Acute Human Immunodeficiency Virus Replication Causes a Rapid and Persistent Impairment of Vγ9Vδ2 T Cells in Chronically Infected Patients Undergoing Structured Treatment Interruption

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T cells expressing Vγ9Vδ2 display lytic and proliferative responses against human immunodeficiency virus (HIV)-infected cells and release antiviral soluble factors. Chronic HIV-positive patients have deep changes in the composition and function of the circulating γδ T cell pool that are restored by highly active antiretroviral therapy (HAART). γδ T cells were analyzed during the rapid plasma HIV RNA rebound in HIV-infected patients undergoing structured treatment interruption (STI). A loss in circulating Vγ9Vδ2 T cells was observed, indicating that acute HIV replication may influence Vγ9Vδ2 homeostasis. These cells were lost among CD45RA CD27 Vγ9Vδ2 T cell effectors, and a significant unresponsiveness, measured as antigen-driven interferon-γ production, was observed during STI. After HAART resumption and consequent inhibition of HIV replication, Vγ9Vδ2 T cell reactivity was restored both quantitatively and functionally. These observations indicate that Vγ9Vδ2 T cells are activated early after active HIV replication but are rapidly lost when viremia is not controlled.

γδ T lymphocytes recognize nonpeptide microbial antigens without antigen processing by professional antigen-presenting cells (reviewed in [1]). Among humans, an apparent homogeneity of circulating γδ T cells is observed; these cells mainly express a paired Vγ9-Vδ2 (Vγ2-Vδ2 in alternate nomenclature) T cell receptor (TCR) [2]. Vγ9Vδ2 T cells are broadly reactive against various intracellular pathogens and in vitro display both proliferative and lytic responses to human immunodeficiency virus (HIV)-infected cells [1]. Similar to NK cells, a large fraction of circulating Vγ9Vδ2 T lymphocytes express major histocompatibility complex class I receptors, mostly the inhibitory CD4/ NKG2 complex [1]. Triggering of CD94/NKG2A down-modulates activation of the γδ TCR by nonpeptide microbial antigens, interfering with the TCR signaling cascade and controlling the cytolytic activity of peripheral blood Vγ9Vδ2 T cells and Vγ9Vδ2 T cell clones against HIV-infected cells [3]. Moreover, activated Vγ9Vδ2 T cells can suppress in vitro HIV replication by releasing soluble factors, including β-chemokines [4].

Effector/memory T cells can be distinguished according to the expression of CD45RA and CD27 molecules: central memory cells are CD45RA CD27+, whereas effector memory cells have lost the expression of CD27 costimulatory molecules and lack the proliferative potential of central memory cells. We recently showed that effector memory γδ T cells have lost CD27 expression and are lacking in immunocompromised hosts [5]. Alterations of γδ T cell distribution were reported elsewhere in the peripheral blood of HIV-infected subjects, including a decrease in both absolute number and function of Vγ9Vδ2 T cells [6]. We showed elsewhere a marked decrease in the functional reactivity of γδ T lymphocytes from HIV-infected persons with opportunistic infections and coinfections. Moreover, highly active antiretroviral therapy (HAART) was able to restore Vγ9Vδ2 T cell number and reactivity [7].

Strong HIV-specific immune responses are detected in subjects who intermittently adhere to HAART regimens [8], suggesting that structured treatment interruption (STI) may be used as a therapeutic strategy to boost HIV-specific immunity. However, such a strategy could be associated with the risk of HIV-mediated loss of CD4+ T cells, severe constitutional symptoms such as those in primary infection, and the emergence of
viral mutations conferring drug resistance [9]. Thus, identification of the immunologic and virologic consequences of STI in HIV-infected patients is crucial for the clinical development of novel long-term treatment strategies.

Patients and Methods

Patient recruitment. In this study, we examined the properties of γδ T lymphocytes in HIV-infected persons who showed a rapid plasma virus rebound after STI. Ten asymptomatic chronically HIV-1-infected patients were recruited from the National Institute for Infectious Diseases “Lazzaro Spallanzani,” Rome. The criteria for study inclusion were ≥2 years of successful HAART, stable CD4+ T cell counts >500 cells/μL for ≥12 months before study entry, undetectable HIV RNA plasma levels (<50 copies/mL by branched DNA assay [bDNA version 3.0; Bayer Diagnostics]) for ≥12 months before entry, and HAART discontinuation for side effects or spontaneous request. The patients underwent a single-cycle STI consisting of ≥1 month of discontinuation. HAART reintroduction was decided upon according to current guidelines on antiretroviral therapy (CD4 T cell count <350 cells/μL and/or HIV RNA level >30,000 copies/mL; at http://www.hivatis.org/trtgdlths.html). In the study patients, a rapid plasma HIV RNA rebound of >30,000 copies/mL was observed after 28.7 ± 1.8 days. Clinical and immunologic follow-up was done when HAART was suspended (T0), 1 month after suspension (T1), at resumption of HAART (T2: 42.3 ± 7.1 days), and 30 days after HAART resumption (T3).

Table 1. Plasma human immunodeficiency virus (HIV) RNA levels and CD4+ and CD8+ cell counts (mean ± SE) in 10 HIV-positive persons with rapid plasma virus rebound after structured treatment interruption (STI).

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<th>T0</th>
<th>T1</th>
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<tbody>
<tr>
<td>Plasma HIV RNA level, copies/mL</td>
<td>&lt;50</td>
<td>145,000 ± 69,000*</td>
<td>186,000 ± 130,000</td>
<td>400 ± 90*</td>
</tr>
<tr>
<td>CD4+ T cell count, cells/μL</td>
<td>698 ± 74</td>
<td>517 ± 68*</td>
<td>489 ± 70*</td>
<td>721 ± 131</td>
</tr>
<tr>
<td>CD8+ T cell count, cells/μL</td>
<td>894 ± 125</td>
<td>1663 ± 242b</td>
<td>1433 ± 188b</td>
<td>1090 ± 132b</td>
</tr>
</tbody>
</table>

NOTE: Data were for T0 (still in treatment but starting STI next day), T1 (1 month after T0), T2 (still in STI from 42.3 ± 7.1 days but restarting antiretroviral treatment next day), and T3 (1 month after T2). Differences between groups were evaluated with the Mann-Whitney U test.

* P < .05, vs. T0.

b P < .01, vs. T0.

In vitro stimulation of Vγ9Vδ2 T cells and effector function assessed. Peripheral blood mononuclear cells isolated from K3-EDTA blood by Ficoll-Paque (Pharmacia) were cultured at 2.0 × 10^6 cells/mL in complete RPMI 1640 medium (supplemented with 10% vol/vol heat-inactivated fetal calf serum, 2 mM L-glutamine, and 10 U/mL penicillin/streptomycin) with 100 U/mL recombinant interleukin (rIL)-2 (Boehringer Mannheim) in 96-well round-bottomed plates (Becton Dickinson Discovery Labware) and stimulated in vitro in the presence or absence of 15 μM isopen-tenyl-pyrophosphate (IPP; Sigma Chemicals) for 7 days. IPP selectively stimulates T cells expressing the Vγ9Vδ2 T cell receptor [11]. Interferon (IFN)-γ level in the culture supernatant at day 7 was assessed by sandwich ELISA by following the manufacturer’s instructions (R&D Systems) and expressed as net IPP-induced IFN-γ production (rIL-2 alone subtracted) related to 10^6 Vδ2 cells.

Statistical analysis. Data are reported as means ± SE. Differences between groups were evaluated by the Mann-Whitney U test. P ≤ .05 was considered to be significant.

Results

Table 1 shows the kinetics of plasma HIV RNA level and circulating CD4+ and CD8+ T cell counts in the study subjects who underwent STI. As expected [12], after 1 month of STI (T1), virus load increased by 3 logs (table 1; P < .05, T1 vs. T0). This plasma HIV-1 RNA increase was accompanied both by a drop in CD4+ T cells (P < .05, T1 vs. T0) and by a near doubling in CD8+ T cells (P < .01, T1 vs. T0). At the end of STI (T2, 42.3 ± 7.1 days), just before HAART resumption, both the virus load and CD4+ and CD8+ T cell levels were similar to those at 1 month after interruption. One month after HAART resumption (T3), the virus load dropped by 3 logs (P < .05, T3 vs. T1), and CD4+ T cell counts were enhanced to approximately the levels before STI (table 1; P > .05, T3 vs. T0). Similar to the HIV RNA level, CD8+ T cells significantly decreased in respect to T1 (P < .05, T3 vs. T1; table 1), reaching a level similar to T0 (P > .05, T3 vs. T0; table 1).

Figure 1A shows the Vγ9Vδ2 T cell counts evaluated at the same time points as in table 1. There was a nonsignificant mean increase in Vδ2 T cells at T1 (after 1 month of STI) that was followed by a significant decrease at the end of STI (T2), just before HAART resumption (P < .05, T2 vs. T1). At T3, after 1 month of HAART, Vδ2 T cells recovered to the level before
IFN-γ release was found soon after treatment interruption at T1 (P < .05, T1 vs. T0; figure 1C), which lasted to T2 (P < .05, T2 vs. T0; figure 1C). The HAART-induced recovery in Vδ2 T cell number and composition also produced fully competent (i.e., IFN-γ-releasing) Vδ2 T cells (P > .05, T3 vs. T0; figure 1C).

Discussion

The identification of immunologic changes during STI is crucial for assessing its clinical value. In this study, we analyzed the influence of plasma HIV RNA rebound induced by STI on Vγ9Vδ2 T cell number and function. As expected, a drop in CD4 T cell count and an increase in CD8 T cell count accompanied the rebound in plasma HIV RNA levels. During STI, a reduction of Vγ9Vδ2 T cell effectors was preceded and accompanied by a functional anergy. Because HAART resumption and virus level reduction were associated with a quantitative and functional restoration of Vγ9Vδ2 T cells, our observations indicate that this cell subset is deeply affected by the acute response to HIV replication.

The changes in Vγ9Vδ2 T cells described indicate a specific alteration of these cells in chronically HIV-infected persons [6]. Because most Vγ9Vδ2 T cells are CD4+ and resistant to HIV infection, their impairment must result from an indirect mechanism triggered by HIV replication. The γδ T cell anergy could be induced by HIV-infected cells through the triggering of the CD94/NKG2-A inhibitory receptors [3] or by negative clonal selection of Vγ9Vδ2 T cells reactive to nonpeptidic microbial phosphoantigens released by cytopathic effects following HIV infection [1]. In this context, the Vγ9Vδ2 T cell loss observed after the STI-induced increase of plasma HIV RNA could reproduce the scenario during the very early phases of HIV infection.

Our data indicate that soon after plasma HIV RNA rebound, these cells first become rapidly anergic and then undergo a profound depletion. Indeed, the cytopathic effect of HIV infection may force Vγ9Vδ2 T cells to enter into an unresponsive or anergic state through stimulation by cell-released phosphoantigens [13]. Furthermore, chronic HIV infection causes selective activation of Vγ9Vδ2 T cells, resulting in the loss by exhaustion of differentiated CD45RA CD27− Vγ9Vδ2 effector T cells [5]. These observations suggest that Vγ9Vδ2 T cell alterations during HIV replication could be induced by massive exposure to antigen stimulation and consequent activation-induced cell death of phosphoantigen-reactive Vγ9Vδ2 T cell effectors [14].

Vγ9Vδ2 T cells suppress HIV replication in vitro by soluble factors that include β-chemokines [4]. Moreover, activated Vγ9Vδ2 T cells produce significant amounts of IFN-γ, which in turn may be important to promote the antiviral immune response. Thus, their loss may represent severe indirect damage that reduces both the effectiveness of cell-mediated immune response and the level of antiviral β-chemokines. In the early...
phases of HIV disease, this may contribute to the establishment of a persistent infection [4]. Increased morbidity and mortality was recently observed in HIV-infected patients who interrupted antiretroviral treatment because of severe side effects [15]. The rapid HIV-induced functional and numeric loss of Vγ9Vδ2 T cells, although rapidly restored by HAART, may contribute to increased risk of opportunistic infections. This underscores the importance of careful application of STI.

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References