Killing of human immunodeficiency virus–infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules

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In the current study, we evaluated whether the capacity of HIV to modulate major histocompatibility complex (MHC) class I molecules has an impact on the ability of autologous natural killer (NK) cells to kill the HIV-infected cells. Analysis of HIV-infected T-cell blasts revealed that the decrease in MHC class I molecules on the infected cell surface was selective. HLA-A and -B were decreased on cells infected with HIV strains that could decrease MHC class I molecules, whereas HLA-C and -E remained on the surface. Blocking the interaction between HLA-C and -E and their corresponding inhibitory receptors increased NK cell killing of T-cell blasts infected with HIV strains that reduced MHC class I molecules. Moreover, we demonstrate that NK cells lacking HLA-C and -E inhibitory receptors kill T-cell blasts infected with HIV strains that decrease MHC class I molecules. In contrast, NK cells are incapable of destroying T-cell blasts infected with HIV strains that were unable to reduce MHC class I molecules. These findings suggest that NK cells lacking inhibitory receptors to HLA-C and -E kill HIV-infected CD8+ T cells, and they indicate that the capacity of NK cells to destroy HIV-infected cells depends on the ability of the virus to modulate MHC class I molecules. (Blood. 2004;104:2087-2094)

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Introduction

Natural killer (NK) cell–mediated cytotoxic responses seem ideal for controlling virus-infected cells during the very early stages of primary HIV infection. NK cells do not require prior recognition of HIV to eliminate the infected cells, whereas adaptive anti-HIV responses require prior sensitization and several days to develop. Moreover, the adaptive immune response does not reach its optimal effect until several weeks after infection. Thus, early effective restraint of the infected cells by NK cells is needed to keep the virus under control until the adaptive immune response has a chance to develop.

Although NK cells are part of the innate arm of the immune response, they are prevented from killing cells that express major histocompatibility complex (MHC) class I molecules, which engage inhibitory molecules on the NK cell surface. Three different classes of NK cell inhibitory receptors regulate the cytotoxic function of NK cells interact with different MHC class I molecules (for a review, see Borrego et al). Despite differences in specificity of the various NK cell inhibitory receptors, they all contain immunoreceptor tyrosine–based inhibitory motifs (ITIMs) in their cytoplasmic tails. The function of ITIMs is to recruit inhibitory phosphatases, such as Src homology domain–containing tyrosine phosphatases (SHP)–1 and SHP-2, which prevent activation and inhibit NK cell cytotoxicity. The signal provided by the inhibitory receptor is dominant over activation signals when activation and inhibitory molecules are engaged simultaneously. Therefore, the presence of MHC class I molecules on the cell surface serves to inhibit NK cell cytotoxicity.

HIV makes use of several mechanisms to evade the potent CD8+ T-cell response that develops soon after infection. One mechanism by which HIV-infected cells escape recognition and, thereby, destruction by CD8+ cytotoxic T-lymphocytes (CTLs) is the ability of the virus to decrease MHC class I molecules on the infected cell surface. Given that HIV-infected cells avoid CD8+ CTL destruction by down-modulating MHC class I molecules on the cell surface and that NK cells are able to kill cells with diminished MHC class I molecules, it follows that HIV-infected cells are susceptible to NK cell–mediated killing. However, we recently demonstrated that NK cells have limited ability to kill HIV-infected cells despite reduced numbers of MHC class I molecules.

A proposed mechanism accounting for the decreased ability of NK cells to kill HIV-infected cells is the selective reduction of HLA-A and -B molecules by HIV while it leaves HLA-C and -E on the infected cell surface. However, several studies suggest that HIV may be unable to regulate all NK cells by leaving HLA-C and -E on the infected cell surface because polyclonal NK cells do not always coexpress multiple MHC class I–specific inhibitory receptors. Even in patients with viremic HIV infection, it has been reported that only 14% of NK cells express inhibitory receptors that recognize HLA-E. Hence, HIV may, at best, be able to prevent NK cells expressing HLA-C and -E inhibitory receptors from destroying the infected cells, but not NK cells lacking these receptors. We determined in our study whether NK cells lacking inhibitory receptors directed to HLA-C and HLA-E are able to kill...
CD4+ T cells infected with HIV in which HLA-A and -B molecules are decreased.

Materials and methods

Human subjects
After informed consent was obtained, venous blood (50 mL) was acquired from healthy HIV-uninfected donors into heparinized (250 units; Sigma Chemical, St Louis, MO) 60-mL syringes (BD Biosciences, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood by Ficoll-Hypaque gradient (Mediatech, Herndon, VA) centrifugation, as described. The institutional review board for the protection of human subjects of State University of New York Upstate Medical University (Syracuse, NY) approved the studies described in this article.

HIV infection of CD4+ T cells
Primary CD4+ T cells were isolated from PBMCs using anti-CD4 antibodies coupled to magnetic beads according to the manufacturer’s instructions (Dynal, Lake Success, NY), resuspended at 3 × 10^6 cells/mL in complete T-cell medium, consisting of RPMI 1640 medium (Mediatech) with 10% heat-inactivated fetal bovine serum (FBS; Mediatech), 2 mM glutamine (Mediatech), 100 μg/mL streptomycin (Mediatech), 100 U/mL penicillin (Mediatech), and 200 U/mL recombinant human interleukin-2 (rhIL-2) (obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH). rhIL-2 from Dr Maurice Gatley, Hoffmann-LaRoche, Nutley, NJ)32 and stimulated with 3 mL of phytohemagglutinin (PHA; Sigma) for 3 days. PHA-stimulated CD4+ T cells were shown to be more than 98% CD4+ and less than 1% CD14+ by flow cytometry. Stimulated CD4+ T cells were spin-infected with 1000 10^6 cells/mL in complete T-cell medium, by negative selection using 15 μL each of PE-conjugated CD158a, CD158b, and CD94 antibodies (Beckman Coulter), 15 μL PE-conjugated anti-CD158a antibody (Beckman Coulter), 15 μL PE-conjugated anti-CD158b antibody (Beckman Coulter), and 15 μL FITC-conjugated CD94 antibody (PharMingen) were added to the cell suspension for 20 minutes on ice. As a negative control, 10 μL each isotype control antibody (PharMingen) was added to a suspension of PBMCs for 20 minutes on ice. Cells were washed twice in flow cytometry buffer and fixed in 500 μL PBS with 1% paraformaldehyde (pH 7.4). CD56+ cells (10^6) were acquired with a flow cytometer (LSRII; BDIS), and the percentage of CD56+ cells stained with various inhibitory receptors was determined using CellQuest Software (BDIS).

Isolation of NK cells and their subpopulations lacking HLA-C- and HLA-E–specific inhibitory receptors
NK cells were isolated from PBMCs obtained from the same HIV-uninfected subjects who donated blood for the generation of HIV-infected T-cell blasts. NK cells were acquired using anti-CD56 antibody coupled to magnetic beads, per the manufacturer’s instructions (StemCell Technologies, Vancouver, BC, Canada). This separation technique resulted in a population of cells that was more than 90% CD56+ (data not shown). NK cells were resuspended in RPMI 1640 medium with 10% heat-inactivated FBS, 2 mM glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin for the 3Cr release assay, added in triplicate in 100-μL aliquots to a 96-well round-bottom plate (Falcon, Franklin Park, NJ) at the proper effector to target cell (E/T) ratios, and placed at 37°C in a 5% CO2 humidified incubator overnight.

Two different approaches were used to isolate the NK cell subpopulations lacking inhibitory receptors to HLA-C and -E. In one study (Figures 5, 6A-B), NK cells were first isolated from PBMCs using anti-CD56 antibody coupled to magnetic beads and a rare earth magnet (StemCell Technologies). CD56+ cells were then depleted of CD94+, CD158a+, and CD158b+ cells (Figures 5 and 6A), or CD158a+, CD158b+, and CD158c+ (Figure 6B) by adding 0.75 μg/mL each purified mouse antibody (Beckman Coulter) per 10^6 isolated CD56+ cells for 30 minutes at 4°C. Antibody-labeled cells were incubated with sheep anti–mouse IgG magnetic beads according to the manufacturer’s instructions (Dynal) and were depleted of inhibitory receptor–positive cells using a magnet provided by the manufacturer (Dynal). In another study (Figure 6C-D), PBMCs were depleted of CD158a+, CD158b+, and CD158c+ by negative selection using 15 μL each of PE-conjugated CD158a, CD158b, and CD158c antibodies (Beckman Coulter) per 10^6 inhibitory receptor–positive PBMCs. PE-positive cells were negatively selected using the EasySep PE selection cocktail according to the manufacturer’s instructions (StemCell Technologies). NK cells were then isolated from the CD158a-, CD158b-, and CD158c-depleted PBMCs (negative fraction) using magnetic beads coupled to anti-CD56 antibody and a rare earth magnet (StemCell Technologies) according to the manufacturer’s instructions. The CD158a-b+ and CD158d-9a-depleted NK cells were shown to be routinely more than 90% CD56+ and less than 5% CD158a-b+ and CD94+ or CD158a-b+ and CD158a- by flow cytometry (data not shown). No difference in percentage of CD94+ and CD158a+ NK cells were then fixed and permeabilized using the Cytofix/Cytoperm kit (PharMingen), resuspended in 100 μL Perm-Wash buffer (PharMingen), and stained with 5 μL human anti-HIV p24 capsid antibody (obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: monoclonal antibody to HIV-1 p24 [clone 71-31] from Dr Susan Zolla-Pazner, New York University, New York, NY),37 followed by 2 μL FITC-conjugated goat anti-human IgG antibody (Beckman Coulter, Miami, FL) or 10 μL PE-conjugated goat anti–human IgG antibody (Caltag). All intracellular staining was performed for 20 minutes on ice, and cells were washed twice with 1 mL Perm-Wash buffer between each staining step. As negative controls for anti-HIV p24 antibody staining, cells were treated with secondary antibody only. Infected cells (106 HIV-1 p24 antigen-positive) or uninfected CD4+ cells (10^6) were acquired using a flow cytometer (LSRII; BDIS, San Jose, CA) and the level of CD4 or MHC class I molecules on the cell surface was determined using CellQuest Software (BDIS).
cells was observed, regardless of the method used to deplete inhibitory receptor–positive cells from the NK cells (data not shown). The inhibitory receptor-depleted NK cells were added in 100-μL aliquots to a 96-well round-bottom plate in RPMI 1640 medium with 10% heat-inactivated FBS, 2 mM glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin and were placed in a 37°C incubator with 5% CO₂ overnight before use in the cytotoxicity assay.

Isolation of HIV-infected primary T-cell blasts for use in the NK cell cytotoxic assay

HIV-infected primary T-cell blasts used as target cells in the 51Cr release assay were isolated as described previously. Briefly, the HIV-infected cells were separated from the uninfected cells using the method described, which is based on the observation that CD4 molecules are down-modulated on HIV-infected cells. Uninfected CD4⁺ T cells were removed with anti-CD4 antibody-conjugated to magnetic beads (Dynal), thus enriching for cells containing HIV (Figure 1). This method allows for the normalization of cells harboring HIV regardless of the percentage of HIV-infected cells acquired after 5-day infection of CD4⁺ cells with HIV. Cells remaining after separation were less than 1% CD4⁺ (Figure 1A). The CD4⁻ cells were shown to express HIV p24 antigen (Figure 1B). If the HIV strain reduced MHC class I molecules, the CD4⁺ and HIV p24 antigen-positive cells had decreased numbers of MHC class I molecules (Figure 1C-D, respectively [mean fluorescence intensity [MFI], 261.34 for uninfected CD4⁺ to 56.63 for HIV-infected or CD4⁻ cells]). HIV-infected (CD4⁻) cells were then washed 3 times in calcium- and magnesium-free Hank's balanced salt solution (HBSS) and used as target cells in a 51Cr release assay.

NK cell cytotoxicity assay

NK-sensitive K562 human erythroleukemia cells (American Tissue Culture Collection, Manassas, VA), uninfected CD4⁺ T-cell blasts, and HIV-infected T-cell blasts were used as target cells in a 4-hour 51Cr release assay, as described. K562 cells were labeled with 100 μCi (3.7 MBq) 51Cr (Amersham Biosciences, Piscataway, NJ) per 10⁶ cells for 2 hours, and uninfected CD4⁺ T-cell blasts and HIV-infected T cells were labeled with 125 μCi (4.63 MBq) 51Cr per 10⁶ cells for 2 hours.

When NK cell inhibitory receptors to HLA-C and -E were blocked (Figure 4), 10 μg/mL purified anti-CD158a, CD158h, and CD159a antibody (Beckman Coulter) was added to the NK cells before target cells were added. The percentage of specific lysis was calculated using the formula: [(sample cpm – mean spontaneous release cpm)/ (mean maximal release cpm – mean spontaneous release cpm)] × 100. The cpm of spontaneous release was typically 10% to 20% of the cpm of maximal release. The 2-tailed Student t test was used to determine statistical differences between the treatment and the control groups.

Results

Ability of HIV to modulate MHC class I molecules on primary T-cell blasts is strain dependent

In this study, we determined whether different HIV strains were able to decrease MHC class I molecules on primary CD4⁺ T cells. We evaluated the expression of MHC class I molecules using an antibody (clone W6/32) that recognized a conserved epitope found on all MHC class I molecules. During peak virus production, after 5-day infection with 1000 × TCID₅₀ of HIV-1SF162, HIV-1SF33, HIV-1SF128A, HIV-1SF2, and the primary HIV isolate HIV-192US712, we determined the extent to which MHC class I molecules were expressed. When we evaluated the expression class I molecules on HIV-infected (HIV p24 antigen-positive) T-cell blasts, we found that HIV-1SF162 was able to decrease MHC class I molecules on the cell surface (mean fluorescence intensity [MFI], 68.55) relative to the MHC class I molecules on uninfected CD4⁺ T cells (MFI, 100.72) (Figure 2A). In contrast to HIV-1SF162-infected cells, primary T-cell blasts infected with HIV-1SF13 (MFI, 110.77) had levels of MHC class I molecules similar to those found in the uninfected controls (MFI, 100.72) (Figure 2A). It should be noted that although there was no decrease in MHC class I molecules on HIV-1SF13-infected cells, there was a decrease in the percentage of infected cells expressing CD4 molecules and an increase in the percentage of HIV p24 antigen-positive cells compared with HIV-1SF162-infected cells (data not shown). Thus, the failure of HIV-1SF13 to decrease MHC class I molecules was not the result of inadequate infection but was inherent in the virus strain used for infection.

We found that the primary T-cell blasts infected with HIV-1SF128A (Figure 2B) and the primary HIV isolate HIV-192US712 (Figure 2C) had decreased expression of MHC class I molecules on the cell surface relative to the MHC class I molecules found on uninfected cells. In contrast, MHC class I molecules on HIV-1SF128A-infected cells were not decreased (Figure 2B). Thus, some strains of HIV down-modulate MHC class I molecules on primary T-cell blasts, whereas other strains are unable to alter MHC class I expression.

HLA-A and -B, but not HLA-C and -E, molecules are decreased on primary T-cell blasts infected with HIV strains that decrease MHC class I molecules

Because some HIV strains decrease MHC class I molecules, we determined whether the reduction in MHC class I molecules was selective or whether all MHC class I molecules were modulated. For this reason, we evaluated the expression of HLA-A, -B, -C, and -E on primary CD4⁺ T cells infected with HIV-1SF162 or HIV-1SF33. We
evaluated the HIV p24 antigen-positive cells for expression of the various HLA-class I molecules during peak virus production (5 days after infection). As seen in Figure 3, HIV decreased the expression of HLA-A2 on HIV-1SF162–infected (HIV p24+/H11001) T-cell blasts (MFI, 109.72) relative to uninfected CD4+/H11001 T cells (MFI, 218.79) (Figure 3A). Reduction of HLA-B7 was also observed on HIV-1SF162–infected (HIV p24+) cells (MFI, 5.70) compared with uninfected cells (MFI, 45.87) (Figure 3B). In contrast to the decreased levels of HLA-A and -B molecules on HIV-infected cells compared with uninfected cells, HLA-C and HLA-E on HIV-infected (HIV p24+) cells (MFI, 68.00 and 116.77, for HLA-C and -E, respectively) were not reduced relative to uninfected controls (MFI, 37.08 and 108.86, for HLA-C and -E, respectively [Figure 3C-D]). In contrast to HIV-1SF162–infected cells, we did not observe modulation of any type of HLA class I molecules on HIV-1SF33–infected cells (p24+) compared with uninfected cells (Figure 3E-H). Thus, when HIV decreased MHC...
class I molecules, the reduction was selective for HLA-A and -B but not for HLA-C and -E.

**Blocking CD158a, CD158b, and CD159a on NK cells from interacting with HLA-C and -E molecules on HIV-infected cells increases the ability of NK cells to kill the infected cells**

Given that HLA-C and -E prevent NK cells from destroying healthy cells,40-41 we determined whether polyclonal NK cells could kill HIV-infected cells directly when the NK cells were treated with blocking antibody to CD158a, CD158b, and CD159a. Although the heterodimer of CD94 and CD159a recognizes HLA-E, the ITIM motifs, important for inhibitory signaling, are contained on the CD159a molecule.40 Hence, in our study, only the interaction between CD159a and HLA-E was blocked. As expected,21 little if any killing of HIV-infected T cells by NK cells occurred, though the same NK cells could kill the NK-sensitive K562 cells (Figure 4). An increase in NK cell–mediated killing of HIV-infected cells was observed (P < .05) when they were treated with antibody to the inhibitory receptors before target cells were added to the effector cells (Figure 4). Although NK cells killed HIV-infected cells when HLA-C and -E inhibitory receptors were blocked, the killing was limited to 15.7% at an E/T ratio of 30:1 and was not statistically different from the response at an E/T ratio of 5:1 (P = .3) (Figure 4B-C).

**Determining whether NK cells lacking inhibitory receptors for HLA-C and -E kill HIV-infected autologous T-cell blasts**

Given that blocking HLA-C and -E and their inhibitory receptors increased NK cell killing (Figure 4), we sought to determine whether NK cells lacking inhibitory receptors to HLA-C and -E are able to destroy autologous CD4+ T cells infected with HIV-1SF162 and HIV-1SF36. We initially evaluated 8 subjects’ peripheral blood NK cells for the expression of inhibitory receptors recognizing HLA-C and -E (Table 1). Either CD158a or CD158b inhibitory receptor recognizes HLA-C molecules. CD94 is paired with CD159a as an inhibitory receptor recognizing HLA-E.40,41 Because CD94 is the marker that appeared to be expressed on the NK cells the least, and because between 92% and 98% of CD159a+ NK cells were CD94+ (data not shown), we evaluated CD94 as a marker for NK cell inhibitory receptors for HLA-E. In Table 1, we illustrate that of the 8 subjects evaluated, on average only 10.31% of the NK cells expressed inhibitory receptors that recognized HLA-C and -E molecules. Within the NK cell population, on average 19.44% expressed inhibitory receptors for HLA-C but not HLA-E, and 39.31% expressed inhibitory receptors for HLA-E but not HLA-C. However, 30.93% of the NK cells did not express CD94, CD158a, or CD158b receptors. Therefore, not all NK cells found in the peripheral blood express inhibitory receptors known to bind to HLA-C and -E.

Figure 4. Blocking the interaction between CD158a, CD158b, and CD159a on NK cells and HLA-C and -E molecules on infected cells increases the ability of NK cells to destroy HIV-infected autologous primary T cells. HIV-1SF162–infected T-cell blasts, uninfected CD4+ T cells, and K562 cells were used as target cells in a 4-hour cytotoxicity assay. Effector cells (NK cells) were isolated from the same donor from whom the CD4+ T cells were isolated and were incubated with or without 10 μg/mL purified anti-CD158, -CD158b, and -CD159a antibody before addition to target cells. Effector cell–target cell ratios of 1:1, 5:1, 10:1, and 30:1 were used. All samples were performed in triplicate. The Student t-test was used to determine the statistical differences between percentage of specific lysis of HIV-infected cells by NK cells in the absence or presence of anti-CD158a, -CD158b, and -CD159a antibody. *P < .05; **P < .01. ND indicates not done.
For this purpose, we evaluated the ability of NK cells lacking CD158a, CD158b, and CD159a from 2 different donors increased killing of HIV-1SF162-infected cells relative to unfractionated controls (Figure 5).

Given that HIV-1SF162 decreases HLA-A and -B but leaves HLA-C and -E on the surface (Figure 3) and that HIV-1SF33 was unable to decrease any of the MHC class I molecules (Figure 3), we compared the ability of NK cells lacking HLA-C and -E receptors to kill HIV-1SF162-infected cells and HIV-1SF33-infected cells. NK cells from 2 different donors lacking CD158a, CD158b, and CD159a/CD94 killed HIV-1SF162 infected cells by 16.5% (Figure 6A) and 10.8% (Figure 6B) at a 5:1 E/T ratio and by 20.7% (Figure 6A) and 16.5% (Figure 6B) at a 10:1 E/T ratio. In contrast, NK cells had little, if any, ability to kill HIV-1SF33-infected cells despite the ability of the same NK cells to kill the HIV-negative sensitive cell line K562 (Figure 6C-D). These studies indicate that the strain of HIV determines the susceptibility of HIV-infected cells to killing by NK cells lacking inhibitory receptors directed at HLA-C and -E.

Table 1. Distribution of NK cells expressing inhibitory receptors for HLA-C and HLA-E

<table>
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<tr>
<th>Subject</th>
<th>CD158a/b ** CD94 **</th>
<th>CD158a/b ** CD94 **</th>
<th>CD158a/b ** CD94 **</th>
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<td>10.31 ± 3.99</td>
<td>30.93 ± 3.83</td>
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PBMCs were isolated from 8 healthy HIV-uninfected subjects and were stained with a PE cyanin 5.1-conjugated antibody directed to the NK cell marker CD56, PE-conjugated anti-CD158a and -CD158b antibodies, and FITC-conjugated anti-CD94 antibodies. Mean ± SD values represent those of the subpopulations of NK cells from all subjects tested.

Discussion

The HIV strains in our study, HIV-1SF162 and HIV-1SF128A, which decreased MHC class I molecules on infected cells (Figure 2), used the CCR5 coreceptor (R5) for viral entry. In contrast, HIV strains (eg, HIV-1SF33) that do not decrease MHC class I molecules (Figure 3) are primary R5 isolates, HIV-1SF128A (Figure 2C), also decreased MHC class I molecules, indicating that reducing MHC class I molecules is an ability not limited to laboratory-adapted strains. The dominant HIV strains in HIV-infected asymptomatic persons are R5 strains. On the contrary, HIV isolated from persons whose disease progresses to AIDS typically has a broad range of coreceptor (ie, R5 and X4) usage, but the strains isolated from AIDS patients are typically X4 strains. Therefore, it would be of interest to determine the ability of primary NK cells to control autologous HIV-infected T cells from infected persons at various stages of disease.

When the different types of MHC class I molecules were evaluated on infected cells, HIV appeared to selectively decrease the CCR5 coreceptor (R5) in infected T-cell blasts used as target cells in a 4-hour cytotoxic assay. NK cells were depleted of CD158a, CD158b, and CD159a/CD94 before their use as effector cells in the cytotoxic assay. Effector cell–target cell ratios of 1:1, 5:1, and 10:1 were used. All samples were performed in triplicate. Student t test was used to determine the statistical differences between percentage of specific lysis of HIV-infected cells by NK cells depleted of cells bearing CD158a, CD158b, and CD159a, with percentage of specific lysis of HIV-infected cells by unfractionated NK cells containing CD158a, CD158b, and CD159a. *P < .05, **P < .01.
HLA-A and -B but not HLA-C (Figure 3A-C). Moreover, the nonclassical HLA-E molecules remained on the surface of the infected cell (Figure 3D). These observations were similar to those of other investigators, who demonstrated that HIV infection led to decreased HLA-A and -B expression but not HLA-C or -E expression.22 It should be noted that this previous study used infectious HIV clones and cell lines in which the CD4 and specific MHC class I molecules were stably transfected. However, our studies used primary PHA-stimulated CD4+ T cells infected with different strains of intact virus originally isolated from infected subjects. In addition, infected cells with relatively high levels of HIV p24 antigen expressed within the cell had greater reductions in HLA-A and -B molecules on the cell surface than infected cells with relatively low levels of HIV p24 antigen (data not shown).

Studies by others indicate that NK cells are unable to destroy HIV-infected cells because HLA-E and -C decrease NK cell responses by receiving inhibitory signals through CD94/CD159a, CD158a, or CD158b.25 However, we demonstrated that only some NK cells expressed inhibitory receptors to HLA-C and -E (Table 1). Furthermore, when we blocked the interaction between the MHC class I molecules and their receptors (Figure 4), we observed increased killing of HIV-infected T-cell blasts by NK cells. Hence, some NK cells may be, but are not exclusively, under the control of HLA-C and -E molecules on HIV-infected cells.

The rate of killing by unenriched NK cells is at best 6.3%. Therefore, it is difficult, even at an E/T ratio of 30:1 (Figure 4), to observe killing of HIV-infected cells in the Cr-release assay if the number of NK cells lacking these receptors make up approximately 30% of the population (Table 1). Enriching a subpopulation of NK cells that lacked inhibitory receptors to HLA-C and -E allowed us to determine whether the remaining NK cells were able to kill HIV-infected cells (Figures 5-6). We observed killing of HIV-infected cells with NK cells lacking HLA-C and -E inhibitory receptors (P < .05) when the virus used to infect CD4+ cells decreased MHC class I molecules.

The strain of HIV infecting CD4+ T cells determines the susceptibility of the infected cells to destruction by NK cells. When the T-cell blasts were infected with an HIV strain that decreased HLA-A and -B (Figure 3A-B), NK cells lacking CD94/CD159a, CD158a, and CD158b were able to destroy the HIV-infected cells (Figure 5A-B) to a greater degree than unenriched NK cells. In contrast, when HLA-A and -B were not decreased (Figure 3E-F), NK cells lacking CD94/CD159a, CD158a, and CD158b were unable to kill the infected cells (Figure 6C-D).

When we exposed the HIV-1SF33-infected cells to anti-HIV gp120 antibody, we observed at best 16.2% lysis of the infected cells by autologous NK cells at an E/T ratio of 10:1, even though 98% of the target cells were HIV-1 p24 antigen-positive (data not shown). No killing of HIV-1SF33-infected cells by NK cells was observed in the absence of anti-gp120 antibody in this same experiment. Thus, even though the killing of the X4 virus–infected cells by autologous NK cells in the presence of anti-HIV gp120 antibody (ie, antibody dependent cell–mediated cytotoxicity) is relatively low, it is comparable to previously observed levels of killing of HIV-1SF162–infected cells in Figure 6 (20.7% lysis). Moreover, other investigators have also shown approximately 30% specific lysis of HIV-1SF162 X4–infected autologous primary T cells by HIV antigen–specific CTL lines.

The variability in the number of HIV-1–infected cells and NK cells lacking HLA-C and HLA-E inhibitory receptors acquired from a subject limited the E/T ratios to 5:1 and sometimes 10:1. However, we observed increased lysis of the HIV-infected autologous T cells by CD158a/b and CD159a/CD94 NK cells over the unenriched population of NK cells, even at relatively low E/T ratios (Figure 5). The relatively low level of NK cell killing of HIV-infected T cells (compared with the killing of K562 cells) was not attributed to a low number of HIV-infected target cells, because the target cells were normalized for CD4-negative–infected cells that were HIV p24 antigen–positive (Figure 1). The best rate we observed of specific lysis of HIV-infected cells by autologous NK cells was 20.7%, at an E/T ratio of 10:1 (Figure 6). This level is nowhere near that observed for NK cell killing of K562 cells. Even though it is difficult to determine whether the level of killing observed in our study was physiologically relevant, NK cells that play a role in controlling herpes virus infection in humans can kill herpesvirus–infected autologous cells at a percentage of specific lysis of 30% to 40% when using NK cell clones as effector cells.45,46

Primary T-cell blasts infected with HIV strains that decrease MHC class I molecules are susceptible to killing by NK cells lacking receptors to HLA-C and -E. However, the killing of these infected cells may not be optimal. Based on our current study, NK cells appear to be important in the control of HIV strains that modulate MHC class I molecules (Figure 6) and may be responsible for curtailing the capacity of the virus to replicate when CTL responses are unable to do so.22,27 However, these responses appear to be limited to NK cells lacking inhibitory receptors specific to HLA-C and -E (Figures 4-6). Even when enriched for cells lacking CD158a/b and CD159a/CD94, the extent to which these NK cells destroyed HIV-infected cells, even under ideal conditions, appears to have been limited (Figures 5-6). NK cell killing of HIV-infected cells in the presence of blocking antibody was not greater with an E/T ratio of 30:1 than it was with an E/T ratio of 5:1 (Figure 4). Increased killing did not result from the ability of anti-CD158a, CD158b, or CD159a antibody to bind to the infected cell surface and to mediate antibody-dependent, cell–mediated cytolysis because we were unable to observe the expression of these molecules on the surfaces of infected T cells or uninfected CD4+ cells.48 Thus, there may be a limit to which NK cells lacking inhibitory receptors to HLA-C and -E can kill HIV-infected cells.

It may be that NK cells lacking inhibitory receptors to HLA-C and -E do not completely kill HIV-infected T lymphocytes because HIV can induce the expression of another nonclassical MHC class I molecule, HLA-G. HLA-G molecule expression on extravillous cytotrophoblasts is important during pregnancy because this molecule modulates maternal NK responses.49 Recent studies from our laboratory demonstrate that HIV can induce the expression of HLA-G on the HIV-infected T-cell surface though uninfected CD4+ T lymphocytes do not express HLA-G (M.I.B. and E.B., unpublished observations, June 2002). Approximately 65% of HIV-1SF365–infected cells expressed HLA-G, whereas approximately 95% of HIV-1SF33–infected cells expressed this nonclassical MHC class I molecule. Unlike the limited expression of HLA-A, -B, -C, and -E–specific inhibitory receptors on NK cells, almost all NK cells express the HLA-G–specific receptor (CD158d).30,31 and most NK cells express the CD158a/h inhibitory receptor, CD85j.32 Thus, HLA-G may limit NK cells lacking CD94/CD159a, CD158a and/or CD158b, or both from killing autologous HIV-infected primary T-cell blasts (Figures 5-6) by triggering CD158d and CD85j. Further studies to evaluate the role of HLA-G in regulating NK cell responses to HIV-infected cells are under way.
References


Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules

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