Virologically Suppressed HIV Patients Show Activation of NK Cells and Persistent Innate Immune Activation


*J Immunol* published online 27 June 2012
http://www.jimmunol.org/content/early/2012/06/27/jimmunol.1200458

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
Virologically Suppressed HIV Patients Show Activation of NK Cells and Persistent Innate Immune Activation


FcRγ is an ITAM-containing adaptor required for CD16 signaling and function in NK cells. We have previously shown that NK cells from HIV patients receiving combination antiretroviral therapy (cART) have decreased FcRγ expression, but the factors causing this are unknown. We conducted a cross-sectional study of cART-naïve viremic patients (ART⁺), virologically suppressed patients receiving cART (ART⁻), and HIV-uninfected controls. CD8⁺ T cells were activated, as assessed by CD38⁺HLA-DR⁺ expression, in ART⁺ patients (p = 0.0001), which was significantly reduced in ART⁻ patients (p = 0.0005). In contrast, CD38⁺HLA-DR⁺ NK cells were elevated in ART⁻ patients (p = 0.0001) but did not decrease in ART⁻ patients (p = 0.88). NK cells from both ART⁺ and ART⁻ patients showed high levels of spontaneous degranulation in ex vivo whole blood assays as well as decreased CD16 expression (p = 0.0001 and p = 0.0025, respectively), FcRγ mRNA (p < 0.0001 for both groups), FcRγ protein expression (p = 0.0016 and p < 0.0001, respectively), and CD16-dependent Syk phosphorylation (p = 0.0001 and p = 0.003, respectively). HIV-infected subjects showed alterations in NK activation, degranulation, CD16 expression and signaling, and elevated plasma markers of inflammation and macrophage activation, that is, neopterin and sCD14, which remained elevated in ART⁺ patients. Alterations in NK cell measures did not correlate with viral load or CD4 counts. These data show that in HIV patients who achieve viral suppression following cART, NK cell activation persists. This suggests that NK cells respond to factors different from those driving T cell activation, but which are associated with inflammation in HIV patients. The Journal of Immunology, 2012, 189: 000–000.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/$16.00

The Journal of Immunology

Published June 27, 2012, doi:10.4049/jimmunol.1200458

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1200458
can also associate with the TCR ζ-chain (TCRζ or CD247), a functional homolog of FcRγ (17). TCRζ can form heterodimers with FcRγ (18); however, at least in murine NK cells, these heterodimers do not appear to associate with CD16 or act as a CD16 chaperone (19).

We have previously shown that expression of FcRγ in NK cells is decreased in HIV-infected individuals receiving cART compared with HIV-uninfected controls (12). We hypothesized that HIV-associated immune activation causes downregulation of FcRγ in NK cells, leading to inhibition of ADCC signaling. In this study, we determine the relationship between NK activation, decreased FcRγ expression, CD16 surface expression, intracellular signaling, and, as a surrogate for ADCC degranulation activity, CD107a mobilization (16). We show that NK cells remain activated in viologically suppressed HIV-infected patients receiving cART and, as a surrogate for ADCC degranulation activity, CD107a mobilization.

Materials and Methods

Study groups

HIV-1-infected male patients were recruited following approval by the Ethics Committee of The Alfred Hospital. The inclusion criterion was: HIV Ab+, >18 y age. Exclusion criteria were: immunomodulatory therapy (IL-2, hydroxyurea or prednisolone, HIV therapeutic vaccine), hepatitis B virus infection, controls, self reporting as being healthy, were age- and sex-matched.

Cell phenotyping

Whole blood was incubated with the following Abs for 30 min, 22°C: CD3-FITC, CD4-PerCP-Cy5.5, CD8-V450, CD16-PE-Cy7, CD38-PE, HLA-DR-PerCP-Cy5.5, CD14-PE, CD16-PerCP-Cy5.5, or PerCP-Cy5.5, CD56-allophycocyanin, CD14-PE, CD16-PerCP-Cy5.5, or FITC (20 min, on ice), washed once, and resuspended in cold FACS buffer (PBS, 2 mM EDTA, 1% newborn calf serum [HyClone]). CD3+CD56+ NK cells were sorted and washed once in cold PBS-. Cells (2 × 10^6) from each population were lysed in 350 μL RNA lysis/binding buffer (100 mM Tris-HCl, 500 mM LiCl, 10 mM EDTA, 5 mM DTT, 1% LiDS). The remaining cells were lysed in 20–50 μL 2.5% Triton X-100–based lysis buffer and snap frozen in liquid nitrogen prior to protein analysis.

mRNA extraction and quantitative real-time PCR

RNA was purified using magnetic bead separation (30 μL RNA beads; GenoPrep) and reverse transcribed using a transcription first strand cDNA synthesis kit (Roche) according to the manufacturer’s protocol. Quantitative real-time PCR was performed using Roche FastStart Universal SYBR Green Master Mix and a Stratagene MX3000P instrument. Primers were designed to allow identical thermal profiles for each product (10 min at 95°C, 40 cycles of 20 s at 95°C, 20 s at 62°C, and 40 s at 72°C, and 1 min at 95°C). The following primer sets were used (including GenBank accession codes, available at http://www.ncbi.nlm.nih.gov/nuccore/): FcRγ, forward, 5'-GATTCCACAGGTGTGCTGACT-3'; reverse, 5'-CCTTCCGACT-TGATCTCTC-3' (accession no. M33196); TCRγ, forward, 5'-AGTTCCA-GCAGGCGCCAGAC-3'; reverse, 5'-ACAGGCTCTGTCGAGGGTC-3' (accession no. DQ072171); DAP12, forward, 5'-GATGGGAGACCT-CCTTTCAC-3'; reverse, 5'-ACATCCGACCTCTTGACACC-3' (accession no. AF019563); GAPDH, forward, 5'-AGTCCACTGGCGCCTTCCACC-3'; reverse, 5'-AGACAGTTGCTGGTGCAACAGAG-3' (accession no. AT340849); CD16, forward, 5'-TGAGGTGTCACAGCTGGAAG-3'; reverse, 5'-GGTTG-ACACTGCAAAACCTT-3' (accession no. NM_00127596). mRNA levels were quantified using the comparative threshold method, with GAPDH mRNA as internal standard.

Protein extraction and immunoblotting

Protein extracts were incubated for 20 min on ice, followed by 5 min at 37°C and then centrifuged at 20,800 × g 4°C for 10 min. Ten micrograms protein per sample was denatured at 95°C for 10 min and separated using 10% Bis-Tris Midi Gels (Invitrogen, catalog no. WG1202BOX) and NuPAGE MES SDS running buffer. Proteins were transferred onto a polyvinylidene difluoride membrane using an iBlot dry blotting system (Invitrogen) at 4°C overnight. After three washes in TBS plus 0.1% Tween 20, membranes were incubated with mouse anti-GAPDH (Santa Cruz Biotechnology) plus rabbit anti-FcRγ polyclonal serum (gift of Mark Hogarth, Burnet Institute) at 4°C overnight. After three washes with TBS plus 0.1% Tween 20, membranes were incubated with secondary Abs (goat anti-mouse Alexa Fluor 680 plus goat anti-rabbit Alexa Fluor 750; Molecular Probes) for 2 h at room temperature. Fluorescence was quantified using an Odyssey infrared imager (LI-COR Biosciences).

Measurement of soluble CD14, soluble CD163, and neopterin

Soluble (s)CD14, sCD163, and neopterin levels were measured in plasma using the Quantikine human sCD14 kit (R&D Systems, Minneapolis, MN), human sCD163 kit (IQ Products, Groningen, the Netherlands), and the neopterin screening EIA kit (Brahms, Berlin, Germany) according to the manufacturers’ instructions. Plasma samples were diluted 1:400 (sCD14), 1:500 (sCD163), and 1:4 (neopterin).

Measurement of plasma LPS and 16S genomic DNA in plasma

Plasma levels of LPS and genomic bacterial DNA encoding the 16S rDNA gene were measured as described in Kramski et al. (20).

Table I. Study population

<table>
<thead>
<tr>
<th>HIV−</th>
<th>HIV+</th>
<th>HIV+</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>n/a</td>
<td>Naive</td>
<td>cART</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Age, y</td>
<td>45.5 (28–64)</td>
<td>40 (26–63)</td>
<td>49 (28–65)</td>
</tr>
<tr>
<td>% CD4+ cells</td>
<td>60.55 (48.8–76.2)</td>
<td>31 (4.57–49.7)</td>
<td>34 (15.3–53.6)</td>
</tr>
<tr>
<td>CD4+ count</td>
<td>ND</td>
<td>427 (41–815)</td>
<td>600 (129–1199)</td>
</tr>
<tr>
<td>Nadir CD4+</td>
<td>379.5 (8–546)</td>
<td>208 (14–403)</td>
<td></td>
</tr>
<tr>
<td>Viral load</td>
<td>n/a</td>
<td>39,050 (&lt;50–&gt;100,000)</td>
<td>&lt;50 (&lt;50–1700)</td>
</tr>
<tr>
<td>Years on ART</td>
<td>n/a</td>
<td>n/a</td>
<td>11.5 (0.25–18)</td>
</tr>
<tr>
<td>Years of virologic suppression</td>
<td>n/a</td>
<td>n/a</td>
<td>2.9 (0.6–7.7)*</td>
</tr>
</tbody>
</table>

All values are given as medians and ranges.

*Data for n = 16 patients; 1 patient in the ART+ group had a detectable viral load (1700).

n/a, Not applicable.
FIGURE 1. NK cell activation phenotype and plasma markers of myeloid activation are not decreased in virologically suppressed HIV patients. (A) Representative dot plots showing gating of CD4 and CD8 T cells, CD56\textsuperscript{dim}CD16\textsuperscript{+} NK cells, and CD56\textsuperscript{bright}CD16\textsuperscript{−} NK cells and their coexpression of CD38 and HLA-DR in a HIV-uninfected control (HIV\textsuperscript{−}), cART-naive HIV-infected patient (ART\textsuperscript{−}), and virologically suppressed HIV-infected individual receiving cART (ART\textsuperscript{+}). Lymphocytes were initially gated in forward and side scatter plots and then events were analyzed on the dot plots as shown. (B) Comparison of the proportion of CD38/HLA-DR double-positive activated lymphocytes between HIV-uninfected controls and HIV-infected subjects stratified for treatment status. KW, Kruskal–Wallis p value. (C) Comparison of levels of plasma markers of myeloid cell activation between HIV-uninfected controls and HIV-infected subjects stratified for treatment status. Differences between groups were assessed using a Kruskal–Wallis test followed by serial Wilcoxon signed rank sum (post) tests. Statistical significance was assumed after adjusting for multiple comparisons when $p < 0.017$. 
FIGURE 2. NK cell degranulation and CD16 expression are not returned to control levels in virologically suppressed HIV patients. (A) CD56<sup>dim</sup>CD16<sup>+</sup> NK cells present within whole blood were gated as shown in the upper panels. Representative dot plots showing isotype control (middle panels) and CD107a (lower panels) staining within the CD3<sup>+</sup>CD56<sup>dim</sup> gate measured after 3 h incubation are shown for an HIV<sup>-</sup> (middle left and lower left panels; both plots showing the 5% isotype gate) and ART<sup>-</sup> individual (middle right and lower right panels; both plots showing the 5% isotype gate). (B) Comparison of the proportion of CD56<sup>dim</sup>CD16<sup>+</sup> NK cells undergoing degranulation in HIV-uninfected and HIV-infected subjects stratified for treatment status. Comparison of CD16 surface expression (C) and mRNA expression (D) on CD56<sup>dim</sup>CD16<sup>+</sup> NK cells. (E) Correlation of CD16 surface and mRNA expression in HIV-uninfected subjects (○), HIV-infected treatment naive (●), and HIV-infected patients currently receiving treatment (□). Differences between groups were assessed using a Kruskal–Wallis test followed by serial Wilcoxon signed rank sum (post) tests. Statistical significance was assumed after adjusting for multiple comparisons when $p < 0.017$. Correlations were assessed using a Spearman rank correlation; regression line and 95% confidence intervals are shown.
Results

NK cell activation is not reduced by ART

We recruited 33 male HIV patients of whom 16 were ART− and 17 were ART+ at enrollment. Twenty age-matched male HIV-uninfected subjects were recruited as controls (Table I). The ART+ patients received antiretroviral therapy for a median time of 11.5 y (range, 0.25–18 y) and had a sustained undetectable viral load for a median time of 2.9 y (range, 0.6–7.7 y) prior to recruitment. We measured cellular markers of immune activation (CD38 and HLA-DR coexpression) on CD56dim and CD56bright NK cells, as well as CD4 and CD8 T cells, using whole blood phenotyping assays (Fig. 1). Representative dot plots for a member of each study group are shown in Fig. 1A. As reported by others (21, 22), both CD4 and CD8 cells showed increased CD38/HLA-DR expression in ART+ patients (p = 0.0007 and p < 0.0001, respectively), which was significantly decreased in ART− patients (p = 0.01 and p = 0.0005, respectively) (Fig. 1B). CD4 T cell activation returned to baseline levels in ART+ patients compared with controls, but CD8 T cell activation did not (p = 0.0008). CD38/HLA-DR coexpression on CD56dimCD16+ NK cells, which in fact reflects changes in HLA-DR expression as NK cells constitutively express CD38, was also significantly increased in ART− patients (p = 0.0001). Surprisingly, and in contrast to T cells, HLA-DR coexpression on NK cells was not reduced following suppression of viremia with ART (p = 0.88).

We did not observe significantly altered expression of HLA-DR in the CD56dimCD16+ NK cell subpopulation (Kruskal–Wallis p = 0.12). We also did not observe significantly altered expression of HLA-DR in the CD56dimCD16− NK cell subpopulation (Kruskal–Wallis p = 0.14), which are already more highly activated as indicated by higher levels of HLA-DR expression compared with the CD56dimCD16+ NK cells (median HLA-DR+ cells, 16 [HIV−], 23 [ART−], and 34% [ART+]). We next measured markers of inflammation and myeloid cell activation in the patient groups. Concentrations of sCD14, neopterin, and sCD163 were elevated in plasma from both ART− and ART+ patients compared with controls (sCD14, p < 0.0001 for both ART− and ART+; neopterin, p < 0.0001 [ART−] and p = 0.019 [ART+]; sCD163, p < 0.0001 [ART−] and p = 0.0012 [ART+]; Fig. 1C). There was no significant difference in plasma sCD14 or neopterin between ART− and ART+ patients (p = 0.16 and p = 0.15, respectively). Thus, these plasma markers of inflammation and myeloid activation showed a similar pattern of increase as does NK cell activation but not T cell activation.

To further examine the activation status of NK cells in HIV patients, we measured their rate of degranulation in whole blood by measuring the mobilization of CD107a in the absence of exogenous targets. CD56dim NK cells in whole blood were gated as shown (Fig. 2A, upper panels), and transient surface expression of CD107a was measured after a 3-h incubation in the absence of added targets. To objectively compare measurements from all study groups, CD107a expression was obtained based on a 5% isotype gate as an internal standard (representative dot plots from HIV-infected and HIV-uninfected donors are shown in Fig. 2A,
CD16 surface expression is modulated in HIV-infected individuals in part by reduced transcription and expression of the chaperone FcRγ

Because CD16 surface expression was decreased in virologically suppressed HIV patients receiving cART, we next measured FcRγ expression, which acts as both a chaperone for CD16 surface expression and as an indispensable transducer of CD16 signaling. NK cells were sorted from patient blood by flow cytometry, and aliquots of purified cells were extracted for protein and RNA preparation. Expression of FcRγ mRNA (Fig. 3A) and protein (Fig. 3B) was significantly decreased in both ART− and ART+ patients compared with HIV-uninfected controls (mRNA, \( p < 0.0001 \) for both ART− and ART+; protein, \( p = 0.0016 \) for ART− and \( p < 0.0001 \) for ART+). Significantly, there was no difference in FcRγ expression between ART+ and ART− patients (mRNA, \( p = 0.14 \); protein, \( p = 0.72 \)). In HIV patients, FcRγ mRNA levels correlated strongly with FcRγ protein expression (Spearman rho = 0.81, \( p < 0.0001 \); Fig. 3C). These data demonstrate that FcRγ protein expression is reduced in HIV-infected individuals at a transcriptional level and is not restored by cART.

Because other ITAM-containing signaling adaptor proteins may potentially compensate for loss of FcRγ, we investigated expression of the adaptor proteins TCRζ and DAP12. Expression of TCRζ mRNA (Fig. 3D) and DAP12 (Fig. 3E) were decreased in both ART− and ART+ patients compared with HIV-uninfected controls (TCRζ, \( p = 0.0012 \) for ART− and \( p < 0.0001 \) for ART+; DAP12, \( p = 0.031 \) for ART− and \( p = 0.0013 \) for ART+). There was no difference in TCRζ or DAP12 expression between ART+ and ART− patients (TCRζ, \( p = 0.53 \); DAP12, \( p = 0.12 \)). To investigate the functional consequences of FcRγ and TCRζ downregulation, signal transduction through CD16 was measured using a whole blood Phosflow assay to quantify CD16-dependent ZAP70/Syk phosphorylation in NK cell subsets. As part of this assay, we cross-linked surface CD16 on CD56dimCD16+ NK cells in whole blood by sequentially adding anti-CD16 primary and goat anti-mouse secondary Abs, as described in detail in Lichtfuss et al. (16). CD56dim and CD56bright NK cells were gated and separately analyzed as shown in Fig. 4A (left and middle panels). In agree-

FIGURE 4. CD16 signaling is not returned to control levels in virologically suppressed HIV patients. (A) Representative histogram showing Phosflow analysis of CD16-dependent Syk/ZAP70 phosphorylation. The approximate position of lymphocytes (L), monocytes (M), and neutrophils (N) are shown in the forward versus side scatter profile (left panel). Events within the lymphocyte gate were plotted on a CD3 versus CD56 profile to define the CD56dim and CD56bright NK cell subsets (middle panel). Histogram overlay depicts isotype control Ab staining (dotted line), unstimulated baseline control staining (dashed line), Syk/ZAP70 phosphorylation in CD56dim cells from an HIV-uninfected subject (light gray histogram), and an ART− HIV-infected subject (dark gray histogram). The proportion of percentage positive cells was calculated in comparison with baseline using a population comparison algorithm. Comparison of Syk/ZAP70 phosphorylation measured 1.5 min after CD16 cross-linking (B) and at baseline (C). Differences between groups were assessed using a Kruskal–Wallis test followed by serial Wilcoxon signed rank-sum (post) tests. Statistical significance was assumed after adjusting for multiple comparisons when \( p < 0.017 \).
mement with our previous findings (16), ZAP70/Syk was not phosphorylated in response to CD16 cross-linking in the CD56<sup>dim</sup> population (data not shown). In contrast, rapid, transient phosphorylation of ZAP70/Syk was observed following CD16 cross-linking in CD56<sup>dim</sup> NK cells, peaking after 1.5 min (16). Compared to HIV-uninfected individuals, CD56<sup>dim</sup> NK cells from HIV-infected individuals showed reduced ability to phosphorylate Zap70/Syk following CD16 cross-linking (exemplified for one HIV-uninfected and one cART-naïve, HIV-infected donor in Fig. 4A, right panel). Phosphorylation was reduced in both patient groups stratified by treatment status, when compared with HIV-uninfected individuals (p = 0.0001 [ART<sup>+</sup>], p = 0.003 [ART<sup>-</sup>]; Fig. 4B). NK cells from ART<sup>+</sup> patients induced significantly more CD16-dependent ZAP70/Syk phosphorylation than those from ART<sup>-</sup> patients (p = 0.013); however, both were still significantly lower than in HIV-uninfected individuals. In contrast, no difference was detected in baseline phosphorylation of Zap70/Syk between groups (Kruskal–Wallis p = 0.36; Fig. 4C).

**NK cell activation and loss of CD16 signaling are not associated with CD4 count or viral load**

To determine which factors may cause NK cell activation and loss of CD16 signaling, we initially correlated these parameters with traditional measures of HIV-1 disease progression. In viremic HIV-infected individuals, there was no correlation between CD16 activation (determined either as the proportion of CD38<sup>+</sup>HLA-DR<sup>+</sup> NK cells or NK cell degranulation) with either CD4 counts or viral load (Table II). In contrast, both CD4 and CD8 T cell activation correlated with viral load as reported by others (23). In the combined group of ART<sup>-</sup> and ART<sup>+</sup> patients, neither NK cell nor T cell activation correlated with CD4 counts. A similar lack of correlation of CD16 expression with either viral load or CD4 count was observed with CD16 expression on CD56<sup>dim</sup> CD16<sup>+</sup> NK cells.

We did not observe any correlation between NK cell or T cell activation and either plasma levels of sCD14, neopterin, endotoxin, or bacterial 16S RNA levels in plasma (Table II).

**Discussion**

We show that NK cells remain activated in patients receiving cART who have received cART for a median duration of 11.5 y (range, 0.25–18 y) and maintained an undetectable viral load for a median time of 2.9 y (range, 0.6–7.7 y). This contrasts with T cell activation, which is reduced by cART in the same group of patients. We also show that activation of NK cells in HIV patients is associated with impaired CD16 signaling owing to loss of CD16 and expression of its chaperone and signaling proteins FcRγ and TCRγ. Significantly, NK cell activation, decreased FcRγ expression, and impaired signaling did not correlate with HIV RNA or CD4<sup>+</sup> T cell counts. Time of undetectable viral load in patients receiving cART did not correlate with measures of the study endpoint FcRγ protein levels (n = 11, Spearman rho = 0.091, p = 0.79), with surface expression of CD16 (n = 8, Spearman rho = −0.32, p = 0.41), or with HLA-DR expression (n = 9, Spearman rho = −0.067, p = 0.86) on CD56<sup>dim</sup> NK cells. However, both HIV-infected study groups showed NK cell activation and also exhibited loss of CD16 signaling and increased levels of plasma neopterin, sCD14, and sCD163, which suggests that these events are linked to myeloid activation and hence activation of the innate immune system. The different responses of NK and T cell activation to cART suggest that they are activated by different factors in HIV patients. Our data suggest that current cART regimens are unable...
to reduce NK cell activation to normal levels and that targeting inflammation, in addition to suppressing viremia, will be required to fully restore NK cell-mediated immune function in these patients.

One potential source of inflammation in HIV patients is chronic endotoxemia arising from impaired barrier function of the gut epithelium caused by destruction of intestinal T cells during acute HIV infection (8). Endotoxin levels are not decreased significantly when patients achieve virologic suppression with cART, and our modeling has shown that decades of cART treatment may be required to reduce endotoxemia in HIV patients (7). We did not observe any correlation, however, between NK cell activation and plasma endotoxin levels or levels of bacterial 16S DNA, which suggests that the relationship between bacterial products in blood and NK cell activation is not direct. A similar conclusion was reached in a study of HIV patients and patients with inflammatory bowel disease, although in this study, which measured activation by expression of the acute activation marker CD69, activation was decreased in patients receiving cART (24). One caveat, however, is that the LAL endotoxin assay is not robust when used for measuring bacterial products in plasma (20). Longitudinal studies are required to establish whether NK activation and function are restored to normal levels after a sufficient duration of suppressive cART, which may also give indications about causality behind the associations we have observed.

HIV-infected individuals showed high basal degranulation of CD56dimCD16+ NK cells in whole blood incubated ex vivo, the levels of which were elevated in both ART+ and ART- patients relative to HIV-uninfected control subjects. This degranulation likely indicates heightened NK cell activity, which has been observed previously by others (25). Our observation that baseline levels of Syk/ZAP70 phosphorylation were not increased in HIV-infected individuals suggests that elevated degranulation is not likely a consequence of high levels of ADCC. We hypothesize that degranulation is due to the presence of targets for NK cell-mediated natural cytotoxicity, which we and others have shown is independent of Syk phosphorylation (16, 26). The existence and nature of these targets remains to be established. It has been shown that HIV-infected T cell blasts are killed by NK cells and inhibit ADCC via activation of NK cell inhibitory receptors through HLA-C and HLA-E expression (27); however, these cells are unlikely to be a significant cause of ADCC loss in virologically suppressed patients receiving cART due to their low prevalence. In contrast, endotoxin, which is elevated in HIV-infected individuals irrespective of cART, is known to upregulate NKG2D ligands such as MIC-A on monocytes (28). Activation of NKG2D is known to induce cross-tolerance, which results in loss of CD16 expression and several ITAM proteins (29).

In HIV-infected individuals, decreased expression of CD16 and its chaperone/signaling adaptor FcγR and signaling adaptor TCRζ resulted in loss of CD16-dependent signaling that is essential for ADCC. Loss of CD16 has been shown to be partially dependent on matrix metalloproteinase induction, which promotes shedding of the extracellular domain of the receptor (30). Our data showing a direct correlation of CD16 mRNA expression with CD16 surface expression indicate that an additional mechanism regulating CD16 surface expression at a transcriptional level is operating in chronic HIV patients. Whether matrix metalloproteinase activity also has an effect on the expression of the FcγR chaperone is not clear. Our finding of reduced CD16 and FcγR mRNA levels in HIV patients implies that additional mechanisms underlie decreased expression of ADCC receptors on NK cells. Furthermore, the association of FcγR expression with CD16 surface expression suggests that the reduced expression of the chaperone is a significant factor.

HIV infection is accompanied by the accumulation of CD56+CD16+ NK cells (31), which may account for early reports of increased numbers of CD56+CD16+ NK cells in HIV-infected patients (25, 32). CD56+CD16+ NK cells are anergic (reviewed in Ref. 32) but retain the ability to synthesize and secrete MIP-1β (25), showing that NK cells lose function in a hierarchical manner in the setting of chronic viral infection. Because we gated specifically on CD3−CD56+ cells in the present study, the decreased expression of signaling molecules that we observed in HIV patients was not due to an increased proportion of CD56+ cells. It is likely that CD56+CD16+ cells arise from maturation of CD56dimCD16+ NK cells. It will be of interest to determine whether CD56+ NK cells expressing low levels of FcγR, TCRζ, and DAP12 represent a precursor stage toward losing CD56, similar to the reduction of SIGLEC-7 expression (33, 34). Consistent with this concept, we have also observed a decreased expression of CD56 (data not shown) in addition to low CD16 expression within CD56+ NK cells from HIV-infected patients both on and off therapy.

Despite its absolute requirement for functional NK cell signaling (15), there have been no data published on FcγR expression in HIV infection or other human diseases. However, reduced expression of TCRζ in T cells and NK cells has been documented in HIV-infected patients before the widespread use of ART (35). Limited data suggest a beneficial effect of ART on TCRζ expression in T cells (36); however, no increase in NK cell TCRζ or FcγR expression at the mRNA level was detected in our ART+ group compared with ART− individuals. This also implies that FcγR loss is not compensated by an increased expression of other homologous ITAM molecules, including DAP12, for which, to our knowledge, we show a pathological dysregulation for the first time in human disease. Whether this is associated with decreased function of NK cell receptors, which use DAP12 for signal transduction, needs to be investigated.

NK cells are able to lyse tumor- and virus-infected cells, but their potential role in preventing non-AIDS comorbidities in patients receiving ART has not been addressed. Non-AIDS cancers are an increasing cause of morbidity and mortality in HIV-infected persons, and continuing defects in NK cells may contribute to their incidence by failing to perform efficient surveillance. In this context, the sensitivity of ADCC signaling in NK cells to inflammation and immune activation suggests it may be a valuable marker determining the long-term consequences of HIV infection on patient morbidity and mortality after viremic suppression with ART.

Acknowledgments

We thank Prof. Mark Hogarth, Center for Immunology, Burnet Institute, for the gift of rabbit anti-FcγR and mouse anti-CD16 Abs; Michael Thomson, Burnet Institute, for support during cell sorting; Dr. Julian Elliott and Cath Downes from the Clinical Research Unit, The Alfred Hospital, for help in patient recruitment; and the patients and control subjects who generously consented to this study.

Disclosures

The authors have no financial conflicts of interest.

References


Downloaded from http://www.jimmunol.org/ by guest on April 12, 2017


