Function of NKT cells, potential anti-HIV effector cells, are improved by beginning HAART during acute HIV-1 infection

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Abstract

NKT cells are a subset of lymphocytes that share features of T cells and NK cells and bridge the innate and adaptive immune responses. They are able to be infected by HIV, but their function in HIV-infected individuals is not known. NKT cell percentage and function was measured in individuals with acute HIV infection before and 1 year into highly active anti-retroviral therapy (HAART). This study demonstrates that percentages of both CD161+ NKT cells and CD161+, CD4+ NKT cells decline within the first few months after HIV-1 infection, but initiating therapy during the acute infection period can prevent a further decline in these NKT cell subsets during the first year. NKT cell function is also impaired during early HIV infection, but significantly improved by effective treatment with HAART. Finally, preservation of NKT cell function may be important in HIV-infected individuals, as NKT cells display an anti-HIV-1 activity in vitro, mediated by IFN-γ secretion.

Introduction

NKT cells are a subset of lymphocytes possessing features of NK cells and αβ T cells that play a key role in the innate immune response. They possess a semi-invariant TCR and are CD1d restricted (1, 2). Upon stimulation, their rapid production of large quantities of both Th type 1 and type 2 cytokines permits them to bridge the innate and adaptive immune responses by activating NK cells, T cells, B cells and dendritic cells (3–8). NKT cells play a role in cancer (9–12) and autoimmune diseases (13, 14). They may also confer protection against viral, bacterial, fungal and parasitic infections (15–20).

NKT cells have been studied in the context of HIV-1 infection only recently. In 2002, Van der Vliet et al. (21) demonstrated that the percentage of NKT cells declined progressively over 5 years of infection. An additional cross-sectional study revealed that the percentage of NKT cells declined progressively over 5 years of infection. An additional cross-sectional study revealed that the percentage of NKT cells declined regardless of whether subjects were receiving highly active anti-retroviral therapy (HAART). While this suggests that suppression of HIV-1 replication with HAART may not prevent NKT cell decline, it can be argued that initiating therapy early in infection may preserve overall immune function. Early initiation of HAART has been shown both to preserve HIV-specific immune responses and to permit a faster and broader reconstitution of conventional CD4+ T cells (22, 23). It has been shown that HIV-1 infects, and preferentially depletes, CD4+ NKT cells in both humans and macaques (24–26). Both CD4+ and CD4− NKT cells can be restored in HIV+ patients receiving a combination of IL-2 with HAART (27).

The effect of HIV-1 infection on NKT cell function has not yet been characterized. HIV-1 infection can cause functional defects of other innate immune cells, including NK cells and γδ T cells, which can be partially restored by HAART (28–37). Different NKT cells subsets, characterized by differing surface markers, have distinct patterns of cytokine secretion and effector function (38, 39). Based on the fact that Vα24+, CD161+ NKT cells are inversely proportional to HIV viral load (25), we hypothesized that the percentage and function of this NKT cell subset may be impaired and may be preserved or restored by initiation of HAART without additional cytokine therapy during the acute infection period.

To test these hypotheses, we studied a cohort of 75 acutely HIV-1-infected subjects. We determined whether early initiation of anti-retroviral therapy can prevent the decline in NKT cell percentage and assessed the effect of HIV-1 infection on NKT cell function. In order to assess the
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relevance of preservation of NKT cell function during HIV-1 infection, we examined whether NKT cells possess anti-HIV-1 activity in vitro.

Methods

Study design and subjects

Analyses were performed on frozen PBMCs from 75 subjects identified and treated during the acute HIV infection period under the National Institutes of Health-sponsored Acute Infection and Early Disease Research Program network. Blood samples were collected at baseline, before initiating HAART, and 12 months into therapy. Inclusion criteria included acute HIV-1 infection, determined by either negative HIV-1 ELISA or negative detuned assay (Blood Centers of the Pacific), initiation of HAART within 1 month of presentation and undetectable plasma HIV-1 RNA 1 year into therapy. Studies were approved by the Rockefeller University Institutional Review Board. All subjects provided written informed consent. Seventy-five HIV-1-uninfected PBMC donors served as controls. PBMCs were isolated from whole blood collected in EDTA by Ficoll-Hypaque density gradient separation, washed with PBS and frozen at −150°C. HIV-1 RNA level was quantified using the Cobas Amplicor system. The New York City Department of Health measured total CD4+ T-cell count.

Quantification of the percentage of CD161+ NKT cells and CD161+, CD4+ NKT cells by FACS analysis

PBMCs (2 × 10^6) were stained with Vα24-FITC (Immunotech clone C15, Fullerton, CA, USA), CD161-PE (Becton Dickinson clone DX12, San Jose, CA, USA) and CD4-CyChrome (BDIS clone RPA-T4, San Jose, CA, USA) in FACS buffer on ice for 45 min, washed, re-suspended and fixed in PFA. Samples were run in duplicate on a BD FACS Calibur and analyzed using CellQuest. A minimum of 500,000 events were analyzed in each sample. Lymphocytes were identified by forward and side scatter. CD161+ NKT cells were defined as percentage of lymphocytes positive for both Vα24 and CD161. Percentage of CD161+, CD4+ NKT cells was determined by quantifying NKT cells expressing the CD4 cell-surface marker. Absolute NKT cell number was defined as total white blood cell count, multiplied by percentage of lymphocytes, multiplied by percentage of NKT cells.

Quantification of the overlap of Vα24+ CD161+ and Vα24+ Vβ11+ lymphocytes by FACS analysis

Initial analyses to determine the NKT cell percentage in HIV-infected individuals were performed using the phenotypic definition of co-expression of CD161 and Vα24 on lymphocytes. The CD161 marker was chosen based on several early studies, including the study by Sandberg et al. (25) demonstrating that Vα24+, CD161+ NKT cells are depleted in HIV with this decrease being inversely proportional to HIV plasma viral load. However, subsequent to initiation of the present study, the definition of type I NKT cells has been amended to include only those lymphocytes that co-express Vα24 and Vβ11. We therefore compared expression of Vα24+ CD161+ with Vα24+ Vβ11+ on the lymphocytes of the 10 HIV-infected individuals on whose lymphocytes were subsequently utilized for functional analyses to ensure these two cell subsets were consistent. PBMCs from a subset of 10 HIV-1-infected subjects with high NKT cell levels were stained with Vα24-PE (Immunotech clone C15), Vβ11-FITC (Immunotech clone C21, Fullerton) and CD161-APC (Becton Dickinson clone DX12, San Jose) to assess the overlap of the Vα24+ CD161+ and Vα24+ Vβ11+ populations after gating on lymphocytes. A minimum of 500,000 lymphocytes were analyzed from each sample. (Supplementary Figure 1, available at International Immunology Online).

Assessment of NKT cell function using an immobilized, α-galactosyl ceramide-loaded, CD1d:lg dimeric protein

Frozen PBMCs from five HIV-1-infected subjects were allowed to recover overnight in culture media + IL-2 (10 IU ml⁻¹) and then depleted of B cells (identified by CD19 expression) and CD8+ T cells with Dynal® magnetic beads (Invitrogen, Carlsbad, CA, USA) to enrich the percentage of NKT cells. Enriched cells were plated at 4 × 10^5 cells per well in a 96-well round-bottom plate, pre-coated with 20 μg ml⁻¹ of a divalent human CD1d-IgG1, fusion protein (Becton Dickinson) loaded with 20 μM excess α-galactosyl ceramide (αGalCer), a specific NKT cell ligand. Enriched cells plated with CD1d:lg dimeric protein in the absence of αGalCer were used as a negative control. In parallel, 5 × 10^6 cells from an NKT cell line were stimulated in the same manner. These cells were generated in our laboratory by isolating Vα24+, CD161+ cells from human PBMCs and co-culturing with irradiated autologous monocyte-derived dendritic cells pulsed with αGalCer. After 48 h of stimulation supernatant was harvested for measurement of IFN-γ and IL-4 via ELISA (eBioscience, San Diego, CA, USA). All wells and samples were run in duplicate.

Determination of anti-HIV activity of NKT cells

Frozen PBMCs from healthy donors were recovered and depleted of CD8+ T cells as above. CD8+ T cells were positively selected for using Detachabead (Dynal, Carlsbad, CA, USA). The CD8+ cells were further enriched for NKT cells by B-cell depletion as above. CD8+ T cells and NKT-enriched cells were plated in 96-well plates at 1 × 10^5 cells per well. CD8+ cells were then stimulated with PHA, while NKT-enriched cells were stimulated with 20 μg ml⁻¹ of the divalent human CD1d:lgG1 dimeric protein, either alone or loaded with 20 μM excess αGalCer as above. NKT cell lines were plated at 5 × 10^4 cells per well and stimulated in the same manner. Some enriched PBMCs and NKT cell lines stimulated with αGalCer-loaded CD1d:lg dimeric protein were also co-incubated with 2.5 μg ml⁻¹ anti-human IFN-γ antibody (R&D Systems, Minneapolis, MN, USA). Supernatants were harvested after 48 h of stimulation. Supernatant from PHA-stimulated CD8+ T cells was used as a positive control for suppression of virus replication. CD4+ cells were isolated from PBMCs from healthy donors by positive bead selection (Dynal). After recovery overnight in culture medium containing IL-2 (10 IU ml⁻¹), cells were plated at 2.5 × 10^5 cells per well and stimulated with PHA (10 μg ml⁻¹) for 24 h. Cells were washed and infected with 100 μl of HIVNL4.3, multiplicity of infection = 0.5. The T-cell tropic, CXCR4-using lab adapted HIV strain NL4.3, was
chosen to minimize destruction of NKT cells as NKT cells are more susceptible to R5-tropic HIV than X4-tropic HIV (24). After 16 h of infection, cells were washed five times and then incubated with 200 μl medium or supernatant as described above. Supernatants were harvested for determination of p24 antigen. All wells were run in duplicate, and the experiment was repeated on three separate occasions. The p24 antigen concentration was quantified by ELISA (Beckman Coulter, Fullerton, CA, USA).

**Statistical analysis**

Values are expressed as mean ± SD. Statistical comparisons of NKT cell percentage and cytokine secretion made between uninfected controls and HIV-1-infected subjects at baseline samples were performed with a two-sample, equal variance t-test. Comparisons of samples from the HIV-infected subjects at baseline and month 12 were performed using a paired t-test.

**Results**

**Subject cohort characteristics**

Table 1 describes the clinical characteristics of 75 HIV-1-infected subjects identified during the acute infection period. At baseline, the mean CD4+ T-cell count of the subjects was 493 ± 241 cells per mm³, and the mean plasma HIV-1 RNA level was 6.4 ± 7.0 log_{10} copies per ml. All subjects responded to 12 months of HAART, with copies of HIV-1 RNA in their plasma falling to undetectable levels (<50 copies per ml). This was associated with an increase in the CD4+ T-cell count to 776 ± 254 cells per mm³, P < 0.0001 (Table 1). None of the HIV-infected subjects had co-morbid conditions such as hepatitis infection. Seventy-five uninfected healthy donors were served as controls (43/75 male, age 18–62, mean 44 years). Although Sandberg et al. (40) described that females have a higher percentage of NKT cells in their series of 75 volunteers, we found no significant differences in NKT cells based on gender within our 75 healthy controls (P = 0.53, data not shown).

**Definition of NKT cells**

The square of the Pearson product moment correlation coefficient (R²) for the two lymphocyte subsets was 0.96. The average percentage of Vα24+ CD161+ cells expressing Vβ11 was 89.5% (range 83.3–93.7%). The average percentage of Vα24+Vβ11+ cells expressing CD161 was 94.9% (range 87.0–98.4%). (Supplementary Table 1, available at International Immunology Online).

**HIV-1 infection is associated with a decrease in both CD161+ NKT cells and CD161+, CD4+ NKT cells**

We compared both the total NKT cell percentage as well as the percentage of NKT cells expressing the CD4R in the peripheral blood of 75 subjects with acute HIV-1 infection versus 75 uninfected control subjects. Flow cytometry was used to quantify lymphocytes expressing both CD161, a cell-surface marker expressed on a subset of NKT cells and Vα24, part of the semi-invariant human NKT cell receptor, with or without expression of CD4 (Fig. 1A and B). As expected, there was a high variability of NKT cells between individuals. Despite this, when compared with uninfected controls, subjects studied during the acute infection period had a significantly lower percentage of CD161+ NKT cells (0.07 ± 0.12% versus 0.04 ± 0.05%, P = 0.032) and CD161+, CD4+ NKT cells (57 ± 28% versus 44 ± 23%, P = 0.003). (Fig. 1C and D) CD161+, Vα24+ cells also declined significantly (20.1 ± 6% versus 17.6 ± 6%, P = 0.02), reflecting a drop in either NK cells and/or CD161+ Vα24+ T cells, whereas CD161−, Vα24+ cells did not significantly differ (0.06 ± 0.05% versus 0.04 ± 0.03%, P = 0.66). There was no significant difference in NKT cell percentage between male and female uninfected controls.

**CD161+ NKT cell and CD161+, CD4+ NKT cell percentages are preserved after 1 year of HAART when initiated during acute infection**

We examined the effect of initiating HAART during the acute infection period on the percentage of both CD161+ NKT cells and CD161+, CD4+ NKT cells in the blood of HIV-infected subjects. Lymphocytes expressing CD161 and Vα24, with and without expression of CD4, were quantified using flow cytometry at baseline before HAART and 12 months into therapy (Fig. 1A and B). We found that a further decline in the percentage of CD161+ NKT cells was prevented by 1 year of therapy (0.04 ± 0.05% versus 0.05 ± 0.04%, P = 0.083). The percentage of CD161+ NKT cells expressing CD4 was not significantly different at baseline and after 1 year of therapy (44 ± 23% versus 44 ± 23%, P = 0.85) (Fig. 1C and D). There was no significant difference in the absolute number of NKT cells at these same time points (121 ± 378 cells per mm³ versus 93 ± 85 cells per mm³, P = 0.53) or the absolute number of CD4+ NKT cells (56 ± 276 cells per mm³ versus 30 ± 24 cells per mm³, P = 0.41). CD161+, Vα24+ cells did not change between months 0 and 12 (17.6 ± 6% versus 16.7 ± 6%, P = 0.16), whereas CD161−, Vα24+ increased, paralleling general T-cell reconstitution (004 ± 0.03% versus 0.05 ± 0.03%, P = 0.002). No significant correlations were observed between the percentage of either, total or CD4+ NKT cells and the plasma levels of HIV-1 RNA, or with total CD4+ or CD8+ T cell count, either at baseline or after 12 months of HAART.
IFN-γ and IL-4 secretion from glycolipid-stimulated, NKT cell-enriched PBMCs of HIV-1-infected subjects is increased after HIV-1 suppressive therapy

We developed a novel assay to assess human NKT cell function in response to stimulation with αGalCer. PBMCs from HIV-1-uninfected controls or from HIV-1-infected subjects were first enriched for NKT cells by depletion of both CD8+ T cells and B cells. The efficiency of each depletion was greater than 99% by flow cytometric analysis. Stimulation of these NKT cell-enriched PBMCs from HIV-uninfected controls or from HIV-infected subjects with increasing concentrations of unloaded CD1d:Ig dimeric protein did not result in detectable IFN-γ secretion. However, loading αGalCer onto increasing concentrations of CD1d:Ig dimeric protein caused a dose-dependent increase in IFN-γ (Fig. 2A). We also observed the αGalCer-specific, dose-dependent secretion of IL-4 in culture (data not shown). This approach has been validated in at least 28 healthy donors to date. The levels of cytokine secretion by NKT cells stimulated with immobilized αGalCer-loaded CD1d dimer were comparable to the levels when using an immobilized anti-Vα24 antibody, which served as a positive control (Fig. 2A). Depletion of Vα24+ cells completely abolished activation (Fig. 2B). We observed the same cytokine secretion profile when an NKT cell line was used as the source of responding cells (Fig. 2C). Together, these results confirm the specificity of NKT cell-restricted stimulation by immobilized αGalCer-loaded CD1d:Ig dimeric protein. There was no IFN-γ secretion from flow-sorted Vα24−, CD8+ B cells, Vα24− CD19+ B cells, and Vα24− CD161+ NK cells.

PBMCs from a subset of five HIV-infected subjects who initiated HAART during acute infection were examined to test whether HIV-1 infection impairs NKT cell function and whether function improves with anti-retroviral therapy. Fig. 3(A) and (B) depict the levels of IFN-γ and IL-4, respectively, secreted by NKT cell-enriched PBMCs from five HIV-1-infected individuals, before (baseline) and 1 year into HAART. In all subjects, both IFN-γ and IL-4 cytokine secretion was significantly greater 1 year after initiation of HAART (P = 0.0346, P = 0.0004, respectively, Fig. 4C and D). IFN-γ and IL-4 secretion from HIV-1-infected donors at baseline was also significantly lower than uninfected controls (P = 0.0272, P = 0.0473), but by month 12, the difference in secretion from uninfected controls was not significant (P = 0.9419, P = 0.4464, respectively) (Fig. 3C and D). IFN-γ and IL-4 secretion in response to stimulation with unloaded CD1d:Ig dimeric protein was modest, <10 pg ml⁻¹ at both time points. Absolute NKT cell number in each well before and after 12 months of HAART were, respectively, 257 versus 302, 219 versus 213, 268 versus 496, 509 versus 194 and 436 versus 299. There was no significant overall difference in absolute NKT cell number across all five donors (337 ± 127 versus 301 ± 120, P = 0.70). Therefore, the increased cytokine secretion at the post-HAART time point was not due to an increase in absolute NKT cell number.

NKT cells display anti-HIV activity in vitro

In three separate experiments, supernatant from CD1d:Ig dimeric protein, alone, did not affect HIV-1 replication in NKT cell-enriched PBMCs as measured by p24 antigen ELISA. In contrast, supernatants from NKT cell-enriched PBMCs stimulated by αGalCer-loaded CD1d:Ig dimeric protein strongly suppressed HIV-1 replication to levels comparable to that suppressed by the supernatant of PHA-activated CD8+ T cells (Fig. 4A). The viability of the cells remained constant across all wells over the course of infection. We also collected supernatants from an NKT cell line. We found that these supernatants inhibited HIV-1 replication more strongly than those from activated NKT cell-enriched populations and from activated CD8+ T cells (Fig. 4B).

In order to determine the role of IFN-γ in mediating the anti-HIV effect of the supernatant obtained from activated NKT cell-enriched PBMCs as measured by p24 antigen ELISA.
IFN-γ is a key mediator of the anti-HIV activity of enriched NKT cells. When we used the supernatants from an NKT cell line activated by αGalCer-loaded CD1d:Ig dimeric protein, we also observed that the anti-IFN-γ antibody completely abrogated the suppressive effect caused by the supernatants of activated NKT cell lines (Fig. 4B), confirming the major role of IFN-γ in mediating the anti-HIV effect displayed by NKT cells.

Discussion

Here we report the effect of HIV-1 infection on NKT cells, one arm of the innate immune system. Previous reports indicate that HIV-1 infection causes a dramatic decline in Va24+ Vb11+ NKT cells in the first year and that treatment with HAART has no effect on NKT cell depletion (21). It has been shown that HIV-1 infects, and preferentially depletes, CD4+ NKT cells (24–27, 41). We studied the effect of beginning anti-retroviral therapy within 3–4 months after initial HIV-1 infection to determine whether this would have an impact on preserving the NKT cell population and its function. In theory, rapid and early reduction of viral replication may minimize the destruction of CD4+ innate immune cells by HIV-1. The diagnosis of acute HIV infection is relatively rare, and many with high initial HIV plasma RNA levels opt to begin therapy. Because the ideal control group of acutely infected individuals with no HAART for 1 year is difficult to study, comparisons were made to the longitudinal cohort study described above (21).

The pre-treatment percentages of both CD161+ NKT cells and CD161+, CD4+ NKT cells were significantly reduced with respect to the uninfected controls, indicating that this depletion occurs quite early in infection, likely due to high levels of viral replication. Interestingly, the quantitative percentages of both the CD161+ NKT cells and CD161+, CD4+ NKT cells were preserved for up to 1 year after HAART therapy. Thus, although HAART cannot restore the percentages of, either, CD161+ NKT cells, or CD161+, CD4+ NKT cells to the levels observed in uninfected controls, it can, at least, prevent their percentages from declining further. Other NKT cell subsets may behave differently (38, 39), although in this study there is a high overlap between Va24+CD161 and Va24+Vb11+ lymphocytes. Similarly, NKT cells in the blood may not reflect activity in other compartments. Another possible caveat is that increased cellular activation induced by HIV infection may down-regulate expression of the invariant TCR, thus precluding detection by cell-surface staining.

However, given that NKT cells, particularly CD4+ NKT cells, are readily infected by HIV-1 (24–26, 41), and that viral burden is high in early infection, the most likely mechanism for depletion is direct infection by HIV-1.

One of the unique and compelling findings from these experiments is that the qualitative function of NKT cells significantly improved with HAART in all subjects. This phenomenon occurred irrespective of change in total NKT cell percentage, indicating this was not merely due to reconstitution of NKT cells. Furthermore, levels of cytokine secretion after 12 months of HAART were no different than in uninfected controls, indicating that anti-retroviral therapy may be able to restore function to pre-infection levels in those

Fig. 2. Use of CD1d dimer loaded with αGalCer to assess NKT cell function from uninfected human PBMCs. (A) Secretion of IFN-γ was measured from supernatant of NKT cell-enriched PBMCs incubated in wells with CD1d:Ig dimeric protein with or without antigen. The first column represents an uncoated well as a negative control. The second bar represents a well pre-coated with anti-human Va24 antibody as a positive control. The subsequent three bars represent wells coated with increasing concentrations of CD1d:Ig dimeric protein without antigen, whereas the final three bars represent wells coated with increasing concentrations of CD1d:Ig dimeric protein loaded with αGalCer. (B) Depletion of Va24+ cells from PBMCs in two separate donors resulted in abrogation of IFN-γ secretion from αGalCer-loaded CD1d-coated wells. (C) A similar result was obtained from stimulation of a purified NKT cell line with αGalCer-loaded CD1d.
subjects treated early in infection. Of note, as these cells were originally collected in EDTA, it is possible that this assay may reflect an underestimate of total NKT cell function. However, this would not influence the change in function before and after anti-retroviral therapy.

The mechanism for this augmentation remains to be determined but may be multifactorial. CD1d is down-regulated on CD14+ monocytes in HIV infection and restored by HAART (42). Differences in responsiveness to αGalCer may therefore be due to altered presentation by antigen-presenting cells. It is also plausible to speculate that, like CD4+ T cells, NKT cell function is compromised in early HIV infection due to increased general immune activation and downstream apoptosis. Finally, given that NKT cells rapidly activate NK cells (43), it is likely that NK cells are in part responsible for the IFN-γ secretion detected in the supernatant. The initial reduced IFN-γ secretion may therefore also reflect loss of NK function in early HIV infection, which has been shown previously (28–33). However, the effect is dependent upon the specific primary stimulation of NKT cells via αGalCer, as demonstrated in this manuscript by the observations that activation with immobilized anti-Vα24 resulted in IFN-γ secretion in PBMCs, that the depletion of Vα24+ cells from PBMCs resulted in abrogation of IFN-γ secreted by PBMCs activated with αGalCer and that αGalCer elicited IFN-γ secretion in a pure NKT cell line.

Immobile, monomeric CD1d has been shown to stimulate murine NKT hybridomas (44). We were able to determine NKT cell function in these patients due to the development of a reliable assay to stimulate the production of cytokines, IFN-γ and IL-4 secreted by human NKT cells present among PBMCs.

In light of the broad range of activity of NKT cells against other pathogens, we hypothesized that NKT cells may play a role in suppressing HIV-1 replication. Although the direct role of NKT cells against HIV had not yet been studied, differing mechanisms had been postulated. NKT cells might either activate T cells to make them more prone to HIV infection or secrete soluble factors to fight HIV infection (45). Our studies indicate that when stimulated by glycolipid, the latter is likely the case as supernatants from a stimulated population of PBMCs enriched for NKT cells caused potent suppression of HIV-1 replication. The NKT cell-enriched population was stimulated in a ligand-specific, CD1d-restricted fashion, indicating that initial responding cells were strictly NKT cells. However, the effector mechanism for this anti-HIV

Fig. 3. NKT cell function is preserved in acutely HIV-1-infected individuals after HAART. Secretion of (A) IFN-γ and (B) IL4 was measured from NKT cell-enriched PBMCs after stimulation with cross-linked CD1d dimer loaded with αGalCer. In both panels, open bars represent HIV-1-infected subjects at pre-treatment baseline and shaded bars represent the same patients after 1 year of HAART. Solid bars represent five HIV-1-uninfected control subjects. Statistical analysis reveals that IFN-γ (C) and IL4 (D) secretion is significantly increased in subjects after 1 year of HAART to levels similar to HIV-uninfected controls, two-sample, equal variance t-test.
suppression may be multifactorial. It has been shown in other diseases that NKT cells facilitate anti-tumor activity by secondary activation of NK cells and other lymphocytes via cytokine activation (10–12). We, therefore, used supernatants from a purified NKT cell line to demonstrate that this anti-HIV effect is primarily caused by NKT cells. The addition of soluble anti-IFN-γ antibody abrogated the effect, indicating that the anti-HIV effect displayed by NKT cells is mediated by IFN-γ. As NKT cell lines secrete at least one log higher IFN-γ than NKT cell-enriched PBMCs (Fig. 2), this explains the fact that the NKT cell line suppressed HIV-1 replication more strongly (Fig. 4B). In addition, it is clear that the effect is not due to direct cell-mediated cytolysis, as only the supernatant was transferred, or due to apoptosis, as the cell viability was constant across all wells.

While the mechanism of the anti-HIV effect of NKT cells may be non-specific, the rapidity and magnitude of IFN-γ response ensures that NKT cells are key players in the initial host immune response. In addition, their role in preventing autoimmunity, protection against neoplasia and in fighting other viral, fungal and parasitic pathogens has been well established (9–20). Therefore, any decrement in NKT cell number or function may have important implications with regard to the health of HIV-1-infected subjects, who are disposed to develop infectious and neoplastic complications.

Further, since NKT cells decline with age (46–49), the aging of the HIV-1-infected population may result in a patient population with severe defects in NKT cell function. Preservation of NKT cell number and improvement of function may, therefore, become important in improved long-term prognosis. Overall, the present data provides support for the role of HAART in the preservation of NKT cell function in persons infected with HIV-1.

Supplementary Table 1 is available at International Immunology Online.

Supplementary data

Supplementary Figure 1 and Table 1 are available at International Immunology Online.

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Abbreviations

αGalCer, αgalactosyl ceramide EIA enzyme immunoassay HAART highly active anti-retroviral therapy
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