation statistics for A and B were analyzed using the Spearman correlation. Statistical comparisons for C were made using the Mann-Whitney U test.

RESULTS

We compared Vγ2Vδ2 T cells from 146 HIV-infected individuals with those from 42 healthy (HIV-uninfected) donors. Consistent with previous studies, both the percentage of Vγ2Vδ2 T cells among total CD3 T cells and absolute Vγ2Vδ2 T cell count were significantly decreased in patients with HIV infection (P < .001) (figure 1). Furthermore, we found a significant positive correlation between Vγ2Vδ2 T cell count and the presence of functional (IPP-responsive) Vγ2Vδ2 T cells (P < .001) (figure 2), indicating that the quantity and quality of Vγ2Vδ2 T cells decreased simultaneously.

Next, we assessed the relationship between Vγ2Vδ2 T cell count, plasma viral load, and CD4 T cell count—the latter 2 parameters being predictors of HIV disease progression. We found a significant positive correlation between CD4 T cell count and the presence of Vγ2Vδ2 T cells (n = 146; P < .001) (figure 3A) and an inverse correlation between viral load and the presence of Vγ2Vδ2 T cells (n = 146; P < .05) (figure 3B). If the HIV-infected participants were divided into 4 groups based on CD4 T cell count (<200 cells/μL, ≥200 but <350 cells/μL, ≥350 but <500 cells/μL, and ≥500 cells/μL) and if Vγ2Vδ2 T cell counts in different subgroups were compared, we found that the subgroups with higher CD4 T cell counts also had higher Vγ2Vδ2 T cell counts. The statistical significance is detailed in figure 3C. The characteristics of the subgroups, such as the number of patients in each subgroup, CD4 T cell counts, CD4:CD8 ratios, and plasma HIV loads, are described in table 1. We also assessed the Vγ2Vδ2 T cell counts in healthy control subjects stratified by CD4 T cell count (table 2). We did not find correlations between CD4 T cell count and Vγ2Vδ2 T cell count in healthy control subjects.

Phosphoantigen stimulation is considered to be a model for the normal response to pathogen infection, because phosphoantigen compounds are present in mycobacterial cell extracts and are also produced as metabolites of stressed cells [12]. Thus, we examined the IPP-responsive Vγ2Vδ2 T cell count measured by IFN-γ expression after IPP stimulation for 66 HIV-infected individuals. We assessed the relationships between IPP-responsive Vγ2Vδ2 T cell count, plasma viral load, and CD4 T cell count. We found a statistically significant pos-
The data presented here show a clear association between Vγ2Vδ2 T cells, their functional response to IPP stimulation, and their use as markers of HIV disease progression. To our knowledge, this is the first reported study of Vγ2Vδ2 T cells among a group of HIV-infected individuals in China, which is the largest single study group analyzed thus far. There was a clear association between advancing disease, measured by CD4 T cell count, and Vγ2Vδ2 T cell count and function. The data on healthy Chinese blood donors agrees with Vγ2Vδ2 T cell counts that have been published for healthy European and North American populations. In terms of HIV-infected groups, the characteristics of this cross-section of HIV-infected individuals are similar to those observed among HIV-infected patients in the United States [18]. The major difference is that...
Figure 4. Functional Vγ2Vδ2 T cell count associated with HIV disease progression. A, Positive correlation between isopentenyl pyrophosphate–responsive functional Vγ2Vδ2 T cell count and CD4 T cell count. B, Inverse correlation between isopentenyl pyrophosphate–responsive functional Vγ2Vδ2 T cell count and viral load. C, Significant differences in functional Vγ2Vδ2 T cell counts between subgroups stratified by CD4 T cell count. Correlation statistics for A and B were analyzed using the Spearman correlation. Statistical comparisons for C were made using the Mann-Whitney U test.

Figure 5. The effect of Vγ2Vδ2 T cell count on CD4 T cell count during HIV disease. CD4 T cell counts in the group with higher Vγ2Vδ2 T cell counts (n = 38) decreased more slowly than those in the group with lower Vγ2Vδ2 T cell counts (n = 50). At each time, the CD4 T cell counts in the group with higher Vγ2Vδ2 T cell counts were significantly higher than those in the group with lower Vγ2Vδ2 T cell counts. Statistical comparisons were made using the Mann-Whitney U test.

The Chinese cohort allows for observation of a group of people infected with a relatively homogeneous virus strain at approximately the same time. Thus, the current studies add to our growing knowledge but also bring an important new dimension to this problem.

These data argue that the Vγ2Vδ2 T cell subset is impacted early during the course of disease, because all HIV-infected individuals showed some degree of Vγ2Vδ2 T cell count decrease or Vγ2Vδ2 T cell damage. Damage to this subset is also progressive, as shown by the decreasing Vγ2Vδ2 T cell counts and function as CD4 T cell counts successively decreased. Damage to the Vγ2Vδ2 T cells is related to viral replication, as shown by the correlation between vRNA levels and Vγ2Vδ2 T cell count or function. However, Vγ2Vδ2 T cells that lack the CD4 receptor for virus are generally considered to be nonpermissive for HIV infection. Thus, the extent of damage depends on the degree of virus replication and occurs by an indirect mechanism that does not involve direct HIV infection and replication in Vγ2Vδ2 T cells.

The mechanism for Vγ2Vδ2 T cell depletion remains unknown. A preliminary study involving simian immunodeficiency virus–infected nonhuman primates revealed an initial increase in γδ T cell count (up to 300% more than the baseline count within a few weeks), followed by the precipitous decrease that characterizes persistent simian immunodeficiency virus or HIV infection [19]. A previous report demonstrated that acute HIV replication causes a rapid and persistent impairment of Vγ2Vδ2 T cells in chronically infected patients undergoing structured treatment interruption [20]. Apparently, viral infection alters cell metabolism or cell surface characteristics that are detected by Vγ2Vδ2 T cells, resulting in their stimulation and proliferation. This is consistent with other examples in which γδ T cells have been among the earliest responders to viral infection. Their ability to secrete cytokines, including IFN-γ [8], provides for immunity against vaccinia infection in mice, in which γδ T cells are the principal mechanism for resistance against fatal disease [21]. Previously, we showed that vaccinia
inhibits human Vγ2Vδ2 T cell responses to phosphoantigens [22]; this type of immune evasion mechanism likely indicates that Vγ2Vδ2 T cells are important for vaccinia resistance in humans, as was seen in mice. During HIV infection, evasion from the Vγ2Vδ2 T cell response appears to occur by specific deletion of this cell population, rendering the cells nonresponsive to phosphoantigen stimulus. Although we cannot yet prove the role of Vγ2Vδ2 T cells in HIV disease, the pattern of deletion, the relationship to disease progression, and the fact that deletion is common in all persons with HIV infection support the idea that Vγ2Vδ2 T cells are part of cellular immunity against HIV.

Studies conducted primarily in mice highlighted several ways that γδ T cells participate in viral immunity. The vaccinia example mentioned above revealed that early γδ T cell responses, with production of IFN-γ, provided resistance to fatal disease in mice [22]. In other studies of vaccinia and vesicular stomatitis virus, γδ T cells were important for developing virus-neutralizing antibodies. When CD4 T cell counts decreased, murine γδ T cells were sufficient for immunoglobulin class switching to produce neutralizing IgG [23]. HIV disease is characterized by a loss of capacity for type-1 immune responses and a failure to produce high titers of neutralizing antibody. The deficit in Vγ2Vδ2 T cells might contribute to both of these important disease mechanisms.

The importance of Vγ2Vδ2 T cells in containing HIV infection and disease progression makes it possible to treat AIDS by recovery of Vγ2Vδ2 T cells. Long-term treatment with HAART led to partial recovery of Vγ2Vδ2 T cells in one study [18] and little or no increase in Vγ2Vδ2 T cell count in another study that used specimens from the Multicenter AIDS Cohort Study [24]. Consequently, studies aimed at stimulating and stabilizing the Vγ2Vδ2 T cell subset might contribute to efforts of reconstituting immunity in persons with HIV infection, in addition to successful control of viremia through HAART. Preliminary studies performed in monkeys suggested a protective effect of activated γδ T cells in simian immunodeficiency virus infection, substantially improving in vivo both clinical and virological parameters [25].

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