Selective Decrease in Circulating Vα24+Vβ11+NKT Cells During HIV Type 1 Infection

Hans J. J. van der Vliet, B. Mary E. von Blomberg, Mette D. Hazenberg, Nobusuke Nishi, Sigrid A. Otto, Birgit H. van Benthem, Maria Prins, Frans A. Claessen, Alfons J. M. van den Eertwegh, Giuseppe Giaccone, Frank Miedema, Rik J. Scheper and Herbert M. Pinedo

J Immunol 2002; 168:1490-1495; doi: 10.4049/jimmunol.168.3.1490
http://www.jimmunol.org/content/168/3/1490

References
This article cites 50 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/168/3/1490.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Selective Decrease in Circulating \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT Cells During HIV Type 1 Infection

Hans J. J. van der Vliet,* B. Mary E. von Blomberg,† Mette D. Hazenberg,§ Nobusuke Nishi,* Sigrid A. Otto,§ Birgit H. van Bentheim,§ Maria Prins,§ Frans A. Claessen,¶ Alfons J. M. van den Eertwegh,* Giuseppe Giaccone,* Frank Miedema,§ Rik J. Schepers,‡*† and Herbert M. Pinedo*

CD1d-restricted NKT cells express an invariant TCR and have been demonstrated to play an important regulatory role in a variety of immune responses. Invariant NKT cells down-regulate autoimmune responses by production of type 1 cytokines and can initiate antitumor and antimicrobial immune responses by production of type 1 cytokines. Although defects in the (invariant) \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cell population have been observed in patients with cancer and autoimmune diseases, little is known regarding the protective role of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells in human infectious disease. In a cross-sectional study in HIV-1-infected individuals, we found circulating numbers of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells were to be reduced, independent of CD4+ T cell counts, CD4:CD8 ratios, and viral load. Because a small minority of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells of healthy donors expressed HIV-1 (co)receptors and the vast majority of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells in HIV-1-infected individuals expressed the Fas receptor, the depletion was more likely due to Fas-mediated apoptosis than to preferential infection of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells by HIV-1. A longitudinal cohort study, in which patients were analyzed before seroconversion and 1 and 5 years after seroconversion, demonstrated that a large proportion of the depletion occurred within the first year postseroconversion. In this longitudinal study no evidence was found to support an important role of \( \text{Va}^{2+}\text{Vb}^{11+} \) NKT cells in determining the rate of progression during HIV-1 infection. *The Journal of Immunology, 2002, 168: 1490–1495.

Natural killer T cells constitute a lymphocyte lineage sharing characteristics of both T cells and NK cells. NKT cells display an extremely restricted TCR repertoire, in humans consisting of a Va24 chain preferentially paired with a Vb11 chain, and recognize Ag in the context of the monomorphic CD1d Ag-presenting molecule (1–3). Although natural ligands are not known, NKT cells have been shown to recognize the α-ano-meric glycolipid α-galactosylceramide (α-GalCer)† when presented by CD1d (4–6). A potential role of NKT cells in the regulation of immune responses has been hypothesized because of their capacity to rapidly release large amounts of IL-4 and IFN-γ upon activation (3, 7). Indeed, NKT cells have now been shown to play crucial roles in various immune responses, including antitumor, autoimmune, and antimicrobial immune responses (summa-

Departments of *Medical Oncology, †Pathology, and ‡Internal Medicine, Free University Medical Center, Amsterdam, The Netherlands; §Department of Clinical Viro-Immunology, CLB, and the Laboratory for Experimental and Clinical Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and ¶Cluster of Infectious Diseases, Municipal Health Service, Amsterdam, The Netherlands

Received for publication September 20, 2001. Accepted for publication December 3, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a Spinoza grant and Grant NR 920-03-142 from The Netherlands Organization for Scientific Research.

2 Address correspondence and reprint requests to Dr. Rik J. Schepers, Department of Pathology, Free University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. E-mail address: rj.schepers@vumc.nl

3 Abbreviations used in this paper: α-GalCer, α-galactosylceramide; HAART, highly active antiretroviral therapy; rh, recombinant human; RH, relative hazard; moDC, monocyte-derived dendritic cell; IQR, interquartile range; FasL, Fas ligand.

Copyright © 2002 by The American Association of Immunologists 0022-1767/02/$02.00
Results

Circulating \( \text{V}_{o24}^+ \text{V}_{b11}^+ \) NKT cells are decreased during HIV-1 infection

Cross-sectional analysis of a group of 50 HIV-1-infected and 46 healthy individuals revealed that circulating numbers of \( \text{V}_{o24}^+ \text{V}_{b11}^+ \) NKT cells (i.e., T cells expressing both the TCR \( \text{V}_{o24} \) and \( \text{V}_{b11} \) chains) were significantly lower in HIV-1-infected individuals (HIV-1 group: median, 71.5; interquartile range (IQR), 25–57; and control group: median, 3461; IQR, 1249–5092; \( p < 0.0001 \); Mann-Whitney \( U \) test, Fig. 1, left panel). Cell numbers were calculated per \( 1 \times 10^9 \) lymphocytes to correct for lymphopenia and lymphocytosis. Although the \( \text{V}_{o24} \) and \( \text{V}_{b11} \) mAb used in our analyses do not molecularly identify the invariant TCR rearrangement of NKT cells, their combined use was shown to be highly specific for invariant NKT cells and allows calculation of circulating cell numbers (8, 21). Circulating numbers of \( \text{V}_{o24}^+ \text{V}_{b11}^+ \) T cells were also reduced (HIV-1 group: median, 1336; IQR, 817–2136; and control group: median, 1512; IQR, 1194–2682; \( p = 0.03 \), Fig. 1, middle panel), but circulating numbers of T cells expressing only the \( \text{V}_{b11} \) chain were comparable in HIV-1-infected individuals and in controls (HIV-1 group: median, 3750; IQR, 2886–5092; and control group: median, 4534; IQR, 3741–5163; \( p = 0.11 \), Fig. 1, right panel). Patients receiving and not receiving HAART had comparable circulating \( \text{V}_{o24}^+ \text{V}_{b11}^+ \) NKT cell numbers (Fig. 1, left panel; \( p = 0.31 \)). Further evaluation revealed that there was no significant correlation between circulating \( \text{V}_{o24}^+ \text{V}_{b11}^+ \) NKT cell numbers and CD4:CD8 ratio (Fig. 2; correlation coefficient \( r = 0.12 \), \( p = 0.42 \), Spearman rank correlation test), CD4+ T cell counts (Fig. 2; \( r = -0.06 \), \( p = \)}
One year postseroconversion, and 5 years postseroconversion. Fig. 3A shows that the frequency of \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells (expressed as a percentage of T cells) significantly decreased during HIV-1 infection. The preseroconversion \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequency (median, 0.03; IQR, 0.01–0.08; \( n = 48 \)) was significantly lower than in our control group (median, 0.08; IQR, 0.04–0.17; \( p = 0.02 \), Mann-Whitney \( U \) test). This frequency decreased to 0.02 (IQR, 0.01–0.04; \( n = 82 \)) and 0.01 (IQR, 0–0.02; \( n = 50 \)) at 1 and 5 years postseroconversion, respectively (preseroconversion vs 1 year postseroconversion, \( p < 0.0001 \) Wilcoxon matched pairs test); preseroconversion vs 5 years postseroconversion, \( p < 0.0001 \). In contrast, the frequency of single \( \text{V}\beta^{11+} \) T cells (expressed as a percentage of T cells) did not significantly change in the course of HIV-1 infection (preseroconversion vs 5 years postseroconversion, \( p = 0.054 \)). Data are expressed as a percentage of T cells to correct for differences in total T cell numbers over time. Fig. 3B shows representative flow cytometric dot plots from one individual.

**Expression of CD4, CCR5, and CD95 on \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells**

One of the potential causes of the selective decrease in the size of the circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell population could be their preferential infection by HIV-1. The HIV-1 virion enters its target cell through a sequence of conformational shifts initiated by binding to CD4. Primary HIV-1 infection is established with non-synctium-inducing CCR5 coreceptor using HIV-1 variants (22–25). First, we studied CD4 expression on \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells of healthy volunteers both before and after activation. CD4 expression was observed on 25 ± 8% (\( n = 5 \); data not shown) of circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells. Although ligand-specific activation of \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells using \( \alpha\)-GalCer-loaded moDC uniformly resulted in the expression of high levels of the activation marker CD25 (data not shown), it did not result in a significant up-regulation of CD4 expression on \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells (40 ± 21%, \( n = 5 \), \( p = 0.21 \), paired Student’s \( t \) test). Furthermore, because only 1.6 ± 1.9% (\( n = 4 \)) of \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells was found to express both CD4 and CCR5, we think that it is unlikely that direct infection accounts for the depletion of the \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell population (Fig. 4A).

Fas/Fas ligand (FasL) interactions represent an important apoptosis-enhancing pathway involved in T cell depletion during HIV infection (26). Therefore, we compared Fas (CD95) expression on \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells and single \( \text{V}\beta^{11+} \) T cells in HIV-1-infected individuals and found that a significantly higher proportion of residual \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells expressed CD95 (93.4 ± 7.4% (mean ± SD) vs 63.4 ± 20.4%, \( n = 7 \), \( p = 0.005 \), paired Student’s \( t \) test). Fig. 4B shows a representative flow cytometric dot plot from one individual.

**Prognostic relevance of the size of the \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell pool on HIV-1 progression**

Because the circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequency showed strong interindividual variability, we evaluated whether the size of the circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell pool could predict disease progression during HIV-1 infection. Participants of the Amsterdam cohort on HIV-1 infection in homosexual men were split into two groups based on whether they had a circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequency above or below the group median. The relative hazard (RH) of the group of participants with above-median circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequencies was then compared with the group of participants with below-median circulating \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequencies with respect to the following AIDS-related endpoints: progression to AIDS (27), death from an AIDS-related cause, CD4+ T cell depletion, 1 year postseroconversion, and 5 years postseroconversion.

---

**DECREASE IN NKT CELLS DURING HIV-1 INFECTION**

---

**Kinetics of \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell depletion during HIV-1 infection**

Because the exact duration of HIV-1 infection had not been recorded in the cross-sectional analysis, we set out to confirm and extend our findings by analyzing the frequency of \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cells in cryopreserved PBMC of the Amsterdam cohort on HIV-1 infection in homosexual men. \( \text{Va}^{24}\text{V}\beta^{11+} \) NKT cell frequencies were determined at three time points: before seroconversion, 1 year postseroconversion, and 5 years postseroconversion.
counts < 200/ml, and conversion to a syncytium-inducing viral variant (SI conversion). Analyses performed preseroconversion, 1 year postseroconversion, and 5 years postseroconversion clearly showed that relatively higher Vα24+ Vβ11+ NKT cell frequencies were not predictive of a better outcome in HIV-1-infected patients (Table I). Similar results were obtained in multivariate analyses where results were corrected for viral HIV-1 RNA load and CD4+ T cell counts (data not shown).

FIGURE 3. Selective depletion of Vα24+ Vβ11+ NKT cells during HIV-1 infection. Cryopreserved PBMC samples of participants from the Amsterdam cohort studies on AIDS and HIV-1 infection in homosexual men were analyzed preseroconversion and at 1 and 5 years postseroconversion. A, A decrease in Vα24+ Vβ11+ NKT cells (preseroconversion vs 5 years postseroconversion, p < 0.0001; Wilcoxon matched pairs test) but not in Vα24+ Vβ11+ T cells (preseroconversion vs 5 years postseroconversion, p = 0.054). Data are expressed as a percentage of T cells; median, IQR, minimum, and maximum are shown. B, Sequential dot plots of CD3+ cells from one individual.

Discussion
Cross-sectional analysis of HIV-1-infected individuals and healthy controls demonstrated a decrease of circulating Vα24+ Vβ11+ NKT cell numbers during HIV-1 infection. This decrease in circulating Vα24+ Vβ11+ NKT cell numbers, which was not associated with either CD4+ T cell count, CD4:CD8 ratios, or viral load, was confirmed in our longitudinal study. The latter analysis also demonstrated the time course of Vα24+ Vβ11+ NKT cell depletion and indicated that a major proportion of the decrease in circulating Vα24+ Vβ11+ NKT cells occurred within the first year postseroconversion.

The disappearance of Vα24+ Vβ11+ NKT cells during HIV-1 infection is not likely to be the result of viral infection per se, because circulating Vα24+ Vβ11+ NKT cell frequencies were recently reported to be normal during hepatitis C viral infection (21). Several factors could contribute to the depletion of the circulating Vα24+ Vβ11+ NKT cell population, including sequestration, preferential infection, and activation-induced cell death. First, the rise in CD4+ and CD8+ T cell numbers during the first 4 wk of HAART is generally believed to be due to redistribution of previously sequestered memory lymphocytes from lymphoid tissues to the circulation (28). No relation between viral load and circulating Vα24+ Vβ11+ NKT cell numbers could be found, and in several patients who showed CD4+ T cell responses upon institution of HAART circulating Vα24+ Vβ11+ NKT cell numbers remained low (data not shown). However, to formally exclude the possibility that the depletion of the Vα24+ Vβ11+ NKT cell population is the result of sequestration in the periphery, we are now systematically evaluating the effects of HAART on circulating Vα24+ Vβ11+ NKT cell numbers. Second, it has been reported that CD4+ T cells can express CD4 upon activation, allowing HIV entry

FIGURE 4. Expression of CD4, CCR5, and Fas on Vα24+ Vβ11+ NKT cells. Shown are representative flow cytometric dot plots of expression of CD4 and CCR5 on Vα24+ Vβ11+ NKT cells of a healthy donor (A) and of Fas expression on Vβ11+ T cells of an HIV-1-infected patient (B).

counts < 200/ml, and conversion to a syncytium-inducing viral variant (SI conversion). Analyses performed preseroconversion, 1 year postseroconversion, and 5 years postseroconversion clearly showed that relatively higher Vα24+ Vβ11+ NKT cell frequencies were not predictive of a better outcome in HIV-1-infected patients (Table I). Similar results were obtained in multivariate analyses where results were corrected for viral HIV-1 RNA load and CD4+ T cell counts (data not shown).

Discussion
Cross-sectional analysis of HIV-1-infected individuals and healthy controls demonstrated a decrease of circulating Vα24+ Vβ11+ NKT cell numbers during HIV-1 infection. This decrease in circulating Vα24+ Vβ11+ NKT cell numbers, which was not associated with either CD4+ T cell count, CD4:CD8 ratios, or viral load, was confirmed in our longitudinal study. The latter analysis also demonstrated the time course of Vα24+ Vβ11+ NKT cell depletion and indicated that a major proportion of the decrease in circulating Vα24+ Vβ11+ NKT cells occurred within the first year postseroconversion.

The disappearance of Vα24+ Vβ11+ NKT cells during HIV-1 infection is not likely to be the result of viral infection per se, because circulating Vα24+ Vβ11+ NKT cell frequencies were recently reported to be normal during hepatitis C viral infection (21). Several factors could contribute to the depletion of the circulating Vα24+ Vβ11+ NKT cell population, including sequestration, preferential infection, and activation-induced cell death. First, the rise in CD4+ and CD8+ T cell numbers during the first 4 wk of HAART is generally believed to be due to redistribution of previously sequestered memory lymphocytes from lymphoid tissues to the circulation (28). No relation between viral load and circulating Vα24+ Vβ11+ NKT cell numbers could be found, and in several patients who showed CD4+ T cell responses upon institution of HAART circulating Vα24+ Vβ11+ NKT cell numbers remained low (data not shown). However, to formally exclude the possibility that the depletion of the Vα24+ Vβ11+ NKT cell population is the result of sequestration in the periphery, we are now systematically evaluating the effects of HAART on circulating Vα24+ Vβ11+ NKT cell numbers. Second, it has been reported that CD4+ T cells can express CD4 upon activation, allowing HIV entry
(31, 32), we found no evidence for a similar activation-induced up-regulation of CD4 expression on Vα24+Vβ11+ NKT cells. Because only 1.6 ± 1.9% of Vα24+Vβ11+ NKT cells expressed both CD4 and the coreceptor CR5, we believe that it is unlikely that physical infection of Vα24+Vβ11+ NKT cells is responsible for the population depletion. Third, several reports indicate that ligation of the Fas receptor by FasL is an important factor involved in HIV-associated lymphocyte depletion. T cells from HIV-infected patients have been reported to exhibit both increased Fas receptor expression and enhanced susceptibility to Fas-mediated death (33, 34). Of note, both FasL expression on PBMC and plasma levels of soluble FasL are increased in HIV-positive patients (35–37). We found the Fas receptor to be expressed by the vast majority of residual circulating Vα24+Vβ11+ NKT cells. Because the proportion of Vα24+Vβ11+ NKT cells expressing the Fas receptor was significantly higher than that of single Vβ11+ T cells, Fas-induced apoptosis might contribute to the observed selective depletion of the Vα24+Vβ11+ NKT cell population. HIV-1 infection has been demonstrated to increase cell division and death rates mainly by causing persistent immune activation (38). Therefore, one could hypothesize that the observed depletion of the Vα24+Vβ11+ NKT cell population is the result of a continuous process of activation-induced cell death. Because renewal of invariant NKT cells was demonstrated to be slow in both mouse and human (39), this could further contribute to the decrease in the size of the Vα24+Vβ11+ NKT cell population.

Infection with HIV-1 has been associated with various autoimmune syndromes and malignancies (40–42). Because defects in the invariant NKT cell population were reported in both animals and patients suffering from autoimmune disease or malignancy (8, 43–46), it is tempting to speculate that the increased frequency of autoimmune phenomena and malignancies during HIV-1 infection is related to a decrease in the size of the Vα24+Vβ11+ NKT cell population. Because activated NKT cells could down-regulate hepatitis B viral replication in mice through production of IFN-α and IFN-γ (11), cytokines previously reported to inhibit HIV replication (19), we hypothesized that Vα24+Vβ11+ NKT cells could slow down progression during HIV-1 infection. However, our data could not demonstrate a statistically significant relation between the circulating Vα24+Vβ11+ NKT cell frequency and several AIDS-related disease endpoints. In contrast to what we expected, individuals with higher preconversion or 1 year postconversion Vα24+Vβ11+ NKT cell frequencies tended to have even higher RHs, suggesting a potential immunosuppressive effect of Vα24+Vβ11+ NKT cells. Recent evidence indeed suggests that the natural role of NKT cells could be immunosuppressive in nature by predominant production of Th2 cytokines (47). Of interest, synthetic glycolipid analogs of α-GalCer were shown to differentially affect the cytokine profile of NKT cells (48). Therefore, because high-affinity TCR ligands preferentially induce Th1-type responses in T cells (49), stimulation of NKT cells by ligands that result in high-affinity interactions could shift NKT cell cytokine production toward a Th1 profile, thereby enhancing the establishment of a proinflammatory immune response (50). This would favor both antiviral and antitumor immune reactivity but could also increase the frequency of autoimmune phenomena during HIV infection. Therefore, although we report in this work that the size of the circulating Vα24+Vβ11+ NKT cell population does not affect the progression rate to several clinical and immunological endpoints, further studies are needed to examine the relationship between the cytokine profile of Vα24+Vβ11+ NKT cells and HIV-1 disease progression.

In conclusion, in this study we demonstrate for the first time the preferential depletion of the immunoregulatory Vα24+Vβ11+ NKT cell population during HIV-1 infection. Although this depletion is likely to contribute to the development of immunodeficiency, our data do not provide evidence to support an important role of Vα24+Vβ11+ NKT cells in determining the rate of progression during HIV-1 infection.

References


Table I. Effect of the size of the Vα24+Vβ11+ NKT cell pool on HIV-1 progression

<table>
<thead>
<tr>
<th></th>
<th>Preconversion</th>
<th>1 Year Postconversion</th>
<th>5 Years Postconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH (95% CI)</td>
<td>p</td>
<td>RH (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>AIDS</td>
<td>1.01 (0.37–2.73)</td>
<td>0.99</td>
<td>1.57 (0.78–3.14)</td>
</tr>
<tr>
<td>Death</td>
<td>1.34 (0.45–3.99)</td>
<td>0.60</td>
<td>1.28 (0.64–2.58)</td>
</tr>
<tr>
<td>CD4 &lt; 200/ml</td>
<td>1.67 (0.68–4.12)</td>
<td>0.27</td>
<td>1.71 (0.84–3.47)</td>
</tr>
<tr>
<td>SI conversion</td>
<td>2.61 (0.24–29.0)</td>
<td>0.24</td>
<td>1.89 (0.66–5.39)</td>
</tr>
</tbody>
</table>

RH, relative hazard; CI, confidence interval.

*At each time point, participants of the Amsterdam cohort studies on AIDS and HIV-1 infection in homosexual men were divided into two groups based on their circulating Vα24+Vβ11+ NKT cell frequency. Univariate RHs are given for those with a Vα24+Vβ11+ NKT cell frequency above the median with respect to the indicated disease endpoints. CI, confidence interval.

Submitted for publication.
25. Doms, R. W. 2000. Beyond receptor expression: the influence of receptor con-