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Cutting Edge: Rapid Recovery of NKT Cells upon Institution of Highly Active Antiretroviral Therapy for HIV-1 Infection

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CD1d-restricted NKT cells play important regulatory roles in various immune responses and are rapidly and selectively depleted upon infection with HIV-1. The cause of this selective depletion is incompletely understood, although it is in part due to the high susceptibility of CD4+ NKT cells to direct infection and subsequent cell death by HIV-1. Here, we demonstrate that highly active antiretroviral therapy (HAART) results in the rapid recovery of predominantly CD4− NKT cells with kinetics that are strikingly similar to those of mainstream T cells. As it is well known that the early recovery of mainstream T cells in response to HAART is due to their redistribution from tissues to the circulation, our data suggest that the selective depletion of circulating NKT cells is likely due to a combination of cell death and tissue sequestration and indicates that HAART can improve immune functions by reconstituting both conventional T cells and immuno-regulatory NKT cells. The Journal of Immunology, 2006, 177: 5775–5778.

Natural killer T cells constitute an evolutionary, highly conserved, immunoregulatory T cell subset that is restricted by the CD1d Ag-presenting molecule. NKT cells display an extremely restricted TCR repertoire in humans consisting of a Vα24 chain preferentially paired to Vβ11 and play crucial roles in various immune responses, including antiviral, autoimmunity, allergic, and antimicrobial immune responses (1, 2).

Infection with HIV-1 results in the rapid and selective depletion of NKT cells (3–6). Although CD4+ NKT cells have been shown to be highly susceptible to direct infection and subsequent cell death by HIV-1, it is important to note that the majority of NKT cells do not express CD4 and that the depletion of NKT cells involves both CD4+ and CD4− subsets (4–7). Therefore, although the preferential infection of CD4+ NKT cells probably contributes to the depletion of NKT cells during HIV-1 infection, it provides an incomplete explanation.

It is well established that treatment of HIV-1 infection with highly active antiretroviral therapy (HAART)3 results in a rapid (2–3 mo) increase in conventional T cell numbers that reflects redistribution of previously sequestered memory lymphocytes from lymphoid tissue to the circulation (8, 9). Because NKT cells express much higher levels of inflammatory lymphocyte chemokine receptors than other memory or effector T cell subsets (10), we hypothesized that tissue redistribution could be a major factor involved in the depletion of NKT cells during HIV-1 infection.

In this study, we evaluated circulating T and NKT cell responses in HIV-1-infected individuals during their first year of HAART. Initiation of HAART resulted in a remarkably similar and rapid recovery in both circulating T and NKT cell numbers, indicating that the depletion of circulating NKT cells during HIV-1 infection is probably at least in part due to tissue sequestration.

Materials and Methods

Patients

Twenty-six male antiretroviral therapy naive HIV-1 infected subjects (mean age, 41 years; range, 27–58); mean CD4+ T cell count 212 cells/μl (range 19–601); median NKT cell count 90 cells/ml (interquartile range, 31–270); and mean viral load, 176,190 copies/ml (range, 13,063–916,000, limit of detection, 50) were studied. The number of patients classified in Centers for Disease Control stages A, B, and C was 14, 7, and 5, respectively. This study is a sub-study of a randomized controlled multicenter trial called MEDICLAS (Metabolic Effects of Different Classes of Antiretrovirals) in which patients received either lopinavir-ritonavir and nevirapine or lopinavir-ritonavir, zidovudine, and lamivudine and included all randomized patients that, at the time of analysis, had received at least one year of HAART.

Flow cytometry and cell culture

Lymphocyte numbers were calculated from heparinized peripheral blood samples using MultiTEST reagents in combination with MultiSET software (BD Biosciences). The following mAbs were used: FITC-labeled Vα24 and PE- and

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3 Abbreviations used in this paper: HAART, highly active antiretroviral therapy; α-GalCer, α-galactosylceramide.
biotin-labeled Vβ11 (ImmunoTech); R-PE-Cy5-labeled streptavidin (DakoCy- 
tomation); PerCP-Cy5.5-labeled CD3, allophycocyanin-labeled CD4, PE-la-
beled IL-4, and IFN-γ (BD Biosciences); and PE-labeled CCRx6 and PE-la-
beled CCR2 (R&D Systems). NKT cells were defined by coexpression of CD3, 
Vα24, and Vβ11, because this combination has been shown to be highly spe-
cific for α-galactosylceramide (α-GalCer)-reactive, CD1d-restricted NKT cells 
(11). The cytokine profile of NKT cells and the expression of CCR2 and 
CCR6 by NKT cells were separately tested in healthy controls and in HIV-1-in-
fected patients who were not included in the randomized controlled trial. The 
cytokine profile of NKT cells was assessed as described previously (12). In short, 
NKT cells were enriched from PBMC by magnetic isolation of Vα24 T 
cells (Miltenyi Biotec) and subsequently cultured for 7 days in the presence of 
α-GalCer-pulsed monocyte derived dendritic cells. Cells were then washed 
and NKT cell cytokine production was determined by intracellular flow cytometry 
after a 5-h coculture with CD1d-transfected HeLa cells in the presence of 100 
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undetectable in 22, 
correlation test.

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ng/ml α-GalCer (KRN700; Kirin Pharmaceutical Research Laboratory) and 1 
µl/ml GolgiPlug (BD Biosciences). Flow cytometry was performed on a FAC-

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Statistical analysis

Statistical analyses were performed using Student t tests and a Spearman rank 
correlation test. p < 0.05 was considered significant.

Results and Discussion

HIV-1 RNA viral load and circulating T and NKT cell num-
bers were evaluated in 26 male HIV-1-infected subjects before 
and after 3 and 12 mo of HAART. Institution of HAART re-
bers were evaluated in 26 male HIV-1-infected subjects before

FIGURE 1. Recovery of circulating CD4⁺ T cells and NKT cells upon in-
stitution of HAART. Bars show fold increase in CD4⁺ T cells (open bars; 1.90 ± 0.18-fold (mean ± SEM) at 3 mo (p < 0.0001) and 2.41 ± 0.33-fold 
at 12 mo (p = 0.0002)) and NKT cells (black bars; 2.07 ± 0.36-fold at 3 mo 
(p = 0.007) and 2.09 ± 0.43-fold at 12 mo (p = 0.02)) after 3 and 12 mo of 
HAART. Bars labeled "pre" represent cells before institution of HAART.

recovery of NKT cells is due to NKT cell neogenesis that may 
follow the HAART-induced reduction of immune activation and the subsequent reduction in activation-induced cell death of 
NKT cells (13). Interestingly, a recent paper by Moll et al. 
(14) demonstrated an increase in circulating NKT cell numbers in 
patients with primary HIV-1 infection treated with a combi-

Although these data demonstrate that HAART can result in 
the recovery of both CD4⁺ T and NKT cells, it is important to 
note that although all patients showed a virologic response 
upon initiation of HAART, not all patients showed a concom-

incomplete immune reconstitution despite successful suppres-

of plasma HIV-1 viremia is well known and has been at-

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FIGURE 1. Recovery of circulating CD4⁺ T cells and NKT cells upon in-
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Contrast, in all patients with a poor initial CD4⁺ T cell numbers at 3 mo (a fold increase of 2.51 ± 0.244 cells/ml (p = 0.0017) and an absolute increase of 126 ± 43 cells/ml (p = 0.009)). In con-
trast, in all patients with a poor initial CD4⁺ T cell response a further decrease in NKT cell numbers was observed (a fold change of 0.54 ± 0.09 (p = 0.006) and an absolute decrease of 345 ± 244 cells/ml (p = 0.23)). In accordance, we found that 
there was a strong correlation between the absolute recovery of CD4⁺ T cells and NKT cells (correlation coefficient (r) = 0.67, 
p = 0.0004; Spearman rank correlation test) as illustrated in 
Fig. 2B. Altogether, these results indicate that the response of 
NKT cells and CD4⁺ T cells to HAART is not only strikingly 
similar in timing but also in occurrence. To evaluate whether the 
reconstituted NKT cells were functional, we analyzed their 
capacity to produce IFN-γ and IL-4 in response to antigenic 
stimulation with the glycolipid α-GalCer and CD1d-trans-
fected HeLa cells. Importantly, the IFN-γ/IL-4 ratio of NKT 
cells from HAART-treated HIV-1-infected patients (5.2 ± 3.2, 
mean ± SD, n = 4) was within the range of that of healthy 
controls (18.2 ± 15.2, n = 3, p = 0.15; unpaired t test (not 
shown)). Furthermore, as CD4⁻ NKT cells were previously re-
ported to be more Th1 biased than CD4⁺ NKT cells (16, 17), 
we also determined the cytokine profile of CD4⁺ and CD4⁻ 
NKT cell subsets and found that the CD4⁻ subset of reconsti-
tuted NKT cells contained similar numbers of IL-4-producing 
cells (9.7 ± 8.3% of CD4⁻ and 14.7 ± 7.6% of CD4⁺ NKT 
cells, n = 4, p = 0.28; paired t test) but significantly more 
IFN-γ producing cells (46.2 ± 13.5% of CD4⁻ and 27.1 ± 1.90/8.3% of CD4⁻ and 14.7 ± 7.6% of CD4⁺ NKT 
cells, n = 4, p = 0.28; paired t test) but significantly more 
IFN-γ producing cells (46.2 ± 13.5% of CD4⁻ and 27.1 ±
increase in NKT cell numbers during HAART resulted from an increase in CD4+ NKT cells (p = 0.03; paired t test), but not in CD4− NKT cells (p = 0.98; Fig. 3). Recently, CXCL16/CXCR6 and CCL2/CCR2 interactions were reported to play a major role in NKT cell trafficking (18–20). Because both CXCL16 and CCL2 are induced under proinflammatory conditions (21, 22), as occurs during HIV-1 infection, and their receptors have been reported to be predominantly expressed by CD4+ NKT cells (23), one could hypothesize that this differential expression of CCR2 and CXCR6 on NKT cell subsets might be involved in the predominant recovery of CD4+ NKT cells during HAART. Therefore, we additionally evaluated the expression of CCR2 and CXCR6 on CD4+ and CD4− NKT cells. In healthy volunteers we found that CCR2 was indeed predominantly expressed by CD4− NKT cells (90.8 ± 9.5% of CD4− and 59 ± 23.1% of CD4+ NKT cells, n = 5, p = 0.03; paired t test), but CXCR6 expression was comparable among both NKT cell subsets (79.2 ± 8.7% of CD4− and 67.6 ± 27.3% of CD4+ NKT cells, p = 0.28). Notably, in HIV-1-infected patients that were not treated with HAART the proportion of NKT cells expressing CCR2, and to a lesser extent CXCR6, was substantially decreased (p < 0.0001 and p = 0.03 respectively; unpaired t test), suggesting that NKT cells expressing these chemokine receptors were selectively depleted from the circulation. However, as the proportion of NKT cells expressing CCR2 or CXCR6 was not significantly higher in HAART-treated compared with HAART-untreated HIV-1-infected patients (p = 0.48 for CCR2 and p = 0.50 for CXCR6; unpaired t test), our data do not indicate that HAART-induced modulation of the ligands of these chemokine receptors plays a dominant role in the reconstitution of NKT cells (Fig. 4).

In conclusion, we demonstrate that HAART results in the rapid recovery of both mainstream T and predominantly CD4+ NKT cells with striking similarities in occurrence and kinetics. As the early recovery of mainstream T cells in response to HAART has been shown to be due to their redistribution from tissues to the circulation, our data suggest that the observed selective depletion of NKT cells during HIV-1 infection is not only caused by the previously reported loss of CD4+ NKT cells as a consequence of cell death after infection with HIV-1 but may also be attributed in part to tissue sequestration

11.7% of CD4+ NKT cells, n = 4, p = 0.04), further supporting the view that the reconstituted NKT cells of HIV-1 infected patients are functional and that subsets have retained their biased cytokine profile.

To evaluate whether the recovery of NKT cells was due to the selective recruitment of CD4+ or CD4− NKT cells, we determined the contribution of both subsets to the total NKT cell pool in five patients that showed a virologic, CD4+ T cell, and NKT cell response to HAART. In this group we noted that the
of NKT cells. Recently, it was shown that levels of CD1d are down-regulated by HIV-1 Nef and gp120 proteins and that HAART-induced viral suppression restored CD1d expression levels (24–26). These data, together with our current data, indicate that the immune reconstitution mediated by HAART is not restricted to conventional CD4+ and CD8+ T cells but also involves reconstitution of an important immunoregulatory axis represented by CD1d and NKT cells.

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Disclosures
The authors have no financial conflict of interest.

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