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Selective Loss of Innate CD4$^+$ Vα24 Natural Killer T Cells in Human Immunodeficiency Virus Infection

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Vα24 natural killer T (NKT) cells are innate immune cells involved in regulation of immune tolerance, autoimmunity, and tumor immunity. However, the effect of human immunodeficiency virus type 1 (HIV-1) infection on these cells is unknown. Here, we report that the Vα24 NKT cells can be subdivided into CD4$^+$ or CD4$^-$ subsets that differ in their expression of the homing receptors CD62L and CD11a. Furthermore, both CD4$^+$ and CD4$^-$ NKT cells frequently express both CXCR4 and CCR5 HIV coreceptors. We find that the numbers of NKT cells are reduced in HIV-infected subjects with uncontrolled viremia and marked CD4$^+$ T-cell depletion. The number of CD4$^+$ NKT cells is inversely correlated with HIV load, indicating depletion of this subset. In contrast, CD4$^-$ NKT-cell numbers are unaffected in subjects with high viral loads. HIV infection experiments in vitro show preferential depletion of CD4$^+$ NKT cells relative to regular CD4$^+$ T cells, in particular with virus that uses the CCR5 coreceptor. Thus, HIV infection causes a selective loss of CD4$^+$ lymph node homing (CD62L$^+$) NKT cells, with consequent skewing of the NKT-cell compartment to a predominantly CD4$^-$ CD62L$^-$ phenotype. These data indicate that the key immunoregulatory NKT-cell compartment is compromised in HIV-1-infected patients.

The importance of innate immune mechanisms in controlling human immunodeficiency virus (HIV) infection and disease is currently an area of intense research interest. Several components of the innate immune system have direct anti-HIV effects, and innate responses can also influence the nature of the subsequent adaptive immune response (23). The interferon (IFN)-producing cells are the major producers of type I IFNs (35), and loss of these cells and alpha IFN (IFN-α) production has been associated with high HIV RNA levels and AIDS (38). NK cells can eliminate HIV-infected cells (1, 3) and are also a source of several cytokines and β-chemokines, which can inhibit HIV replication in vitro (12). In addition, chemokines produced by γδ T cells located at mucosal surfaces (27), as well as the antigen-independent CD8$^+$ T-cell antiviral factor (24), are components of the innate anti-HIV mechanisms. These innate responses may play a critical role in reducing transmission, limiting plasma viremia and contributing to lower viral set points.

Natural killer T (NKT) cells, a rare subset of CD3$^+$ T lymphocytes, are considered part of the innate immune system (13). They posses a semi-invariant T-cell receptor with uniform expression of the α-chain variable gene segment 24 (Vα24), preferentially paired with the β-chain variable gene segment 11 (Vβ11), and express CD161, a marker commonly found on NK cells (8, 28). NKT cells recognize self or nonself glycolipids presented by CD1d and respond by producing high levels of the immunoregulatory cytokines interleukin-4 (IL-4) and gamma IFN (IFN-γ) (11, 29).

The primary role for Vα24 NKT cells appears to be in regulation of immune tolerance and control of autoimmunity. Functional skewing or depletion of these cells has been observed in patients with autoimmune diabetes (44), systemic sclerosis (40), and multiple sclerosis (18). The analogous murine Vα14 NKT cells can protect against diabetes in the nonobese diabetic mouse (16, 17, 22, 34) and may also be necessary for induction of peripheral tolerance at the immune-privileged site of the eye (37). In addition, NKT cells can be cytotoxic against tumor cell lines in vitro and play a role in rejection of metastases in vivo (25, 36), possibly via the IL-12-mediated activation of NK cells (4). Activated intrahepatic Vα14 NKT cells clear hepatitis B virus infection by producing large quantities of IFN-γ (20), and *Mycobacterium bovis* bacillus Calmette-Guérin infection in mice activates intrahepatic Vα14 NKT cells, suggesting that these cells may respond against mycobacteria (10).

The NKT-cell ligand CD1d is expressed by dendritic cells (DCs), and the interaction between Vα24 NKT cells and DCs seems to be important for the immunoregulatory function of NKT cells. Vα24 NKT-cell recognition of CD1d-presented antigens on myeloid-derived DCs activates the DCs to produce IL-12, which enhances cellular immune responses (21). The myeloid-derived DCs can also be targets for lysis by NKT cells. This may be a regulatory feedback mechanism, because these DCs preferentially induce differentiation of naïve CD4$^+$ T cells into Th1 cells (33, 45). Vα24 NKT cells develop into IL-4$^-$ IFN-γ$^+$ NKT cells when cultured with myeloid-derived DCs,

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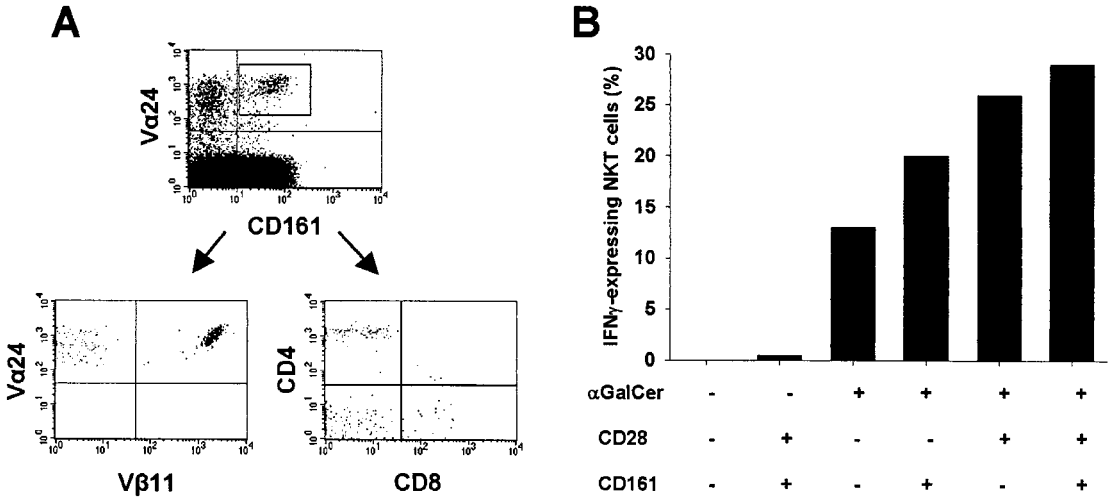

FIG. 1. NKT cells defined by Vα24 and CD161 express IFN-γ in response to αGalCer ex vivo. (A) Vα24 NKT cells identified by expression of Vα24 and CD161 coexpress Vβ11 and are $CD4^+$, $CD4^-$ $CD8^{/-}$, or $CD8^+$ as determined by four-color flow cytometry. (B) Production of IFN-γ in Vα24 NKT cells directly measured ex vivo by intracellular staining and flow cytometry after stimulation with αGalCer in the presence of anti-CD28 and anti-CD161 as indicated.

while lymphoid-derived DCs induce polarization into $IL\text{-}4^+$ $IFN\text{-}\gamma^-$ NKT cells (19).

The effects of HIV type 1 (HIV-1) on Vα24 NKT cells and the functions of these cells in the defense against HIV and associated opportunistic infections are unknown. We hypothesized that HIV infection causes depletion of Vα24 NKT cells. Loss of these cells could contribute to HIV pathogenesis because of their role in directing both adaptive and innate immune responses, because of their role in controlling peripheral tolerance and autoimmunity, and because of the possible involvement in tumor rejection and defense against mycobacteria (13). To test this hypothesis, we investigated the expression of receptors for HIV on the surface of Vα24 NKT cells and assessed the levels of NKT cells in HIV-infected patients and the sensitivity of these cells to HIV infection in vitro.

MATERIALS AND METHODS

Patient samples and viral load measurements. Heparinized whole-blood samples were obtained from 57 HIV-infected pediatric subjects, 10 healthy children, and 68 healthy adult subjects according to local institutional review board-approved protocols. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque PLUS density gradient centrifugation (Amersham Pharmacia Biotech, Uppsala, Sweden). Plasma HIV-1 RNA was measured with the Amplicor HIV-1 Monitor (Roche Diagnostic Systems, Branchburg, N.J.).

Flow cytometric assessment of NKT-cell numbers and phenotype. Circulating Vα24 NKT-cell frequency and expression of surface markers were assessed in fresh PBMCs by four-color flow cytometry. The following reagents were used: anti-Vα24 PE and anti-Vα11 biotin were from Immunotech (Marseilles, France); streptavidin-allophycocyanin (APC) was from Molecular Probes (Eugene, Oregon); and anti-CD3 fluorescein isothiocyanate (FITC), anti-CD4 APC, anti-CD8 PerCp, anti-CD11a FITC, anti-CD16 FITC, anti-CD27 FITC, anti-CD28 FITC, anti-CD45RO FITC, anti-CD45RA FITC, anti-CD56 FITC, anti-CD62L FITC, anti-CD94 FITC, anti-CD161 FITC, anti-CD161 APC, anti-CCR5 APC, and anti-CXCR4 APC were from BD Pharmingen (San Diego, California). Samples were analyzed on a FACSCalibur (Becton Dickinson) instrument using CellQuest software. Lymphocyte events were collected (2×10^5 events per sample).

NKT-cell cytokine expression assay. For activation of Vα24 NKT cells, PBMCs were incubated for 7 h in RPMI 1640 medium supplemented with 15% fetal calf serum, in the presence of αGalCer (1 μg/ml) and brefeldin A (10 μg/ml), which blocks transport through the Golgi apparatus. Purified anti-CD28 (3 μg/ml) and anti-CD161 (3 μg/ml) (BD Pharmingen) were included as indicated for additional costimulation. Cells were then washed and permeabilized with fluorescence-activated cell sorter permeabilizing solution (Becton Dickinson) for 15 min at room temperature. Cells were then washed twice and incubated with antibody against intracellular IFN-γ (BD Pharmingen) for 30 min at 4°C, washed three times, and analyzed by flow cytometry. Between 1×10^5 and 2.5×10^5 lymphocytes were analyzed in each sample.

In vitro infection assay. PBMCs from healthy donors were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum in the presence of anti-CD3 (1 μg/ml). After 2 days, the activated PBMCs were washed three times and infected with the CXCR4-using NL4-3 HIV strain or the NL4-3 chimeric virus 81A that contains the Ba-L V1-V3 region and uses the CCR5 coreceptor (42), or were mock infected for 3 h. p24 concentrations of virus stocks were 204 ng/ml for 81A and 165 ng/ml for NL4-3. The infected PBMCs were washed and moved to a 24-well plate at 2×10^6 PBMCs/ml in 1 ml of culture medium. Five days later cells were collected; washed; stained with anti-Vα24, anti-CD161, anti-CD4, and anti-CD3; and analyzed by four-color flow cytometry in triplicate.

Statistical analysis. The flow cytometry and clinical data were analyzed by descriptive statistics, linear regression, *t* test, and the Mann-Whitney rank sum test with Sigma Stat software (SPSS, Chicago, Ill.).

RESULTS

NKT cells defined by Vα24 and CD161 produce IFN-γ in response to αGalCer ex vivo. Human CD1d-restricted NKT cells uniformly express the Vα24 T-cell receptor α-chain segment and the NK marker CD161, and these two markers can be used to identify $V\alpha24^+$ $CD161^+$ NKT cells in peripheral blood. We first stained PBMCs for Vα24, CD161, CD4, and Vβ11 or CD8 and examined them by four-color flow cytometry (Fig. 1A). Circulating NKT cells in healthy subjects ($n = 68$) were diverse in their expression of CD4 and CD8. On average, 59% ± 21% were CD4 single positive, 15% ± 13% were CD8 single positive, and 25% ± 20% were CD4-CD8 double negative (data not shown) (values are means ± standard deviations unless otherwise noted).

Long-term Vα24 NKT-cell clones respond to the CD1d-presented glycolipid compound αGalCer in vitro by producing cytokines, including IFN-γ (11). To verify that the cells iden-

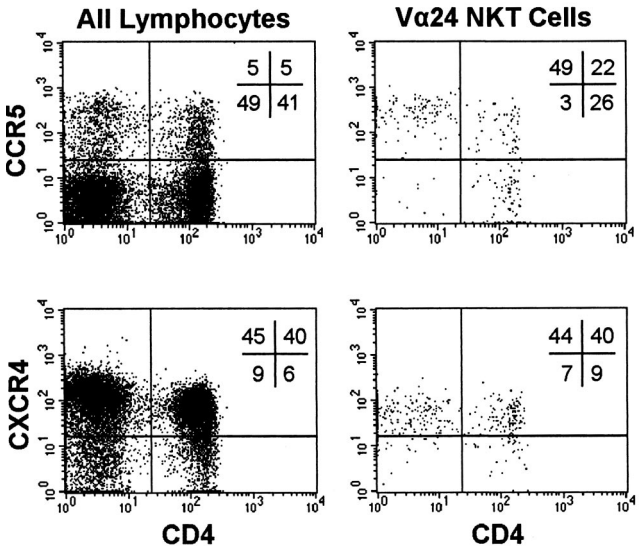

FIG. 2. Vα24 NKT cells express the HIV receptors CXCR4 and CCR5. Expression of CXCR4 and CCR5 in all lymphocytes (left panels), or in lymphocytes from a healthy adult subject gated on Vα24 and CD161 (right panels), was determined by four-color flow cytometry.

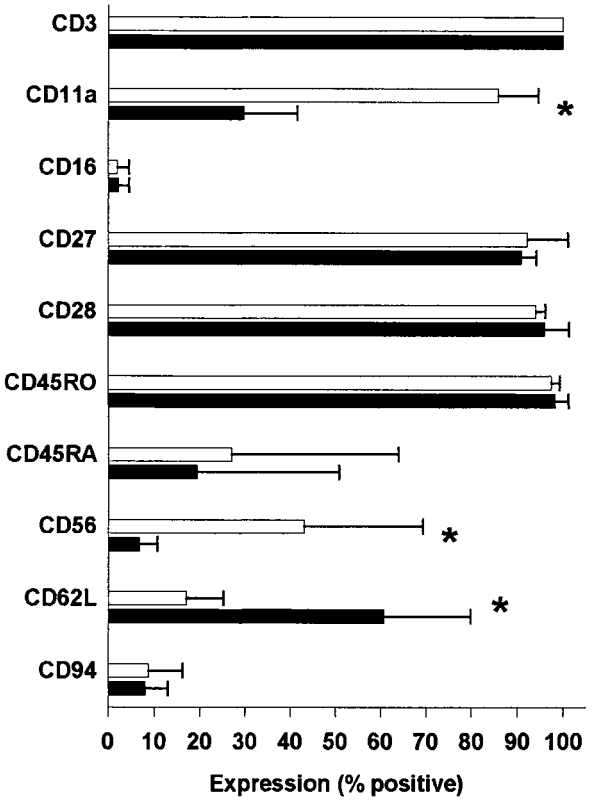

FIG. 3. Differential expression of adhesion and homing receptors on $CD4^+$ and $CD4^-$ subsets of NKT cells. Expression of cell surface markers on $CD4^-$ Vα24 NKT cells (white bars) and $CD4^+$ Vα24 NKT cells (black bars) was determined by four-color flow cytometry. Results are means and standard deviations (error bars) of data from five healthy adult subjects. A star indicates a statistically significant difference between subsets as determined by the *t* test. Bars for CD11a represent the percentage of cells in the two groups that are CD11a high.

tified by Vα24 and CD161 expression are functional NKT cells, we stimulated PBMCs from healthy subjects in a direct ex vivo assay for 7 h with αGalCer in the presence of brefeldin A and measured the production of IFN-γ by intracellular cytokine flow cytometry (Fig. 1B). IFN-γ expression was clearly detectable in Vα24 and CD161 double-positive cells and was further enhanced by the addition of anti-CD28 and anti-CD161 antibodies (Fig. 1B). This cytokine was detected in $CD4^+$ as well as in $CD4^-$ subsets (data not shown). Thus, the cells identified by Vα24 and CD161 expression are functional NKT cells that produce IFN-γ when activated.

Vα24 NKT cells express the HIV receptors CXCR4 and CCR5. Because HIV-1 usually requires the CD4 receptor, and an additional chemokine receptor for target-cell entry, we investigated the expression of CXCR4 and CCR5 on the NKT cells in healthy subjects. CXCR4 was expressed by almost all NKT cells, as is the case with T cells in general (Fig. 2). However, the $CD4^+$ and $CD4^-$ NKT subsets differed in that close to all $CD4^-$ NKT cells (93.4% ± 2.5%) and 33.3% ± 14% of $CD4^+$ NKT cells expressed CCR5 ($n = 6$) ($P < 0.001$) (Fig. 2). Furthermore, the high prevalence of CCR5 expression on $CD4^+$ NKT cells was in sharp contrast to the overall population of peripheral $CD4^+$ T cells, in which only 7.6% ± 4.7% of cells had CCR5 on the surface ($P = 0.003$). The finding that both CXCR4 and CCR5 are common on $CD4^+$ NKT cells suggested that these cells might be targets for strains of HIV utilizing either of these receptors and thus be early targets for HIV transmission and subject to rapid depletion in infected individuals.

$CD4^+$ and $CD4^-$ NKT cells differ in their expression of homing receptors. Because both $CD4^+$ and $CD4^-$ NKT cells were identified in all healthy subjects tested, we were next interested in the possibility of phenotypical differences between these subsets (Fig. 3). Both $CD4^+$ and $CD4^-$ NKT cells consistently expressed CD3 and were negative for the NK cell marker CD16, while the NK cell receptor CD94 was found on a small number of cells in both subsets. Most interestingly, $CD4^-$ NKT cells were largely CD11a high and CD62L negative, indicating that cells in this subset are homing to the peripheral tissues. $CD4^+$ NKT cells on the other hand, displayed the opposite pattern (i.e., CD11a low and CD62L positive), indicating preferential homing to the secondary lymphoid tissues. In line with this opposite homing pattern of the $CD4^-$ and $CD4^+$ NKT-cell subsets, a larger fraction of $CD4^-$ NKT cells expressed the CD56 marker that can be associated with an effector-like phenotype in T lymphocytes (26). Almost all NKT cells expressed the CD27 and CD28 costimulatory receptors and the CD45RO isoform, indicating prior activation (43). These data indicate that the two subpopulations of NKT cells have different homing properties in vivo, with $CD4^+$ NKT cells preferentially recirculating through the lymph nodes and $CD4^-$ NKT cells going to peripheral tissues.

Loss of Vα24 NKT cells in HIV-infected subjects. To investigate the impact of HIV on Vα24 NKT cells, we analyzed peripheral blood samples from 57 children with vertically transmitted HIV-1 infection. We anticipated that this large cohort of HIV-infected subjects would allow dissection of the

effects of HIV on Vα24 NKT cells, despite the low numbers of these cells in peripheral blood. The subjects ranged in age from 1 to 18 years old, 54% were female, and their ethnic origins were 46% Hispanic and 54% non-Hispanic black. Of the subjects, 79% were receiving highly active antiretroviral therapy at the time of the study, 14% were receiving dual or single antiretroviral drugs, and 7% were on no medications. Symptomatic HIV disease, plasma viremia, and $CD4^+$ T-cell counts varied widely in this group, indirectly reflecting the problem of adherence to complex antiretroviral drug regimens and the cumulative affect of drug resistance, viral diversification, and duration of infection on $CD4^+$ T-cell depletion and disease progression.

We first investigated the size of the circulating pool of Vα24 NKT cells in these subjects in relation to that of healthy control subjects. The HIV-infected pediatric subjects had significantly lower numbers of NKT cells in PBMCs ($n = 57$), compared to healthy pediatric control subjects ($n = 10$) (0.037% ± 0.004% [mean and standard error] and 0.059% ± 0.009%, respectively; $P = 0.013$) and healthy adult subjects ($n = 68$) (0.066% ± 0.005%; $P < 0.001$). We next investigated the size of the circulating pool of Vα24 NKT cells in these patients in relation to HIV load. The absolute counts of Vα24 and CD161 double-positive cells correlated inversely with viral load ($R = 0.33$; $P = 0.012$) (Fig. 4A). There was also a strong direct correlation between the loss of Vα24 NKT cells and $CD4^+$ T-cell depletion in these patients ($R = 0.53$; $P < 0.001$) (Fig. 4B). As expected, overall $CD4^+$ T-cell counts correlated inversely with viral load ($R = 0.41$; $P = 0.002$), although some subjects maintained high numbers of circulating $CD4^+$ T cells despite high viral loads (Fig. 4C). These data indicate that Vα24 NKT cells are lost in HIV-infected subjects with uncontrolled viremia and marked $CD4^+$ T-cell depletion.

Differential impact of HIV on Vα24 NKT-cell subsets in vivo. Because the Vα24 NKT-cell population contained both $CD4^+$ and $CD4^-$ subsets, which differed in their expression of chemokine and homing receptors, we next investigated whether these subsets were differentially affected in the HIV-infected subjects. The circulating numbers of Vα24, CD161, and CD4 triple-positive NKT ($CD4^+$ NKT) cells correlated inversely with viral load ($R = 0.49$; $P < 0.001$) (Fig. 5A). In contrast to $CD4^+$ NKT cells, the $CD4^-$ NKT-cell subset showed no significant correlation with viral load ($R = 0.14$; $P = 0.28$) (Fig. 5B). These data show that $CD4^+$ Vα24 NKT cells are lost in subjects with uncontrolled HIV replication, while $CD4^-$ Vα24 NKT cells are relatively less affected.

Evidence that $CD4^+$ NKT cells are preferential targets for HIV. Our finding that $CD4^+$ NKT cells commonly express CCR5 suggests that they are preferential targets for infection by HIV (Fig. 2). To assess this possibility, PBMCs from healthy donors were activated with anti-CD3 in vitro for 2 days and then exposed to the CXCR4-using HIV strain NL4-3 and the NL4-3 chimeric virus 81A that contains the Ba-L V1-V3 region and uses the CCR5 coreceptor (42). Five days later, the percentage of $CD4^+$ NKT cells was severely reduced by 81A infection ($P < 0.001$), and also by NL4-3 infection, although not as dramatically ($P = 0.003$) (Fig. 6A). $CD4^-$ NKT-cell numbers, on the other hand, were not significantly affected in the infected cultures (Fig. 6B). In comparison to $CD4^+$ NKT cells, drops in regular $CD4^+$ T-cell numbers were much less

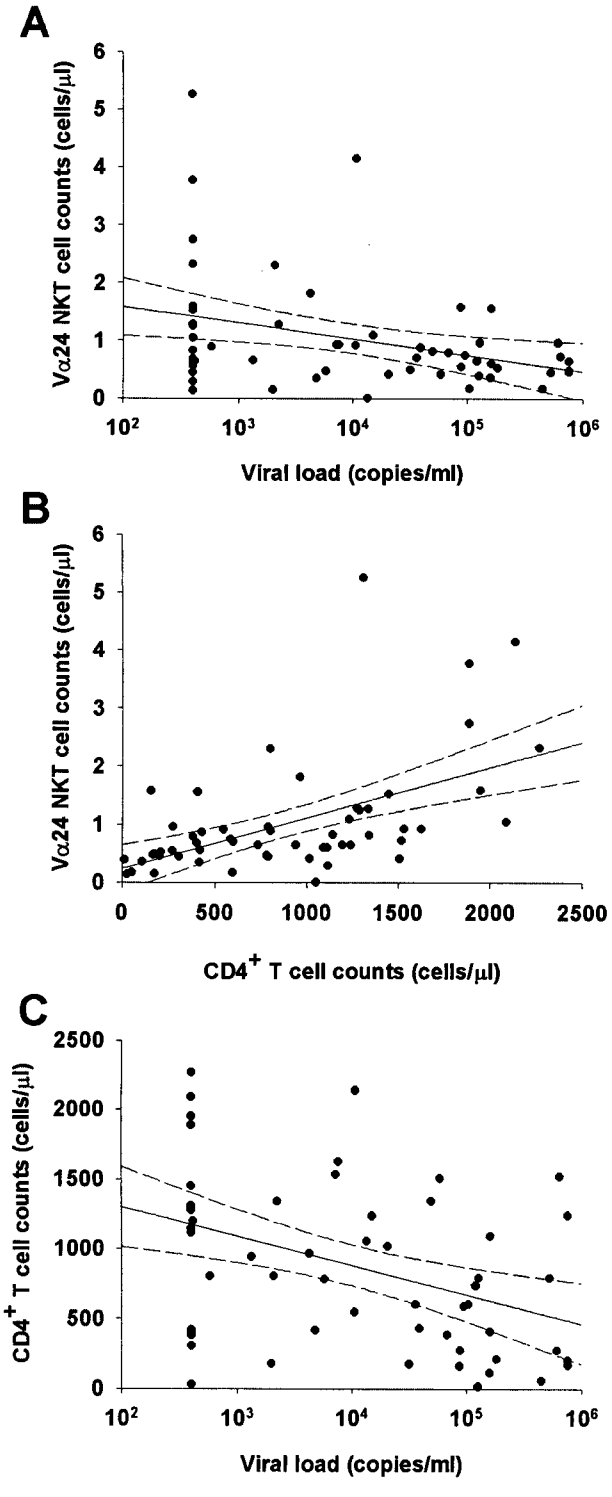

FIG. 4. The number of circulating $Vα24^+$ $CD161^+$ NKT cells correlates with viral load and $CD4^+$ T-cell counts in HIV-infected patients. (A) Levels of Vα24 NKT cells in 57 HIV-infected pediatric patients in relation to HIV viral load. The solid line shows linear regression of all data in the plot by the least-squares method ($R = 0.33$; $P = 0.012$). (B) Vα24 NKT cell numbers in relation to $CD4^+$ T-cell count with linear regression of all data in the plot ($R = 0.53$; $P < 0.001$). (C) $CD4^+$ T-cell counts in relation to HIV viral load ($R = 0.41$; $P = 0.002$). Dashed lines represent 95% confidence intervals.

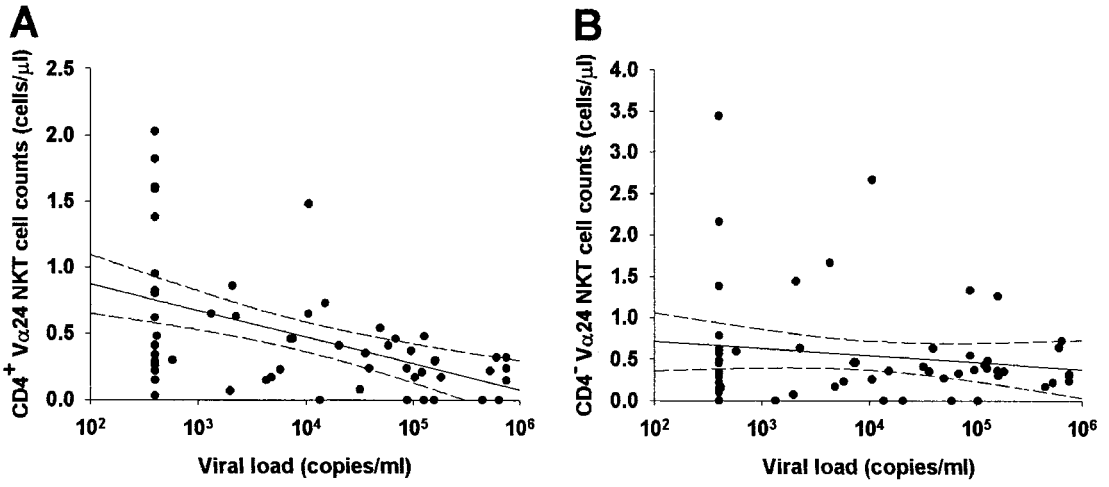

FIG. 5. Increase in HIV load correlates with loss of $CD4^+$ NKT cells. (A) The $CD4^+$ subpopulation of NKT cells plotted against HIV load. The solid line represents linear regression ($R = 0.49$; $P < 0.001$). (B) $CD4^-$ NKT cells plotted against viral load ($R = 0.14$; $P = 0.28$). Dashed lines represent 95% confidence intervals.

pronounced (Fig. 6C). These findings show that $CD4^+$ NKT cells are very vulnerable to HIV-induced depletion in vitro, while $CD4^-$ NKT cells appear resistant. Furthermore, the data suggest that $CD4^+$ NKT cells are more susceptible to HIV-induced depletion than regular $CD4^+$ T cells. This is particularly striking with CCR5-using virus, which predominates over most of the course of HIV-1 infection in humans.

DISCUSSION

Here we report that the innate CD1d-restricted Vα24 NKT cells are reduced in HIV-infected patients with uncontrolled viremia and marked $CD4^+$ T-cell depletion. The Vα24 NKT cells can be subdivided into $CD4^+$ or $CD4^-$ subsets that differ in their expression of CD62L and CD11a homing receptors. Interestingly, HIV has an asymmetric impact on the two subsets such that $CD4^+$ NKT cells are lost in patients who have high viral loads, whereas $CD4^-$ NKT cells are relatively less affected. Also, $CD4^+$ NKT cells are more vulnerable to HIV than regular $CD4^+$ T cells, as indicated by more-frequent expression of the HIV coreceptor CCR5 and a much more pronounced loss of these cells in PBMC cultures infected with HIV in vitro. The susceptibility of $CD4^+$ NKT cells to HIV is particularly clear when cultures are infected with virus that uses CCR5. The sensitivity of $CD4^+$ NKT cells to HIV infection is further supported by the observation that the inverse correlation between the number of $CD4^+$ NKT cells and viral load in vivo is somewhat stronger than the relationship between overall $CD4^+$ T-cell counts and viral load. These data also suggest that $CD4^+$ NKT cells are preferentially targeted during acute and early HIV infection, since CCR5-using strains of virus are responsible for transmission in most cases and dominate in early infection (2, 5).

We find that a majority of $CD4^+$ NKT cells express the CD62L receptor for homing to lymph nodes, while the $CD4^-$ subset expresses little CD62L but more CD11a for infiltration into tissues. Our results therefore indicate that the NKT cells that survey the secondary lymphoid organs are lost in individuals with high HIV load. This likely disrupts the interaction between the NKT-cell compartment and DCs, which is important for the immunoregulatory function of NKT cells (19, 21), because the lymph nodes are sites of interaction between lymphocytes and DCs (31). Also, $CD4^+$ and $CD4^-$ NKT cells in humans differ in their cytokine expression patterns ($CD4^+$ NKT cells produce up to five times more IL-4 [7, 41]), and the loss of $CD4^+$ NKT cells may therefore lead to a shift in the cytokines produced by the NKT-cell compartment. Interestingly, this is reportedly the case in autoimmune diabetes, in which NKT-cell clones fail to produce IL-4 (44). Thus, selective loss of the $CD4^+$ subpopulation of NKT cells may have significant effects on the function of the NKT-cell compartment in HIV-infected individuals.

Loss of $CD4^+$ NKT cells and the consequent skewing of the NKT-cell compartment in HIV-infected individuals could contribute to autoimmunity and autoimmune-like conditions, as well as impaired tumor immunity in these individuals. Autoantibodies of the IgM isotype against DNA, as well as against endogenous proteins, occur in both HIV-infected humans and in an HIV transgenic mouse model (30, 32). There is also an increase in circulating cardiac-specific autoantibodies in HIV-positive individuals, supporting a role for autoimmunity in the pathogenesis of HIV-related heart muscle disease (6). With regard to tumor immunity, deficient immune-control of tumors most probably contributes to the increased prevalence of Kaposi's sarcoma and non-Hodgkin's lymphoma in AIDS patients (14).

Are the $CD4^-$ NKT cells that persist in the face of high viral load useful, and can they be artificially activated by vaccination to augment host immunity in these patients? These cells are potent producers of cytokines and have profound regulatory effects despite their low numbers in circulation (13). They may also be considerably more numerous in organs, such as the liver (9). αGalCer activates a large fraction of Vα24 NKT cells both in vitro and in vivo and could potentially be used to activate and expand NKT cells in HIV patients. Data obtained in mouse models suggest that αGalCer may be used to treat

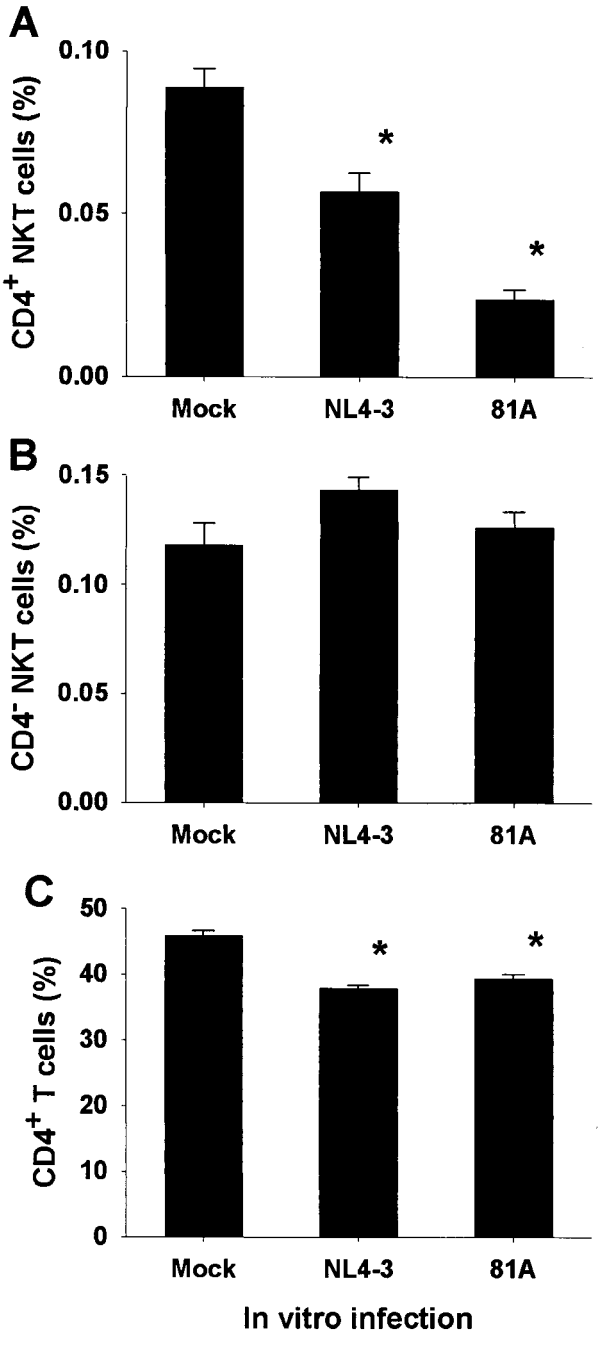

FIG. 6. $CD4^+$ NKT cells are lost from HIV-infected cultures in vitro. PBMCs from a healthy subject were activated by anti-CD3 stimulation and infected with the HIV strains 81A and NL4-3 or mock infected. Subpopulations of cells were determined 3 and 5 days after infection by flow cytometry. Data from day 5, in triplicate, are shown. (A) $CD4^+$ subset of NKT cells. (B) $CD4^-$ subset of NKT cells. (C) Regular $CD4^+$ T cells. Data are expressed as a percentage of all lymphocytes in culture. One representative experiment out of three experiments is shown. A star indicates that the difference between the HIV-infected sample and the mock-infected sample is significant as determined by the *t* test ($P < 0.05$).

hepatitis B virus infection and liver metastases (20, 25), suggesting that activation of NKT cells may benefit HIV patients with liver disease. In relation to autoimmune-like conditions in HIV patients, it is interesting that treatment of nonobese diabetic mice with αGalCer can prevent the development of diabetes (17, 34).

Glycolipid antigens from mycobacteria are presented by CD1a, CD1b, and CD1c molecules and are recognized by αβ T cells (39), and such T-cell responses occur in HIV-infected patients (15). Although there is little evidence for Vα24 NKT-cell recognition of mycobacterial antigens presented by CD1d in humans, bacillus Calmette-Guérin vaccination in mice can activate the homologous murine Vα14 NKT cells (10). Opportunistic mycobacterial infections occur in HIV patients, and one could speculate that Vα24 NKT-cell expansion might occur in that setting. Alternatively, opportunistic mycobacterial infection could result secondary to depletion of $CD4^+$ NKT cells. We have observed disseminated or pulmonary tuberculosis in six patients and disseminated *Mycobacterium avium-Mycobacterium intracellulare* in two patients in the cohort, and $CD4^+$ NKT cells were lower in these patients compared to patients with no known opportunistic infection, although the difference was not statistically significant (data not shown). However, the possible relationship between opportunistic infections and loss of $CD4^+$ NKT cells is interesting and needs to be further investigated.

In this work, we have addressed the putative impact of HIV on the immunoregulatory Vα24 NKT-cell compartment. We demonstrate that HIV infection causes loss of $CD4^+$ Vα24 NKT cells, and results in a skewing of the NKT cells in the blood, and we propose that this may contribute to HIV pathogenesis given the important immunoregulatory functions of these cells. Subversion of innate immune functions by HIV probably plays an important role in HIV pathogenesis and in the development of AIDS.

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