Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection

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In this study, we demonstrate that the in vitro interactions between a CD56^{neg}/CD16^{pos} (CD56^{neg}) subset of natural killer (NK) cells and autologous dendritic cells (DCs) from HIV-1–infected viremic but not aviremic individuals are markedly impaired and likely interfere with the development of an effective immune response. Among the defective interactions are abnormalities in the process of reciprocal NK–DC activation and maturation as well as a defect in the NK cell–mediated editing or elimination of immature DCs (iDCs). Notably, the lysis of mature DCs (mDCs) by autologous NK cells was highly impaired even after the complete masking of major histocompatibility complex I molecules, suggesting that the defective elimination of autologous iDCs is at the level of activating NK cell receptors. In this regard, the markedly impaired expression/secretion and function of NKp30 and TNF-related apoptosis–inducing ligand, particularly among the CD56^{neg} NK cell subset, largely accounts for the highly defective NK cell–mediated lysis of autologous iDCs. Moreover, mDCs generated from HIV-1 viremic but not aviremic patients are substantially impaired in their ability to secrete interleukin (IL)–10 and –12 and to prime the proliferation of neighboring autologous NK cells, which, in turn, fail to secrete adequate amounts of interferon–γ.

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Abbreviations used: ART, anti-retroviral therapy; iDC, immature DC; iMDDC, immature MDDC; iNKR, inhibitory NK receptor; KIR, killer cell Ig-like receptor; MDDC, monocyte-derived DC; mDC, mature DC; TRAIL, TNF-related apoptosis-inducing ligand.

DCs and NK cells are important elements of the innate immune system in its relationship to the ultimate response of the adaptive immune system to inflammatory insults. The first contact between immature DCs (iDCs) and antigens occurs in peripheral tissue at sites of inflammation where iDCs are recruited from the bloodstream through cytokine and chemokine signals produced by resident DCs and other cell types (1, 2). After antigen uptake, iDCs undergo a maturation process that allows the resulting mature DCs (mDCs) to migrate to secondary lymphoid tissues, where they prime an antigen-specific T cell response.

In the past few years, NK cells have been found to be extremely important in optimizing the differentiation of mDCs. This process requires both NK cell–DC (NK–DC) cellular interactions and NK cell secretion of specific cytokines. The final outcome of this cross talk is the coordination and optimization of both the innate and adaptive immune responses (1–6).

DCs undergoing maturation secrete several cytokines, such as IL-12 and –15, that act as potent inducers of NK cell activation and proliferation. In turn, once activated, NK cells produce IFN-γ, TNF-α, and GM-CSF, three important antiviral cytokines that are directly involved in completing the maturation program of DCs (1–3, 5, 7–10). The incomplete or aberrant maturation of DCs is highly undesirable because antigen presentation by these cells

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might lead to T cell tolerance or apoptosis and, therefore, to a greatly impaired adaptive immune response (11, 12). NK cells further optimize conditions for subsequent T cell priming by eliminating DCs that have failed to mature properly. iDCs express remarkable amounts of classic HLA-A/-B/-C molecules but at considerably lower levels compared with mDCs (13, 14). Moreover, very low levels of expression of non-classic HLA-E on surfaces of iDCs compared with mDCs have also been reported (15). The attenuated expression of MHC-I molecules, the natural ligands for the inhibitory NK receptors (iNKRs), makes iDCs uniquely susceptible to NK cell lysis (1, 16). NK cell–mediated killing of iDCs likely occurs to a substantial degree only when these two cell types colocalize in sufficient numbers at sites of tissue infection/inflammation (2, 5, 12, 17). Therefore, in their interactions with DCs, NK cells can act as regulatory cells as well as effectors to ensure an appropriate activation of adaptive immunity (12).

Several lines of evidence suggest that normal NK–DC interactions are aberrant in HIV-1 infection. A recent study showed that the autologous NK cell lysis of immature monocyte-derived DCs (MDDCs [iMDDCs]) generated from HIV-1–infected individuals was defective; however, the mechanisms underlying this phenomenon have not yet been elucidated (18). In addition, different studies showed that the expression and function of several important activating and inhibitory

NK cell receptors are altered in viremic HIV-1–infected individuals (19–22). Furthermore, NK cells isolated from viremic patients exhibit defective cytotoxicity (20, 21) and cytokine secretion (23) after activation. All of these phenotypic and functional NK cell abnormalities are particularly pronounced in an unusual CD56neg/CD16pos (CD56neg) NK cell subset that is preferentially expanded in HIV-1–infected individuals with high levels of active viral replication and are rarely represented in aviremic patients or in healthy donors (20, 23–25).

Both ex vivo and in vitro studies have shown that during the course of HIV-1 infection, several functional defects are associated with mDCs, although their surface expression of maturation markers appears to be normal. These defects include an impaired ability to prime autologous CD4$^+$ T cells and a decreased secretion of several cytokines and chemokines despite a retained antigen uptake capacity (26–30). These phenotypic and functional perturbations observed in HIV-1–exposed NK cells and DCs strongly suggest that there might be abnormalities in the interactions between these two important members of the innate immune system (31).

This study characterizes the highly impaired bidirectional NK–DC cross talk in chronically HIV-1–infected viremic individuals compared with HIV-1 seropositive patients whose viremia had been suppressed to undetectable levels by anti-retroviral therapy (ART) and with normal uninfected control
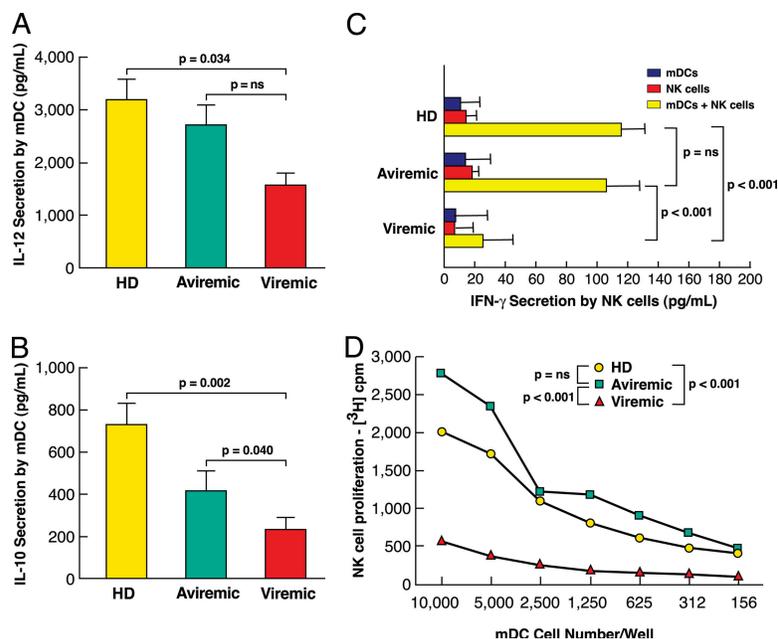

Figure 1. mDC functional profiles and ability to prime autologous NK cells. (A and B) Levels of IL-12p70 (A) and IL-10 (B) secreted by mDCs generated from healthy donors (HD; yellow bars), HIV-1–infected aviremic (green bars) patients, and viremic (red bars) HIV-1–infected patients. (C) Levels of IFN-γ secreted by freshly purified NK cells cocultured with autologous mDCs (yellow bars) for 24 h. mDCs alone (blue bars) and NK cells alone (red bars) served as negative controls. The mDC/NK cell ratio was 1:10. (D) mDC-mediated priming of NK cells; fresh NK cells (responders) purified from HIV-1–infected viremic (red triangles) and aviremic (green squares) individuals and from uninfected subjects (yellow circles) were cocultured with autologous mDCs (stimulators) at different ratios. Cells were harvested after 4 d of coincubation, and the proliferation of NK cells was measured by ^{3}[H]thymidine incorporation. All data are presented as the median (± SD [error bars] for A–C) of experiments conducted on 15 healthy donors and 15 HIV-1 viremic and aviremic infected patients.

subjects. We also demonstrate that the phenotypic and functional characteristics of the CD56^{neg} NK cell subset present at high frequencies in viremic individuals underlie the highly dysfunctional interactions between NK cells and DCs.

RESULTS

Phenotypic correlates of DC maturation

Previous studies have demonstrated that the exposure of iDCs generated in vitro from healthy donors to various strains of HIV-1 or to HIV-1–Gp120 envelope before exposure to maturation stimuli results in the impairment of several mDC functions despite the normal expression of surface markers associated with a mature phenotype (28–30). On the basis of these studies, we sought to determine whether iDCs obtained from HIV-1–infected patients expressed normal levels of those surface markers that usually define an mDC when exposed to strong maturation stimuli. The evaluation of CD40, CD80, CD83, CD86, MHC-I, and MHC-II surface expression on in vitro–generated iDCs after 24 h in culture with LPS (the time frame within which maturation occurs) did not reveal substantial differences among viremic and aviremic HIV-1–infected patients or uninfected individuals (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20060894/DC1). Similar experimental results were obtained when triggering the maturation of iDCs with sCD40 ligand (unpublished data).

Functional correlates of mDCs

Despite the expression of cell surface markers associated with a mature phenotype, we found that mDCs derived from viremic patients exhibited several functional defects that could potentially impair the mDC-mediated activation of NK cells. In vitro matured DCs from viremic HIV-1–infected subjects secreted a markedly lower amount of IL-10 as well as IL-12, which is an important cytokine for the activation of NK cells (7–9, 32), compared with mDCs of HIV-1–infected aviremic patients who were receiving ART (for IL-10) or with healthy donors (for IL-10 and -12; Fig. 1, A and B). However, the production of IL-15 and -18 by mDCs did not substantially differ among the three study groups (unpublished data).

To determine the capacity of mDCs to activate immunoregulatory functions of NK cells, we measured the amount of IFN-γ secreted by NK cells in the presence of autologous mDCs. As shown in Fig. 1 C, resting NK cells from HIV-1–infected aviremic patients released IFN-γ in amounts comparable with those from uninfected donors. In contrast, freshly purified NK cells cocultured with autologous mDCs derived from viremic individuals showed a statistically significant lower secretion of IFN-γ (P < 0.001). Moreover, within the same group of viremic individuals, the ability to secrete IFN-γ was markedly lower (P < 0.001) in freshly purified CD56^{neg} NK cells compared with CD56^{pos}/CD16^{pos} (CD56^{pos}) NK cells (unpublished data). We could not detect substantial differences in the levels of TNF-α and GM-CSF secreted by NK cells or NK cell subsets primed with autologous mDCs among the three study groups (unpublished data).
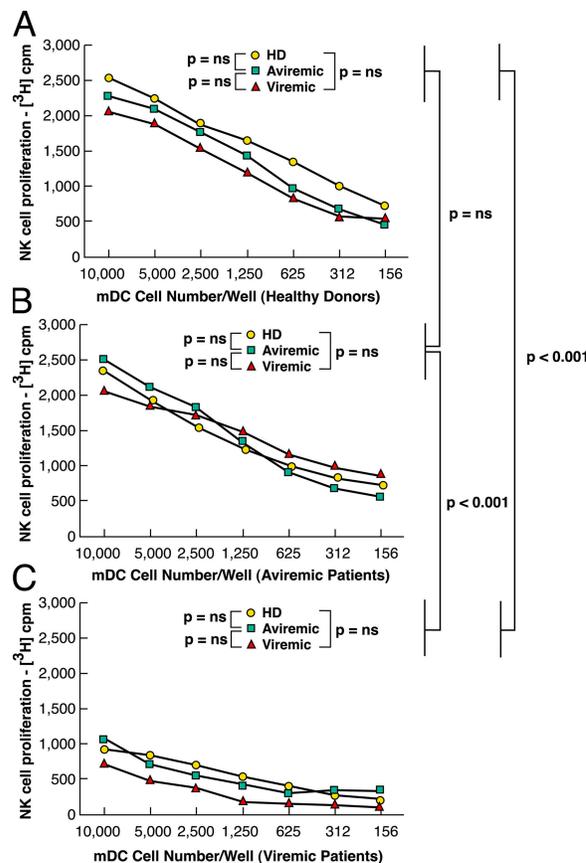

Figure 2. mDC priming of heterologous NK cells. Fresh NK cells (responders) purified from HIV-1–infected viremic (red triangles) and aviremic (green squares) individuals and from healthy donors (HD; yellow circles) were cocultured with heterologous mDCs (stimulators) generated from healthy donors (A) as well as aviremic (B) and viremic (C) HIV-1–infected individuals at different ratios. Cells were harvested after 4 d of coincubation, and the proliferation of NK cells was measured by ³[H]thymidine incorporation. Data are presented as the median of experiments conducted on 15 healthy donors and 15 HIV-1 viremic and aviremic infected patients.

As previously described, an important function of mDCs is to trigger the proliferation/activation of autologous resting NK cells (1–3, 5). In coculture experiments, the ability of mDCs generated from viremic patients to induce the proliferation of autologous NK cells was significantly diminished compared with that of either HIV-1–infected aviremic individuals (P < 0.001) or uninfected donors (P < 0.001; Fig. 1 D). This result was not likely caused by an inherent defect in the proliferative capacity of NK cells isolated from viremic patients because these cells displayed a normal proliferation in response to mDCs derived from aviremic HIV-1–infected or healthy subjects in heterologous cocultures. Furthermore, NK cells isolated from all HIV-1–infected or uninfected subjects exhibited significantly lower rates of proliferation when cultured with heterologous mDCs generated from viremic subjects compared with proliferation rates triggered by exposure to heterologous mDCs generated from healthy donors

(P < 0.001) or HIV-1–infected aviremic individuals (P < 0.001; Fig. 2). Therefore, these coculture experiments confirm that the defective NK cell proliferation observed in HIV-1–infected viremic patients was not caused by a primary impairment of NK cell responsiveness but by an impairment of the triggering of NK cells by mDCs.

To address whether mDCs had any role in the expansion of the CD56^neg NK cell subset, we analyzed the distribution of CD56 and CD16 on the surface of NK cells cocultured with heterologous mDCs. Under all combinations of DC and NK cell cocultures, mDCs were not able to modify the relative distribution of NK cell subsets. In particular, heterologous cocultures of DCs generated from HIV-1–infected viremic patients and NK cells purified from healthy donors did not result in the expansion of the CD56^neg NK cell subset (unpublished data).

NK cell–mediated killing of autologous iDCs

A recent study showed that iMDDCs from HIV-1–infected patients are relatively resistant to killing by autologous NK

cells (18). To ascertain whether this phenomenon pertains to all HIV-1–infected individuals or is restricted to those with high levels of chronic HIV-1 viremia, we analyzed the baseline lysis of autologous iDCs by rIL-2–activated, unfractionated NK cells isolated from HIV-1 viremic and aviremic patients. The degree of NK cell–mediated lysis of autologous iDCs was calculated using the traditional Cr^{51} release assay and was also visualized by fluorescence microscopy (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20060894/DC1). Spontaneous killing of autologous iDCs by activated NK cells isolated from aviremic patients did not substantially differ from that observed with NK cells purified from healthy donors. In contrast, the elimination of autologous iDCs by NK cells isolated from HIV-1–infected viremic individuals was markedly reduced compared with that from either aviremic HIV-1–infected subjects or healthy controls. This result was not likely caused by an increased resistance of iDCs to the elimination by NK cells from HIV-1–infected viremic patients. As shown in Fig. 3 A, the susceptibility to NK cell–mediated killing of heterologous
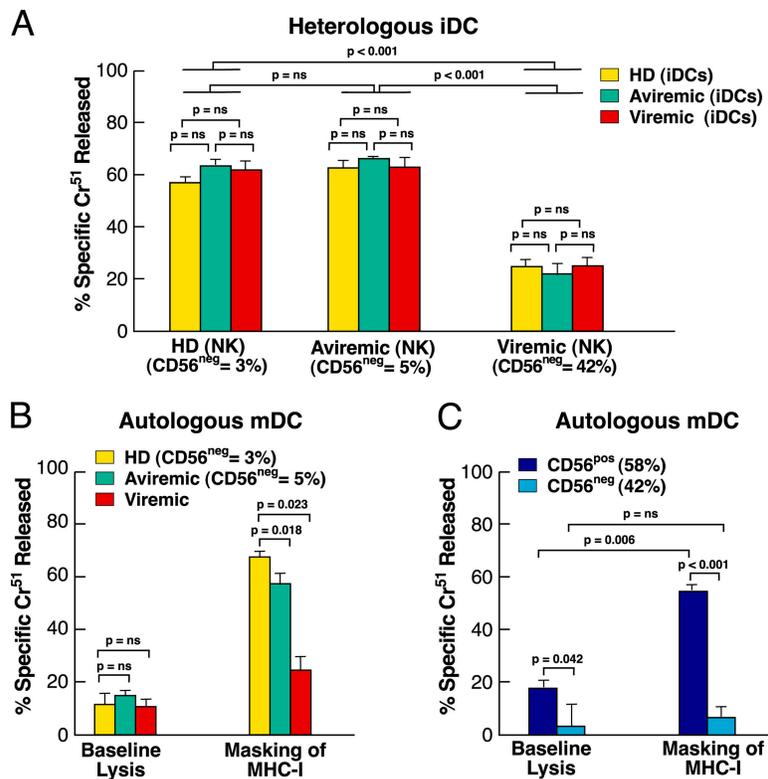

Figure 3. NK cell–mediated killing of heterologous iDCs and autologous MHC–I masked mDCs. (A) Cytolysis exerted by unfractionated rIL-2–activated NK cells purified from a healthy donor, an aviremic, and a viremic HIV-1–infected individual against heterologous iDCs generated from a healthy donor (HD; yellow bars), an aviremic (green bars), and a viremic (red bars) HIV-1–infected patient. (B) Autologous mDC cytolysis exerted by unfractionated rIL-2–activated NK cells purified from a healthy donor (yellow bars), an aviremic (green bars), and a viremic (red bars) HIV-1–infected individual. (C) Comparison of autologous mDC killing exerted by

rIL-2–activated CD56^pos- (dark blue bars) and CD56^neg (light blue bars)-derived NK cell subsets purified from the same HIV-1–infected viremic subject shown in B. NK cells were incubated either in the absence (baseline lysis) or presence of specific mAbs masking classic HLA-A/-B/-C and nonclassic HLA-E. Data were obtained from single experiments performed in triplicate (± SD [error bars]) and are representative of results obtained using cells isolated from 15 viremic and 15 aviremic HIV-1–infected individuals and from 15 healthy donors. The NK cell/iDC ratio in all experiments was 10:1.
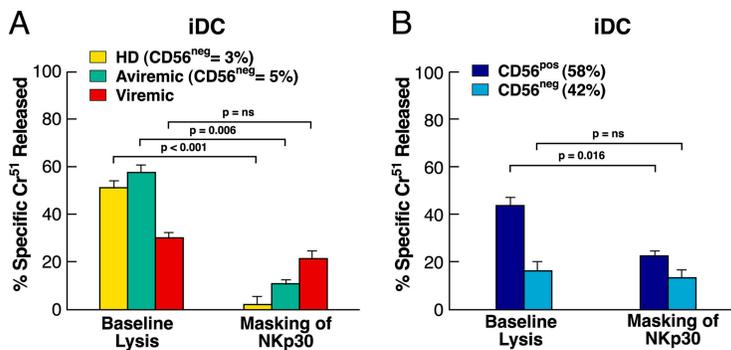
Figure 4. NK cell–mediated killing of autologous iDCs: masking of NKp30. (A) Autologous iDC cytolysis exerted by unfractionated rIL-2–activated NK cells purified from a healthy donor (HD; yellow bars), an aviremic (green bars), and a viremic (red bars) HIV-1–infected individual. (B) Comparison of autologous iDC killing exerted by rIL-2–activated CD56^{pos}- (dark blue bars) and CD56^{neg} (light blue bars)-derived NK cell subsets purified from the same HIV-1–infected viremic subject shown in A.

NK cells were incubated either in the absence (baseline lysis) or presence of a specific mAb masking NKp30. Data were obtained from single experiments performed in triplicate (± SD [error bars]) and are representative of results obtained using cells isolated from 15 viremic and 15 aviremic HIV-1–infected individuals and from 15 healthy donors. The NK cell/iDC ratio in all experiments was 10:1.

iDCs isolated from all HIV-1–infected or uninfected subjects did not differ if cocultured with NK cells purified either from aviremic or healthy individuals. Only NK cells purified from viremic patients displayed a markedly defective killing of heterologous iDCs isolated from all HIV-1–infected or uninfected donors.

Using NK cells isolated from viremic individuals, we further characterized the defect in NK cell–mediated lysis of iDCs by comparing rIL-2–activated CD56^{neg}– and CD56^{pos}–derived NK cell subsets for their ability to eliminate autologous iDCs. The iDC killing appeared to be relatively intact for the CD56^{pos} NK cell subset but was highly impaired for the CD56^{neg} subpopulation (Fig. S2).

Cellular mechanisms of the impaired NK cell–mediated killing of autologous iDCs

For an NK cell to exert lytic activity, an inhibitory signal must be weak or lacking, and an activating signal must be present. The interactions between MHC-I molecules and iNKRs trigger an inhibitory signal and result in the abrogation of NK cell lytic activity even if activating NK cell receptors are triggered. Two major classes of MHC-I–specific surface inhibitory receptors have been identified on NK cells: (a) the killer cell Ig-like receptors (KIRs), which bind HLA/-B/-C alleles, and (b) CD94/NKG2A, which binds to nonclassic HLA-E alleles (33–35). In this regard, the lysis of autologous iDCs is confined to the subpopulation of NK cells that express CD94/NKG2A and lack KIRs (36). Moreover, NKp30, one of the three natural cytotoxicity receptors, has been shown to be primarily responsible for the NK cell–mediated killing of autologous iDCs (16).

Role of MHC-I molecules

Under physiological conditions, the maturation of DCs leads to the elevated surface expression of classic and nonclassic MHC-I molecules that render them resistant to NK cell–mediated

cytolysis (1, 2, 5). As expected, the normal levels of HLA-A/-B/-C and HLA-E (Fig. S1) expressed by mDCs generated from donors in all three study groups made them similarly resistant to lysis by activated NK cells. Given the importance of interactions between MHC-I and iNKRs in inhibiting the NK cell–mediated killing of autologous mDCs (1, 2, 5), the masking of HLA molecules using specific mAbs would theoretically enhance the susceptibility of mDCs to lysis by NK cells in vitro. Despite the complete blocking of all HLA-I alleles with specific mAbs, activated NK cells from viremic patients were still highly impaired in their cytolytic activity against autologous mDCs compared with NK cells from aviremic patients and uninfected individuals. Moreover, the cytotoxicity of the rIL-2–activated CD56^{neg}–derived NK cell subset against autologous HLA-I–masked mDCs was virtually undetectable compared with that of activated CD56^{pos}–derived NK cells (Fig. 3, B and C). These masking experiments performed on mDCs clearly indicate that NK cells from viremic patients are defective in their lysis of mDCs when the inhibitory effects of MHC-I interactions with KIRs are abolished by masking the MHC-I molecules. Therefore, these data strongly suggest that the defect in iDC lysis of NK cells from viremic patients is at the level of the activating receptors on the NK cell itself.

Role of NKp30

The surface expression of NKp30 is decreased on fresh and rIL-2–activated NK cells from viremic HIV-1–infected individuals compared with cells from aviremic and uninfected individuals (20, 21). Therefore, we analyzed the contribution of NKp30 to the cytolysis of autologous iDCs in the three study groups. The masking of NKp30 with a specific mAb induced a statistically significant reduction in the killing of iDCs by activated NK cells derived from both uninfected donors (P < 0.001) and HIV-1–infected patients with undetectable viral load (P = 0.006), confirming the leading role of NKp30 in this process (16). As expected, blocking NKp30

had almost no effect in further reducing the already impaired rIL-2–activated NK cell–mediated lysis of iDCs in the cohort of HIV-1–infected viremic individuals because the expression of NKp30 on NK cell surfaces was already very low (20, 21). Moreover, the masking experiments highlighted that the impairment in NKp30 activity was much greater in the rIL-2–activated CD56^{neg}-derived subset compared with the CD-56^{pos}-derived NK cell subset (Fig. 4). The reduced ability of the CD56^{neg} NK cell subset to lyse autologous iDCs correlated well with its weak NKp30 surface expression (23).

It has been reported that the secretion of IL-15 and -12 by mDCs normally plays an important role in triggering the activation of autologous NK cells (7). Unlike IL-12, the production of IL-15 by mDCs did not substantially differ among the three study groups, prompting us to test whether NK cells stimulated in vitro with rIL-15 had an increased NKp30 surface expression and NKp30-dependent killing of autologous iDCs. As shown in Fig. S3 (available at http://www.jem.org/cgi/content/full/jem.20060894/DC1), NK cells

cultured in the presence of rIL-15 did not show any considerable increase in NKp30 surface expression on either CD56^{pos} or CD56^{neg} NK cell subsets compared with freshly purified and rIL-2–activated cells from HIV-1–infected viremic individuals. In contrast, both rIL-2– and -15–activated NK cells expressed markedly higher levels of NKp30 compared with freshly purified NK cells from either HIV-1–infected aviremic patients or normal donors. Consistent with the similar expression of NKp30 on both rIL2– and -15–activated NK cells from each donor within the three study groups, we did not observe differences in the NKp30-dependent killing of autologous iDCs between rIL-2– and -15–activated NK cells from HIV-1–infected patients or healthy donors (Fig. S4).

Role of TRAIL

TNF–related apoptosis–inducing ligand (TRAIL) is a member of the TNF ligand family that signals apoptosis via the death domain–containing receptors TRAIL-R1 (DR4) and
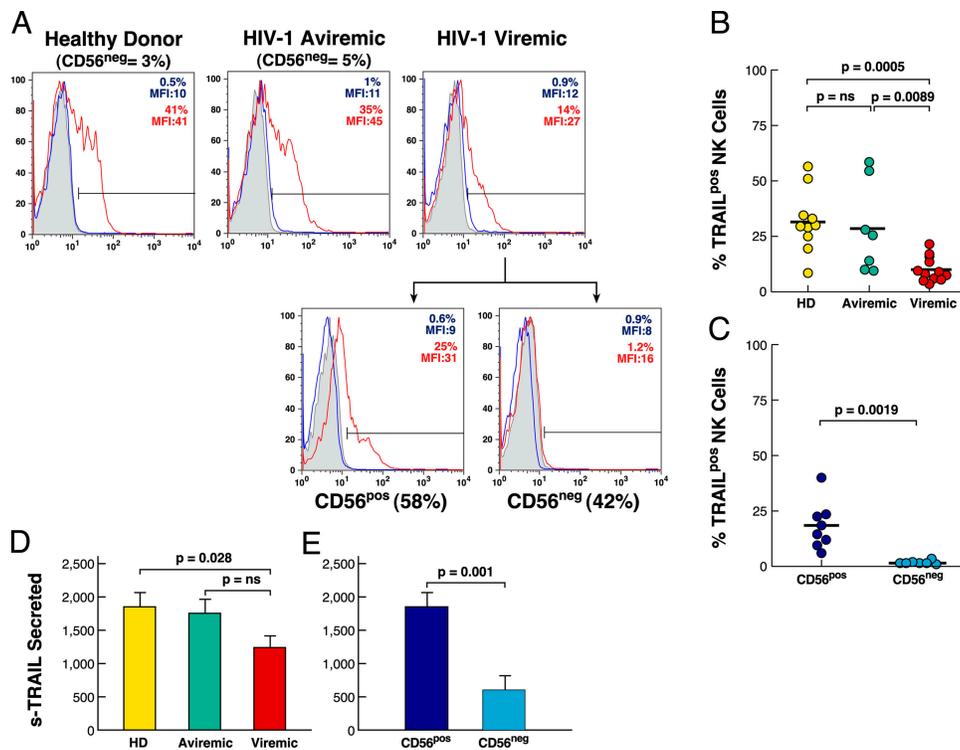

Figure 5. TRAIL expression and s–TRAIL secretion by rIL–2–activated NK cells. (A) Surface expression of TRAIL by NK cells in representative examples for a healthy donor, an aviremic, and a viremic HIV-1–infected individual and on CD56^{pos} and CD56^{neg} NK cell subsets purified from the same HIV-1–infected viremic subject. Histogram graphs show the percentage and geometric mean fluorescence intensity (MFI) of freshly purified (blue lines) and rIL–2–activated (red lines) cells. Gray shaded histograms represent the negative controls stained with the second reagent alone. (B and C) Summary graphs of statistical dot plots with medians (horizontal bars) showing the percentages of unfractionated rIL-2 NK TRAIL^{pos} cells purified from healthy donors (yellow

circles), aviremic (green circles), and viremic (red circles) HIV-1–infected patients and from rIL-2–activated CD56^{pos} (dark blue circles) and the CD56^{neg} (light blue circles)-derived NK cell subsets purified from viremic HIV-1–infected individuals. (D and E) Levels of sTRAIL secreted by unfractionated rIL-2–activated NK cells purified from healthy donors (yellow bar), HIV-1–infected aviremic (green bar), and viremic subjects (red bar) and by rIL-2–activated CD56^{pos}– (dark blue bar) and CD56^{neg} (light blue bar)-derived NK cell subsets purified from viremic HIV-1–infected individuals. Data are presented as the median ± SD (error bars) of experiments conducted on 15 healthy donors and 15 HIV-1 viremic and aviremic infected patients.

TRAIL-R2 (DR5). It is primarily expressed as a type II membrane protein and is also secreted in a soluble form (sTRAIL) only by activated T and NK cells. Moreover, in the mouse system, TRAIL contributes substantially to NK cell–mediated killing of iDCs in vivo and plays an important role in antiviral responses (37, 38). Therefore, we analyzed the surface expression and secretion of TRAIL from NK cells stimulated with rIL-2 for 6 d. The percentage of TRAIL^{pos} NK cells and the amount of sTRAIL released in the culture supernatant did not substantially differ between HIV-1–infected aviremic patients and healthy donors. In contrast, both the levels of TRAIL surface expression and sTRAIL secretion were highly reduced in NK cells from HIV-1–infected viremic patients compared with healthy controls. Moreover, within the cohort of viremic HIV-1–infected individuals, the frequency of rIL-2–activated NK cells expressing TRAIL was markedly lower in the CD56^{neg} subpopulation compared

with the CD56^{pos} subset. Furthermore, the concentration of sTRAIL in the culture supernatant of rIL-2–stimulated CD56^{neg} NK cells was considerably lower compared with that from activated CD56^{pos} cells (Fig. 5).

To understand whether the decreased expression/secretion of TRAIL by activated NK cells from viremic subjects affects NK cell function in the context of the elimination of autologous iDCs, we repeated the masking experiments using a specific anti-TRAIL mAb. Blocking TRAIL induced a statistically substantial reduction of the NK cell–mediated iDC cytolysis in both HIV-1–infected patients with undetectable viral load and healthy donors, which is in line with the high percentage of NK cells expressing and secreting TRAIL. In contrast, among viremic individuals, blocking TRAIL had almost no effect in further reducing the already low cytolysis of iDCs by NK cells. Again, this correlated with the low levels of TRAIL expression and secretion by NK cells isolated from
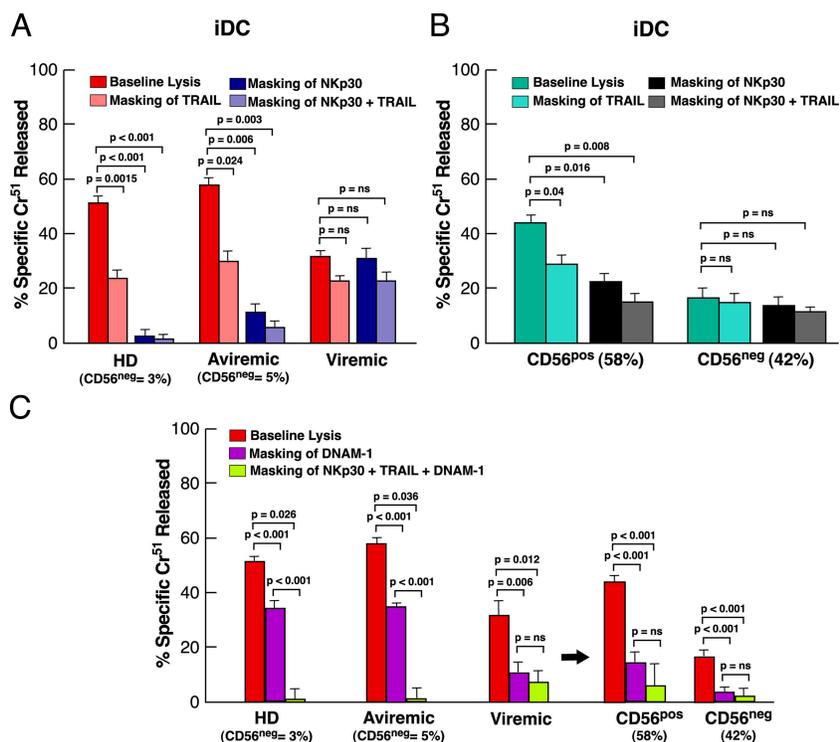

Figure 6. NK cell–mediated killing of autologous iDCs: masking of TRAIL and NKp30. (A) Autologous iDC cytolysis exerted by unfractionated rIL-2–activated NK cells purified from a healthy donor (HD), an aviremic, and a viremic HIV-1–infected individual. NK cells were incubated either in the absence (baseline lysis; red bars) or presence of a mAb-neutralizing TRAIL (pink bars). Experiments were also performed masking the effectors with an anti-NKp30–specific mAb either in the absence (blue bars) or presence (purple bars) of a specific anti-TRAIL mAb. (B) Comparison of autologous iDC killing exerted by rIL-2–activated CD56^{pos}- and CD56^{neg}-derived NK cell subsets purified from the same HIV-1–infected viremic subject shown in A. Cells were incubated either in the absence (baseline lysis; green bars) or presence of a mAb-neutralizing TRAIL (light blue bars). Experiments were also performed masking the effectors with an anti-NKp30–specific mAb either in the absence (black bars)

or presence (gray bars) of a specific anti-TRAIL mAb. (C) Autologous cytolysis of iDCs exerted by unfractionated rIL-2–activated NK cells from a representative healthy donor, an HIV-1–infected aviremic and viremic individual (bars on the left side of the arrow), and by CD56^{pos}- and CD56^{neg}-derived NK cell subsets purified from the same viremic patient (bars on the right side of the arrow). NK cells were incubated either in the absence (baseline lysis; red bars) or presence of a specific mAb-neutralizing DNAM-1 (purple bars). Experiments were also performed masking the effectors with anti-NKp30, anti-TRAIL, and DNAM-1 mAbs together (yellow bars). Data were obtained from single experiments performed in triplicate ($\pm$ SD [error bars]) and are representative of results obtained using cells isolated from 15 viremic and 15 aviremic HIV-1–infected individuals and from 15 healthy donors. The NK cell/iDC ratio in all experiments was 10:1.

this study group. Even the simultaneous masking of TRAIL and NKp30 did not result in any substantial reduction of iDC cytolysis by NK cells isolated from viremic patients, whereas the concomitant blocking of NKp30 and TRAIL virtually eliminated the NK cell–mediated lysis of autologous iDCs in the cohorts of HIV-1–infected aviremic individuals and healthy donors. Moreover, the impairment of TRAIL activity was much greater among the activated CD56^{neg}-derived NK cells compared with the CD56^{pos}-derived NK cell subset either in the absence or presence of NKp30 masking (Fig. 6, A and B). This reflected the negative or very low surface expression and secretion of TRAIL from the rIL-2–stimulated CD56^{neg} NK cell subset. Finally, we detected similar DR4 and DR5 expression on the surface of iDCs generated from viremic and aviremic infected patients compared with iDCs of healthy donors (unpublished data).

Role of DNAM–1

The aforementioned masking experiments clearly confirmed the crucial role of NKp30 in the NK cell–mediated lysis of autologous iDCs in healthy donors and in aviremic HIV-1–infected subjects. Moreover, they also delineated the substantial contribution of TRAIL to autologous iDC elimination by human activated NK cells purified from the same study groups. In contrast, the function of both of these molecules appeared to be highly impaired in NK cells isolated from viremic HIV-1–infected subjects. Nevertheless, NK cells from this latter cohort still exerted a considerable level of residual iDC cytolysis (Fig. 6 A). Therefore, we investigated the role of another activating NK cell receptor, DNAM-1, which contributes to the NK cell–mediated killing of iDCs (39). The addition of a blocking mAb specific for DNAM-1 together with TRAIL and NKp30 reduced the lysis of autologous iDCs by unfractionated NK cells or NK cell subsets purified from viremic patients to almost undetectable levels (Fig. 6 C). Moreover, only in this latter cohort of patients, the masking of DNAM-1 alone similarly reduced the NK cell–mediated lysis of autologous iDCs to levels comparable with those achieved by simultaneously blocking NKp30, TRAIL, and DNAM-1. This phenomenon demonstrated the compensatory role of DNAM-1 in iDC lysis among HIV-1–infected viremic patients. In this regard, unlike NKp30 and TRAIL, DNAM-1 was expressed at normal levels on all NK cell subpopulations isolated from viremic subjects (unpublished data), which is in line with its conserved functional activity. Moreover, similar levels of expression of poliovirus receptor (CD155) and Nectin-2 (CD112), the two natural ligands for DNAM-1 (39), were detected on the surface of iDCs generated from all three study groups (unpublished data).

DISCUSSION

This study demonstrates that numerous components of the bidirectional NK–DC cross talk between a CD56^{neg} subset of NK cells and autologous DCs is highly impaired in cells from viremic HIV-1–infected individuals compared with those from healthy donors and aviremic HIV-1–infected individu-

als who have been receiving ART for 2 yr or longer. As illustrated in the in vitro model proposed in Fig. S5 (available at http://www.jem.org/cgi/content/full/jem.20060894/DC1), actively replicating HIV-1 in peripheral tissue is associated with a markedly dysfunctional maturation of DCs, although these DCs appear to be phenotypically mature on the basis of the expression of several costimulatory markers and MHC class I and II molecules. These abnormally matured DCs are substantially impaired in their ability to secrete IL-10 and -12 and to prime the proliferation of neighboring autologous NK cells, which, in turn, fail to secrete adequate amounts of IFN-γ. It has been previously reported that NK cells from HIV-1–infected individuals are defective in eliminating autologous iDCs (18). In this study, we show that NK cells purified from HIV-1–infected individuals with high levels of ongoing viral replication but not from aviremic patients are also markedly impaired in their ability to eliminate autologous iDCs. This phenomenon is largely caused by the high frequency of a dysfunctional CD56^{neg} NK cell subset, whose surface expression/secretion and function of NKp30 and TRAIL molecules are either extremely low or absent.

Under physiological conditions, the maturation of DCs is induced directly by microbial signals as well as by activated NK cells performing a regulatory role (12). Consistent with our results, several studies have demonstrated that in vitro exposure of iDCs to various isolates of HIV-1 or HIV-1 envelope interferes with the complete functional maturation of DCs, although the surface expression of markers associated with the DC mature phenotype is not affected (28–30). We show here that the secretion of IFN-γ, a potent inducer of DC differentiation (3), is highly impaired when NK cells are exposed to autologous mDCs generated from viremic but not aviremic HIV-1–infected individuals. It was recently demonstrated that the ability to promote DC maturation was essentially confined to NK cells expressing a KIR[neg]/NKG2A[dull]/NKp30^{pos} phenotype (40). We previously reported that freshly purified NK cells from HIV-1 viremic individuals expressed increased levels of KIRs, whereas they expressed extremely low or negative levels of NKG2A and NKp30 (20), particularly among the CD56^{neg} NK cell subset (23). Given the association of these defects with the viremic state, together, these data suggest that HIV-1 directly or indirectly negatively interferes with the maturation process of DCs. In this regard, it remains to be determined whether the reduced NK cell activation, as observed in vitro in this study, plays a role in generating functionally impaired myeloid or plasmacytoid DCs freshly isolated from HIV-1–infected viremic patients as previously reported (27).

The priming of NK and T cells by autologous mDCs represents two fundamental steps in the natural process that links the innate to the adaptive immune response (1, 2, 4–6). In this study, autologous mDCs generated from viremic HIV-1–infected subjects clearly exhibited a markedly impaired capacity to induce both NK cell expansion and the secretion of IFN-γ. IL-12 is an important cytokine for the activation and proliferation of NK cells (7–9, 32). Thus, the

reduced IL-12 production by mDCs generated from viremic HIV-1–infected individuals, as demonstrated in this study, may partially account for their defective priming of NK cells. In this regard, others have previously reported that MDDCs that had been infected in vitro with HIV-1 fail to produce IL-12 in response to CD40 ligand stimulation (29).

Here, we demonstrate that mDCs generated from HIV-1–infected viremic individuals secreted a markedly lower amount of IL-10 compared with mDCs from aviremic patients. Although not addressed directly in this study, the lower secretion of IL-10, an HIV-1 inhibitory cytokine in most systems (41, 42), by mDCs as well as the reduced cytolytic activity of NK cells (20, 21) from HIV-1–infected viremic patients may impair the inhibition of HIV-1 replication in peripheral tissues. Furthermore, it has been shown that IL-10 inhibits the expression of DC costimulatory molecules (43), and, therefore, the weak production of IL-10 by mDCs generated from viremic HIV-1–infected individuals may partly contribute to the mature phenotype of the markedly dysfunctional mDCs.

Whether DCs from HIV-1–infected individuals are also impaired in their ability to prime an effective T cell response remains controversial (44). Nevertheless, several studies have reported a highly impaired capacity to activate autologous and heterologous T cells either by in vitro HIV-1–exposed mDCs generated from healthy individuals or by myeloid or plasmacytoid DCs freshly isolated from HIV-1–infected viremic individuals (27, 28, 45). Moreover, the in vitro observation that iDCs exposed to HIV-1 achieve an mDC phenotype, express high amounts of CCR7, and are able to chemotax in response to MIP-3β (CCL19) indicates that they are able to migrate to secondary lymphoid tissues, where they can participate in the priming of T cells (30). It has also been shown in vitro and ex vivo that these phenotypically mature but functionally immature DCs are infected by HIV-1 (46, 47) and are also able to transmit the virus to autologous T cells (30, 47, 48). Collectively these data suggest that these phenotypically mature but dysfunctional DCs would generate a suboptimal adaptive T cell response and might also be able to spread the infection.

A potentially important mechanism through which NK cells are thought to impact the quality of DCs undergoing maturation in response to antigen uptake is by killing iDCs (1, 2, 4–6). It is likely that this phenomenon occurs primarily at sites of tissue inflammation and appears to be dependent on the NK cell/DC ratio (1, 2, 5, 6). Low ratios of NK cells/DCs lead to DC survival and maturation, whereas high ratios of NK cells/DCs result in the elimination of DCs and the inhibition of DC maturation (17). A recent study showed that iMDDCs generated from HIV-1–infected patients can escape lysis by autologous NK cells (18), but the underlying causes of this escape have not been identified. We address some of the cellular mechanisms that may account for impaired NK cell–mediated lysis of autologous iDCs and show that this phenomenon appears to be restricted to those HIV-1–infected individuals with persistent high viremia. Our data demonstrate that the markedly impaired expression/secretion and function of NKp30 and TRAIL largely account for the highly defective NK cell–mediated lysis of autologous iDCs generated from viremic HIV-1–infected subjects. Moreover, we show that within this study group, the overrepresentation of CD56^{neg} NK cells expressing the unusual KIR^{pos}/NK-G2A^{neg}/NKp30^{neg}/TRAIL^{neg} phenotype (23), a subset present at very low frequencies in aviremic HIV-1–infected patients as well as in healthy donors (20), likely explains why this defective elimination of autologous iDCs by NK cells is confined to HIV-1–positive viremic patients.

DCs are distributed within all lymphoid and nonlymphoid tissues and in humans are classified into at least two major types, myeloid and plasmacytoid, which differ in terms of function and phenotype (49). Plasmacytoid DCs, regardless of their state of differentiation/activation, are poorly susceptible to NK cell–mediated lysis (12) and do not efficiently present antigens to T cells, whereas myeloid DCs are potent inducers of adaptive immunity (47, 49). It has been reported recently that freshly isolated myeloid DCs from healthy individuals were susceptible to NK cell–mediated lysis, although to a lesser degree than were iMDDCs (50). However, the NK cell/DC ratios found in that study were possibly too low, and it is conceivable that the observed differences between freshly isolated myeloid DCs and iMDDCs with regard to the susceptibility to lysis may have been reduced at higher NK cell/DCs ratios. In addition, the heterogeneity of freshly isolated myeloid DCs in their surface expression of MHC-I molecules and costimulatory markers reflects differences in the maturation or activation state (49, 50) and, therefore, may impact NK cell–mediated killing. Although MDDCs differ from freshly isolated myeloid DCs in certain respects, previous studies have found that MDDCs are very similar both phenotypically and functionally to myeloid DCs present in the blood (47, 49). Therefore, we feel that MDDCs are appropriate surrogates for freshly isolated myeloid DCs and represent a practical tool in vitro for investigating the role of NK–DC cross talk in the pathogenesis of human diseases.

The conserved numbers and phenotype of circulating monocytes among viremic patients compared with aviremic and healthy subjects (unpublished data) are consistent with previous studies showing that a very small proportion of blood monocytes harbor HIV-1 throughout the course of infection (51, 52). Nonetheless, it has been demonstrated that the infection of monocytes with HIV-1 can affect important functions of monocytes, such as chemotaxis and phagocytic capacity, suggesting that viral replication in monocytes/macrophages may contribute to the establishment and persistence of HIV-1 infection and may also activate and infect surrounding T cells (51, 52).

In summary, certain bidirectional NK–DC interactions that normally occur after an inflammatory insult are affected during HIV-1 infection, resulting in the abnormal maturation of DCs, impaired activation of NK cells, and deficient killing of unnecessary iDCs by NK cells. As demonstrated in this study, these defects are associated with an overrepresented

CD56^{neg} subset of NK cells observed only in viremic and not in aviremic HIV-1–infected individuals or normal controls. Additional studies will be needed to verify the in vivo significance of this markedly defective NK–DC cross talk among cells from HIV-1–infected viremic individuals to fully appreciate the potential negative impact on the immune-mediated control of HIV-1.

MATERIALS AND METHODS

Study subjects. Two cohorts of 15 viremic and 15 aviremic HIV-1–infected patients were studied (Table S1, available at http://www.jem.org/cgi/content/full/jem.20060894/DC1). The viremic group was composed of HIV-1–infected individuals who were either naive to therapy or had formerly been receiving ART but whose treatment regimen had been discontinued at the time of our study. The aviremic group had been receiving ART for at least 24 mo, and viremia remained undetectable over this time period. PBMCs were obtained by leukapheresis performed after obtaining signed consent forms and in accordance with clinical protocols approved by the Institutional Review Board (IRB) of the University of Toronto (13 patients) and of the National Institute of Allergy and Infectious Diseases (NIAID; 17 patients). As negative controls, PBMCs from 15 healthy donors seronegative for HIV-1 were obtained by apheresis provided by the Transfusion Medicine Department of the Mark O. Hatfied Clinical Research Center National Institutes of Health as a part of IRB-approved clinical studies (Table S1). All experiments performed in this study using cells derived from HIV-1–infected individuals and from healthy donors have been approved by the IRBs of the University of Toronto and the NIAID.

Isolation and culture of NK cells and generation of MDDCs. PBMCs were isolated over Ficoll-Hypaque gradients (lymphocyte separation medium; MP Biomedicals). NK cells were freshly isolated by negative selection (StemCell Technologies Inc.; reference 20). Purified NK cells contained ≤3% contamination with other PBMC subsets as determined by the expression of CD3, TCR-α/β, TCR-γ/δ, CD19, or CD14. CD56^{pos} or CD56^{neg} NK cell subsets were separated by a magnetic cell-sorting technique (Miltenyi Biotec; reference 23). The purities of CD56^{pos} or CD16^{neg} NK cell fractions were consistently >95 and 90%, respectively. Polyclonal NK cells and NK cell subsets were activated in vitro with recombinant IL-2 (rIL-2; Roche) at 200 UI/ml or with recombinant IL-15 (rIL-15; R&D Systems) at 10 ng/ml for 6 d (23).

To generate iDCs, monocytes were isolated from PBMCs by immunomagnetic selection (Miltenyi Biotec). The purity of CD14^{pos} and CD1a^{neg} monocytes was ≥98%. iMDDCs were then obtained by culturing the highly purified CD14^{pos} cells at 10[6] cells/ml in complete media plus IL-4 at 200 U/ml (PeproTech) and GM-CSF at 200 ng/ml (Leukine Liquid Sargramostin). After 6 d of stimulation in culture, CD14[dim-neg] and CD1a^{pos} iMDDCs were induced to undergo maturation by incubation with LPS at 1 µg/ml (Sigma-Aldrich) or sCD40 liter at 2 µg/ml (Biosource International) for 24 h.

mAbs. The following mAbs were used in this study: mAbs 289 (IgG2a anti-CD3), C218 (IgG1 anti-CD56), KD1 (IgG2a anti-CD16), Gl183 (IgG1 anti-p58.2/KIR2DL2), 11pb6 (IgG1 anti-p58.1/KIR2DL1), Z27 (IgG1 anti-p70/KIR3DL1), F278 (IgG1 anti-LIR1/ILT2), Z270 (IgG1 anti-NKG2A), Xa185 (IgG1 anti-CD94), Bab281 and KL247 (IgG1 and IgM anti-NKp46, respectively), Az20 and F252 (IgG1 and IgM anti-NKp30, respectively), Z231 and KS38 (IgG1 and IgM anti-NKp44, respectively), Kra236 and F5 (IgG1 and IgM anti-DNAM, respectively), L95 (IgG1 anti-poliovirus receptor), L14 (IgG2a anti-Nectin-2), and A6-136 (IgM anti-HLA-A/-B/-C). FITC- or PE-labeled anti-CD4 (IgG1), anti–TCR-α/β (IgG1), anti–TCR-γ/δ (IgG1), anti-CD19 (IgG1), anti-CD56 (IgG2b), anti-CD14 (IgG1), anti-CD1a (IgG1), anti-CD40 (IgG1), anti-CD80 (IgM), anti-CD83 (IgG1), anti-CD86 (IgG1), anti–HLA-A/-B/-C (IgG1), anti–HLA-DP/-DQ/-DR (IgG2a), and anti-TRAIL (IgG1) were purchased from BD Biosciences. The anti–Trail-R1, -R2, -R3, and -R4 mAbs were

purchased from R&D Systems, and the anti–HLA-E mAbs (IgG1) were purchased from Novus Biologicals.

Flow cytometry and cytolytic activity. For one- or two-color cytofluorimetric analysis (FACSCalibur; BD Biosciences), rIL-2–activated NK cells, iDCs, and mDCs were stained with the appropriate mAbs followed by PE- or FITC-conjugated isotype-specific goat anti–mouse second reagent (Southern Biotechnology Associates, Inc.). Second appropriate antiisotypic mAbs stained with PE and/or FITC were used as negative controls. The data were analyzed using CellQuest software (BD Biosciences). After 6 d of activation with rIL-2, NK cells were tested for cytolytic activity in a 4-h Cr[51] release assay as described previously (20). Saturating concentrations (10 µg/ml) of specific mAbs blocking NK cell receptors were added for the masking experiments performed with autologous iDCs. The NK cell/iDC ratio was 10:1.

Fluorescence microscopy. rIL-2–activated NK cells were stained with cell tracker CM-DiI (Invitrogen) and autologous iDCs with Hoechst 33342 (Invitrogen). Fluorescent cells were coincubated for 2 h in 1640 RPMI medium supplemented with 10% FCS in the presence of FLICA reagent (Invitrogen) on fibronectin-coated chamber slides (LAB-TEK) at 37°C and 5% CO[2] atmosphere. The NK cell/iDC ratio was 10:1. NK cells and iDCs were then washed with PBS, suspended in medium, and sealed on the slides with coverslips. Snap-shot images were captured on an inverted epifluorescence microscope (Axiovert 135; Carl Zeiss MicroImaging, Inc.) equipped with a filter for red, green, and blue dyes using a 20× plan-Apochromat objective. Imaris 4.04 software (Bitplane AG) was used for image processing.

NK cell proliferation assay. Freshly purified NK cells were cryopreserved until required as responders. Experiments were performed in triplicate in 96-well round plates with complete medium. NK cells were cocultured at a constant concentration of 2×10^5 NK cells/well with autologous and heterologous mMDDCs (stimulators) in serial dilutions ($10–1.56 \times 10^3$ cells/well). After 4 d, NK cell proliferation was measured by [^{3}H]thymidine uptake (16 h).

Cytokine secretion. The levels of IL-12p70 (IL-12), IL-10, -15, and -18 secreted by mDCs and the level of sTRAIL secreted by r-IL2–activated NK cells were measured from cell culture supernatant by ELISA (R&D Systems and Biosource International). To detect the production of IFN-γ, freshly purified NK cells were cryopreserved until required and cocultured with autologous LPS or sCD40L matured DCs in 96-well round-bottom plates with complete medium (9). The mDC/NK cell ratio was 1:10. The supernatant of the cultures was collected after 24 h and assayed by ELISA (BD Biosciences).

Statistical analysis. The distributions of each immune response variable were compared between uninfected and HIV-1–infected viremic and aviremic individuals using the Mann-Whitney test. For each individual, the differences between the CD56^{neg} and CD56^{pos} NK cell subsets were evaluated using the Wilcoxon signed ranks test. All p-values are two sided.

Online supplemental material. Fig. S1 shows the surface expression of maturation markers on iDCs and mDCs generated in vitro from representative donors within the three study groups. Fig. S2 shows the degree of NK cell–mediated lysis and CD56^{pos} and CD56^{neg} NK subset–mediated lysis of autologous iDCs from representative donors within the three study groups using the traditional Cr[51] release assay and fluorescence microscopy. Fig. S3 shows the NKp30 surface expression on freshly purified rIL-2– and -15–activated NK cells from representative donors within the three study groups. Fig. S4 shows the NKp30-dependent killing of autologous iDCs by rIL-2– and -15–activated NK cells from representative donors within the three study groups. Fig. S5 shows our proposed model of the impaired interactions between NK cells and DCs in HIV-1–infected viremic patients. Table S1 provides data on the profiles of viremic and aviremic HIV-1–infected patients and healthy donors. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20060894/DC1.

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