Functional advantage of educated KIR2DL1+ natural killer cells for anti-HIV-1 antibody-dependent activation

Running head: NK cell education and ADCC

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Abstract

Evidence from the RV144 HIV-1 vaccine trial implicates anti-HIV-1 antibody-dependent cellular cytotoxicity (ADCC) in vaccine-conferred protection from infection. Amongst effector cells capable of mediating ADCC are natural killer (NK) cells. The ability of NK cells to be activated in an antibody-dependent manner is reliant upon several intrinsic cellular factors. In general, NK cell mediated antibody-dependent activation is most robust in terminally differentiated CD57\(^+\) NK cells, as well as NK cells educated through ontological interactions between inhibitory killer immunoglobulin-like receptors (KIR) and their major histocompatibility complex class I (MHC-I or HLA-I) ligands. With regards to anti-HIV-1 antibody-dependent NK cell activation, previous research has demonstrated the epidemiological relevant KIR3DL1/HLA-Bw4 receptor/ligand combination confers enhanced activation potential. In the present study we assessed the ability of the KIR2DL1/HLA-C2 receptor/ligand combination to confer enhanced activation upon direct stimulation with HLA-I-devoid target cells or antibody-dependent stimulation with HIV-1 gp140-pulsed CEM.NKr-CCR5 target cells in the presence of an anti-HIV-1 antibody source. Amongst donors carrying the HLA-C2 ligand for KIR2DL1, higher IFN\(\gamma\) production was observed within KIR2DL1\(^+\) NK cells than in KIR2DL1\(^-\) NK cells upon both direct and antibody-dependent stimulation. No differences in KIR2DL1\(^+\) and KIR2DL1\(^-\) NK cell activation were observed in HLA-C1 homozygous donors. Additionally, higher activation in KIR2DL1\(^+\) than KIR2DL1\(^-\) NK cells from HLA-C2 carrying donors was observed within less differentiated CD57\(^-\) NK cells, implying observed differences were due to education and not an overabundance of KIR2DL1\(^+\) NK cells within the differentiated CD57\(^+\) NK cell population. These observations are relevant for optimizing antibody-based HIV-1 vaccines.
Introduction

A prophylactic vaccine is desired to reduce the number of new HIV-1 infections. Anti-HIV-1 antibodies capable of triggering antibody-dependent cellular cytotoxicity (ADCC) might be important to elicit through vaccination. Following binding of viral epitopes on the surface of HIV-1-infected cells, the constant regions of ADCC antibodies engage the CD16 constant region receptor on innate immune cells, such as NK cells and monocytes. Engagement of CD16 can result in the lysis of the HIV-1-infected target cell [1-3]. Additionally, NK cells activated upon stimulation through CD16 release chemokines and cytokines [4, 5]. Chemokines produced by activated NK cells can directly inhibit HIV-1 replication [6]. In the context of vaccination, ADCC would allow for the elimination of autologous cells that become infected upon HIV-1 exposure, as well as HIV-1-infected allogeneic cells delivered within infected bodily fluids. The modestly successful RV144 vaccine trial has indicated a role for ADCC-competent antibodies in vaccine-conferring protection against HIV-1 infection [7-9]. Indeed, ADCC antibodies were associated with a lower likelihood of infection in vaccinees that carried low levels of anti-envelope IgA, which could compete with IgG for antigen binding and block anti-HIV-1 ADCC [7, 9]. This observation highlights the need for further research into the factors regulating the ability of innate immune effector cells to mediate antibody-dependent functions. An improved understanding of the factors regulating NK cell-mediated anti-HIV-1 antibody-dependent functions will facilitate utilizing the full potential of ADCC antibodies in HIV-1 vaccine development.

The ability of NK cells to mediate anti-HIV antibody-dependent functions is dependent upon several factors intrinsic to the NK cell, including the education status and state of differentiation of the cell [10, 11]. The education status of an NK cell is determined through interactions of activating and inhibitory NK cell receptors with self-major histocompatibility complex class I (MHC-I or HLA-I) ligands, which tunes the functional potential of the NK cell [12-14]. In general, NK cells that express inhibitory receptors capable of binding self-HLA-I are tuned for higher functional potential; whereas, NK cells lacking inhibitory receptors or expressing inhibitory receptors that do not recognize self
HLA-I are tuned for reduced functional potential [12, 14]. Furthermore, NK cells expressing activating receptors capable of binding self HLA-I are tuned for lower functional potential [13]. The education status of NK cells is linked to their ability to become activated by antibody-dependent and independent stimuli [12]. With regards to anti-HIV antibody-dependent NK cell activation, our group has previously demonstrated that educated NK cells expressing the inhibitory killer immunoglobulin-like receptor 3DL1 (KIR3DL1), derived from donors carrying its HLA-Bw4 ligand, exhibit a functional advantage over autologous KIR3DL1+ NK cells and allogeneic KIR3DL1+ NK cells, derived from donors lacking its HLA-Bw4 ligand [11]. In addition to education, NK cells independently undergo a differentiation process whereby they phenotypically progress from CD56brightCD16−CD57− to CD56dimCD16−CD57−, and finally develop into CD56dimCD16+CD57−NK cells [15]. Along with changes in phenotype, the functional profile of NK cells is also altered by differentiation. Indeed, NK cells expressing the CD57 differentiation marker exhibit more robust activation upon stimulation through CD16 [10, 16]. Differentiated CD57+ NK cells are also more likely to express inhibitory KIRs [16]. Although differentiated NK cells expressing inhibitory KIR for self HLA-I would be educated for higher functional potential, the contributions of NK cell education and differentiation to NK cell antibody-dependent functional potential appear to be, at least partially, distinct [10].

Our previous research regarding the contribution of NK cell education to the antibody-dependent functional potential of NK cells focused upon the KIR3DL1/HLA-Bw4 receptor/ligand combination [10, 11, 17]. The results of those KIR3DL1/HLA-Bw4 studies corroborate epidemiological studies that have linked allelic combinations of this receptor/ligand pair to protection from HIV-1 infection and progression to AIDS [18, 19]. Several recent studies have now suggested that the inhibitory KIR2DL1 receptor might also be important for understanding susceptibility to HIV-1 infection and HIV-1 pathogenesis. The KIR2DL1 receptor binds a subset of HLA-C alleles, termed HLA-C2, which are characterized by the presence of lysine at amino acid position 80 [20]. Non-HLA-C2 alleles are termed HLA-C1 and are characterized by the presence of asparagine at amino acid position 80. Evidence for a potential role of KIR2DL1/HLA-C2 in protection from HIV-1 infection has
been provided by the observation that exposed-uninfected Senegalese carry the education-competent KIR2DL1/HLA-C2 combination, while their partners lack HLA-C2 ligands that could inhibit the recognition and cytolysis of HIV-1-infected allogeneic leukocytes [21]. Suggestive of a role for KIR2DL1 in understanding HIV-1 disease progression is the demonstration by Korner et al. that KIR2DL1+ NK cells are expanded in primary HIV-1 infection in individuals carrying the HLA-C2 ligand [22]. These expanded KIR2DL1+ NK cells also exhibited a functional advantage over KIR2DL1− NK cells upon stimulation with HLA-I-devoid target cells. Interestingly, and further indicative of importance for understanding HIV-1 pathogenesis, the frequency of KIR2DL1+ NK cells appears to wane during chronic HIV-1 infection. Indeed, this phenomenon has been demonstrated in HIV-1 clade C-infected South Africans and clade A and D-infected Ugandans [23, 24]. Additional research has demonstrated that while the functional advantage of NK cells expressing the HLA-C binding KIR2DL1/2/3 receptors upon stimulation with HLA-I-devoid target cells is observed in HIV-1-uninfected donors, this advantage is not present in chronically HIV-1-infected donors [25].

Given that recent evidence has implied that educated KIR2DL1+ NK cells might play a role in protection from HIV-1 infection and suppressing viral replication during primary HIV-1 infection, we set forth to assess if education of NK cells through KIR2DL1/HLA-C2 interactions enhanced the ability of NK cells to become activated in an anti-HIV antibody-dependent manner. We now present data demonstrating that education of NK cells through KIR2DL1/HLA-C2 interactions enhances NK cell activation upon exposure to antibody-dependent and antibody-independent stimuli. Furthermore, we demonstrate, through assessing CD57+ NK cells, that the functional advantage of educated KIR2DL1+ NK cells upon antibody-dependent stimulation is not a bystander effect of NK cell differentiation. These results enhance our understanding of the regulation of ADCC effector cells and will be important for understanding how to optimize vaccines that induce ADCC-competent antibodies.
Materials and methods

Participants

Blood was collected from 13 HIV-1-uninfected donors by forearm venepuncture into vacuettes containing sodium heparin anti-coagulant. Ficoll Paque PLUS (GE Healthcare Life Sciences) density gradient centrifugation was employed to obtain PBMCs from whole blood. These PBMCs were utilized as effector cells in NK cell activation assays. As a source of anti-HIV-1 antibodies, plasma was obtained from an HIV-1-infected client of the Melbourne Sexual Health Centre. This HIV-1-infected donor’s plasma has previously been shown to carry antibodies capable of activating NK cells in an anti-HIV-1-dependent manner [26]. All donors provided informed consent prior to collection of biological samples and the ethics committees of the participating institutions approved all performed experiments.

Cell lines

The CD4+ CEM.NKr-CCR5 T-cell line was obtained from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. The HLA-I-devoid 721.221 cell line was kindly provided by Dr. Andrew Brooks (Department of Microbiology and Immunology, University of Melbourne).

HLA-C typing and KIR2DL1 expression

The Victorian Transplantation and Immunogenetics Service at The Australian Red Cross Blood Service performed sequence-based typing of HLA-C alleles to four-digit resolution for all 13 donors. Expression of KIR2DL1 by NK cells from all 13 donors was demonstrated by staining with FITC-conjugated anti-KIR2DL1 antibody (clone: 143221; R&D Systems) and detection by flow cytometry using a BD LSR Fortessa. This antibody clone specifically detects KIR2DL1 and exhibits no cross-reactivity with other KIR2D or KIR3D gene products. Flow Jo Version 9.2 (Tree Star) was utilized for analysis of flow cytometry data.
**NK cell activation assays**

To study HIV antibody specific NK cell activation, we used a previously described flow cytometric assay to detect NK cell IFN\(\gamma\) expression [17]. Briefly, CEM.NKr-CCR5 target cells were prepared by coating with HIV-1 gp140\(_{AD8}\) (3\(\mu\)g/1.0X10\(^6\) cells in 1ml of solution) for 90 minutes at 4\(^\circ\)C. The HIV-1 gp140\(_{AD8}\) was prepared as previously described [27]. Next, PBMC effector cells were combined with gp140-pulsed CEM.NKr-CCR5 target cells at a 10:1 effector to target ratio in the presence or absence of a 1:2000 final dilution of plasma from an HIV-1-infected donor, Brefeldin A (5\(\mu\)g/ml) (Sigma) and monensin (6\(\mu\)g/ml) (BD). Co-cultures were incubated for five hours at 37\(^\circ\)C. Following incubation, cells were surface stained with Per CP-conjugated anti-CD3 (clone: SK7; BD), PE-Cy7-conjugated anti-CD56 (clone: NCAM16.2; BD), FITC-conjugated anti-KIR2DL1 (clone: 143221; R&D Systems) and Pacific Blue-conjugated anti-CD57 (clone: HCD57; Biolegend) antibodies. Next, cells were fixed in formaldehyde, permeabilized with 1X Perm solution (BD) and stained with Alexa Fluor 700-conjugated anti-IFN\(\gamma\) antibody (clone: b27; BD). Lastly, cells were fixed in formaldehyde and acquired using a BD LSR Fortessa (BD). Data was analysed with FlowJo Version 9.2. Antibody-independent NK cell activation was also assessed in an identical manner as anti-HIV antibody-dependent NK cell activation above, except PBMCs were cultured with the HLA-I-devoid 721.221 cell line in the absence of any antibody sources.

**Statistics**

GraphPad Prism version 4.0 was used for statistical analyses. Within group differences were compared using the Wilcoxon matched pairs test. Data throughout the manuscript is presented in the [median (range) vs. median (range)] format.
Results

Direct and anti-HIV-1 antibody-dependent activation of NK cells educated through KIR2DL1

The functional advantage of educated KIR2DL1+ NK cells over the KIR2DL1− population, which contains both uneducated NK cells and cells educated through other HLA/KIR combinations, has been observed upon direct stimulation, for both HIV-1-infected and uninfected donors, and non-HIV-1 antibody-dependent stimulation, for HIV-1-uninfected donors [12, 22, 28]. The role of education through KIR2DL1 on anti-HIV antibody-dependent activation potential, however, has not yet been investigated. To address this void we stimulated NK cell effectors within PBMCs, obtained from eight HLA-C2 carrying donors and five donors homozygous for HLA-C1 alleles (Table 1), with HIV-1AD8 gp140-coated CEM.NKr-CCR5 T-cells in the presence of plasma from an HIV-1-infected donor. Simultaneously, in order to demonstrate that the utilized HLA-C2 carrying donors, but not the HLA-C1 homozygous donors, exhibit the previously reported functional advantage within the educated KIR2DL1+ population upon direct stimulation, we stimulated NK cells within PBMC with the HLA-I-devoid 721.221 cell line. Following stimulation, samples were assessed by flow cytometry. The gating procedure used to identify KIR2DL1+ and KIR2DL1− NK cells, as well as the percentage of NK cells within each population that became activated to produce IFNγ is depicted in Figure 1A. As expected, upon stimulation with 721.221 targets the percentage of NK cells activated to produce IFNγ was higher in the KIR2DL1+ population than in the KIR2DL1− population for HLA-C2 carrying donors [16.2% (3.6-28.9%) vs. 10.4% (3.4-12.9%), p=0.0078] (Figure 1B). No differences in IFNγ production was observed between these NK cell populations in donors homozygous for HLA-C1 alleles [10.7% (5.2-16.9%) vs. 8.5% (7.3-14%), p=1.00] (Figure 1B). Similarly, when NK cells were stimulated in an anti-HIV-1 antibody-dependent manner, HLA-C2 carrying donors exhibited higher percentages of IFNγ producing NK cells in the KIR2DL1+ population than the KIR2DL1− population [6.6% (1.9-16.2%) vs. 3.5% (0.9-5.7%), p=0.0078] (Figure 1C). No differences were observed between these NK cell populations upon anti-HIV-1 antibody-dependent stimulation in donors homozygous for HLA-C1
alleles [6.2% (3.0-9.0%) vs. 6.2% (2.8-6.9%), \( p=0.6250 \)] (Figure 1C). These data reaffirm a role for NK cell education through KIR2DL1 in determining the ability of NK cells to exhibit activation upon direct stimulation. Further, the data suggest that the role of education in determining NK cell functional potential extends to the ability of NK cells to exhibit anti-HIV-1 antibody-dependent NK cell activation.

**Impact of NK cell differentiation on differences in activation between KIR2DL1⁺ and KIR2DL1⁻ NK cells**

The ability of NK cells to exhibit activation upon stimulation through CD16 is higher in NK cells expressing the CD57 differentiation marker [10, 16]. As higher percentages of CD57⁺ NK cells express KIRs, the relative contributions of education and maturation to antibody-dependent NK cell activation can become unclear upon assessments of the total NK cell population [10, 16]. As such, we next sought to assess the influence of KIR2DL1 expression on the function of both CD57⁺ and CD57⁻ NK cells within our donors. Coinciding with previously published data, we observed higher anti-HIV-1 antibody-dependent NK cell activation in the CD57⁺ NK cells than the CD57⁻ NK cells in all 13 donors [6.5% (1.4-13%) vs. 2.4% (0.6-4.4%), \( p=0.0002 \)] (Figure 2A) [10]. Additional assessments of CD57⁺ and CD57⁻ NK cells revealed a higher frequency of KIR2DL1 expression in the CD57⁺ population across all 13 donors [25.3% (8.4-50.4%) vs. 12.7% (3.5-32.1%), \( p=0.0002 \)] (Figure 2B). Lastly, we sought to confirm that the differences in anti-HIV-1 antibody-dependent activation observed between KIR2DL1⁺ and KIR2DL1⁻ NK cells in HLA-C2 carrying donors were due to education and not only as a result of NK cell differentiation. As such, we compared the anti-HIV-1 antibody-dependent activation of CD57⁺KIR2DL1⁺ and CD57⁻KIR2DL1⁻ NK cells within HLA-C2 carrying donors and HLA-C1 homozygous donors. As depicted in Figure 2C, within HLA-C2 carrying donors KIR2DL1⁺ NK cells within the CD57⁺ population exhibited higher levels of IFNγ production upon anti-HIV-1 antibody-dependent stimulation than the KIR2DL1⁻ population [4.6% (0.8-9.4%) vs. 2.2% (0.4-3.0%), \( p=0.0156 \)]. No differences were observed between the CD57⁺KIR2DL1⁺ and CD57⁻KIR2DL1⁻ populations in donors homozygous for HLA-C1 [3.1% (0.1-3.5%) vs. 2.4% (1.3-4.5%), \( p=0.3125 \)].
(Figure 2C). These data further reaffirm a role for NK cell education through KIR2DL1 in determining the ability of NK cells to exhibit anti-HIV-1 antibody-dependent activation.
Discussion

The data presented in this manuscript provide the first demonstration that education of NK cells through KIR2DL1/HLA-C2 combinations enhances the ability of NK cells to respond upon anti-HIV-1 antibody-dependent activation. Additionally, and in concordance with previous studies, we provide data demonstrating an activation advantage of the KIR2DL1+ NK cell population upon antibody-independent stimulation with HLA-I-devoid target cells [12, 22]. These observations are interesting in the context of recent studies implicating KIR2DL1/HLA-C2 combinations in providing protection from HIV-1 infection, or contributing to inhibiting viral replication during primary HIV-1 infection [21, 22]. Indeed, the data presented in the current manuscript could be of importance for understanding mechanisms contributing to protective outcomes upon HIV-1 exposure or infection.

Jennes et al recently put forth the notion that KIR2DL1/HLA-C2 combinations could contribute to protection from HIV-1 infection [21]. In a cohort of Senegalese couples concordant and discordant for HIV-1 infection the authors observed cognate ligand matches between inhibitory KIR in HIV-1 recipients of concordant couples and HLA-I in the HIV-1 donors. In the discordant couples, cognate ligand mismatches were observed between the inhibitory KIR of the uninfected partner and the HLA-I of the infected partner. The HIV-1 uninfected partners were observed to carry the education-competent KIR2DL1/HLA-C2 combination, while their HIV-1 infected partners tended to be HLA-C1 homozygous. The authors further demonstrated that NK cells carrying KIR mismatched to HLA-I expressed on CD4+ T-cells were capable of killing allogeneic CD4+ T-cells, suggesting that lack of ligands for inhibitory KIRs can result in enhanced direct recognition of HIV-1-infected allogeneic target cells and offer protection from HIV-1 infection. The data presented in the current manuscript, showing that educated KIR2DL1+ NK cells have an activation advantage for anti-HIV-1 antibody-dependent activation, might further explain the protection observed in Senegalese serodiscordant couples. Although HIV-1 exposed uninfected individuals do not carry anti-HIV-1 IgG within their sera, it has recently been shown that antibodies passively provided by HIV-1-infected mothers to their
children via breast milk can protect against virus transmission [29]. Others and we have recently observed anti-HIV-1 antibodies capable of activating NK cells and/or triggering ADCC in seminal plasma and vaginal fluids [30, 31]. As these antibodies are exchanged between HIV-1-infected donors and their uninfected partners upon exposure to HIV-1, it is possible that the NK cells within the exposed individual could utilize these antibodies to eliminate infected allogeneic lymphocytes or autologous lymphocytes infected early upon exposure.

Although the prospect of eliminating HIV-1-infected allogeneic lymphocytes via ADCC is supported by the observation that KIR/HLA-I mismatched NK cells can directly kill allogeneic CD4+ T-cells, the notion that autologous infected CD4+ T-cells can be targeted is complicated by the presence of cognate HLA-I ligands on CD4+ T-cells for the inhibitory KIRs expressed by educated NK cells. Indeed, Ward et al. demonstrated that the presence of HLA-C and HLA-E on HIV-1-infected CD4+ T-cells inhibits NK cell-mediated ADCC via triggering inhibitory signals through KIR2DL1/2/3 and NKG2A [32]. Despite this observation, several additional studies assessing ADCC or antibody-dependent NK cell activation, triggered by polyclonal anti-HIV-1 antibodies or therapeutic anti-tumor monoclonal antibodies, have demonstrated that antibody-dependent NK cell stimulation can at least partially overcome inhibitory signals through HLA-I/KIR combinations [11, 17, 33, 34]. Indeed, we have recently demonstrated that educated KIR3DL1+ NK cells from HLA-Bw4 carrying donors exhibit higher anti-HIV-1 antibody-dependent activation than KIR3DL1- NK cells upon stimulation with allogeneic CD4+ T-cells expressing HLA-Bw4 [17]. Unfortunately, the data in the current manuscript does not address if anti-HIV antibody-dependent activation of KIR2DL1+ NK cells overcomes the inhibitory signals that the presence of HLA-C2 would initiate, as the utilized CEM.NKrf-CCR5 target cells have been shown to be HLA-C1 homozygous (Dr. Nicole Bernard, McGill University, Personal communication). Further research is required to resolve why previous research indicated inhibition of anti-HIV-1 antibody-dependent NK cell functions occur through HLA-C and HLA-E interactions with KIR2DL1/2/3 and NKG2A, while we have reported anti-HIV-1 antibody-dependent stimulation at least partially overcomes inhibition through HLA-B and KIR3DL1 combinations. While these
differences could reflect intrinsic signalling intensity differences between KIR3DL1 and KIR2DL1/2/3 or NKG2A receptors, it is perhaps most likely that methodological differences account for the discrepancy. To assess anti-HIV-1 antibody-dependent responses Ward et al. utilized a pool of four monoclonal antibodies, while our studies implemented polyclonal anti-HIV-1 sera [11, 17, 32]. Smalls-Mantey et al. demonstrated that pooled monoclonal anti-HIV-1 antibodies mediate poor anti-HIV-1 ADCC compared to the polyclonal mixtures found in sera [35]. We hypothesize that the strength of the signal through CD16 determines the susceptibility of antibody-dependent NK cell responses to inhibition via inhibitory NK cell receptors.

In addition to possibly contributing to HIV-1 susceptibility, the enhanced function of educated KIR2DL1⁺ NK cells have been suggested to contribute to control of HIV-1 replication during primary HIV-1 infection [22]. Indeed, educated KIR2DL1⁺ NK cells are expanded during primary HIV-1 infection, and these cells exhibit potent activation, compared to KIR2DL1⁻ NK cells, upon direct stimulation with HLA-I-devoid 721.221 target cells. Future research should assess the ability of expanded educated KIR2DL1⁺ NK cells from primary HIV-1 infection to mediate anti-HIV-1 antibody-dependent activation. As anti-HIV-1 antibodies capable of activating NK cells are detected during primary HIV-1 infection, the anti-HIV-1 antibody-dependent activation potential of educated KIR2DL1⁺ NK cells might play a role in controlling early viral replication and establishing viral set point [36].

The data presented in this manuscript adds to a growing body of literature on the importance of NK cell education and the interplay of activating and inhibitory receptors in determining the potential of NK cells to mediate anti-viral functions. Although likely important for assisting with understanding HIV-1 susceptibility and pathogenesis, the role of NK cell education in tuning anti-HIV-1 antibody-dependent activation potential might also be useful for designing anti-HIV-1 vaccines. Indeed, understanding the regulation of effector cells mediating anti-HIV-1 antibody-dependent responses will assist with optimizing antibody-based vaccination protocols.
References


27. Center RJ, Wheatley AK, Campbell SM, Gaeguta AJ, Peut V, Alcantara S, Siebentritt C, Kent SJ, Purcell DF. Induction of HIV-1 subtype B and AE-specific neutralizing antibodies in mice and


Figure 1. Direct and anti-HIV-1 antibody-dependent activation of KIR2DL1⁺ and KIR2DL1⁻ NK cells from HLA-C2⁺ and HLA-C1 homozygous donors. (A) Direct and anti-HIV-1 antibody-dependent activation of NK cell effectors within PBMC was accomplished by stimulation with the HLA-I-devoid 721.221 cell line or HIV-1AD8 gp140-pulsed CEM.NKr-CCR5 in the presence of anti-HIV-1 antibodies, respectively. Following stimulation PBMCs were stained with fluorochrome-conjugated antibodies and assessed by flow cytometry. The FACs plots depict progressive gating upon lymphocytes (Top Left), CD3⁻CD56⁺ NK cells (Top Middle), KIR2DL1⁺ and KIR2DL1⁻ NK cell subsets (Top Right), and the assessment of gated cells for IFNγ production in the non-stimulated (Bottom Left), 721.221 stimulated (Bottom Middle) and anti-HIV-1 antibody dependent stimulated conditions (Bottom Right). (B) Graphs depict the relative activation of the KIR2DL1⁺ and KIR2DL1⁻ NK cell subsets upon activation by the 721.221 cell line in eight donors carrying HLA-C2 alleles (Left) and five HLA-C1 homozygotes (Right). (C) Graphs depict the relative activation of the KIR2DL1⁺ and KIR2DL1⁻ NK cell subsets upon anti-HIV-1 antibody-dependent activation in eight donors carrying HLA-C2 alleles (Left) and five HLA-C1 homozygotes (Right).

Figure 2. The anti-HIV-1 antibody-dependent activation advantage of differentiated NK cells and the role of differentiation in the activation advantage of educated KIR2DL1⁺ NK cells. (A) The FACs plot depicts the gating utilized to identify the differentiated CD57⁺ and less differentiated CD57⁻CD56dim NK cell populations. The graph highlights the relative ability of CD57⁺CD56dim and CD57⁻CD56dim NK cells from all 13 donors to exhibit anti-HIV-1 antibody-dependent activation. (B) The graph depicts the relative percentages of CD57⁺CD56dim and CD57⁻CD56dim NK cells expressing the KIR2DL1 receptor in all 13 donors. (C) The graphs depict the relative anti-HIV-1 antibody-dependent activation of KIR2DL1⁺ and KIR2DL1⁻ NK cells within the CD57⁺CD56dim NK cell population in eight HLA-C2⁺ donors (Left) and five HLA-C1 homozygotes (Right).
Table 1. HLA-C typing of KIR2DL1 carrying effector cell donors.

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Figure 1 A.
B. 721.221 Stimulation

C. Anti-HIV Antibody-Dependent Stimulation
Figure 2 A.

SL_CEM GrBread, <710-50Blue-A>, <780-60Yellow-A> subset

p<0.01
C.

p<0.05

ns