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Conferral of Enhanced Natural Killer Cell Function by KIR3DS1 in Early Human Immunodeficiency Virus Type 1 Infection▽

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A flurry of recent reports on the role of activating and inhibitory forms of the killer cell immunoglobulin-like receptors (KIR) in natural killer (NK) cell activity against human immunodeficiency virus type 1 (HIV-1) have yielded widely divergent results. The role of the activating NK receptor encoded by the *KIR3DS1* allele and its putative ligands, members of the HLA class I *Bw4Ile80* cluster, in early HIV-1 disease is controversial. We selected 60 treatment-naïve adults for study from the OPTIONS cohort of individuals with early HIV-1 infection in San Francisco. We performed NK cell functional assays measuring gamma interferon (IFN-γ) and CD107a expression by NK cells in the unstimulated state and after stimulation by the major histocompatibility complex class I-deficient 721.221 B-lymphoblastoid cell line. In addition, we measured CD38 expression (a T-cell activation marker) on T and NK cells. Persons who have at least one copy of the *KIR3DS1* gene had higher IFN-γ and CD107a expression in the unstimulated state compared to those who do not possess this gene. After stimulation, both groups experienced a large induction of IFN-γ and CD107a, with *KIR3DS1* carriers achieving a greater amount of IFN-γ expression. Differences in effector activity correlating with *KIR3DS1* were not attributable to joint carriage of HLA *Bw4Ile80* and *KIR3DS1*. We detected a partial but not complete dependence of KIR3DS1 on the members of B*58 supertype (B*57 and B*58) leading to higher NK cell function. Possessing *KIR3DS1* was associated with lower expression of CD38 on both $CD8^+$ T and NK cells and with a loss or weakening of the known strong associations between $CD8^+$ T-cell expression of CD38 mean fluorescence intensity and the HIV-1 viral load. We observed that possessing *KIR3DS1* was associated with higher NK cell effector functions in early HIV-1 disease, despite the absence of HLA *Bw4Ile80*, a putative ligand of KIR3DS1. Carriage of *KIR3DS1* was associated with diminished $CD8^+$ T-cell activation, as determined by expression of CD38, and a disruption of the traditional relationship between viral load and activation in HIV-1 disease, which may lead to better clinical outcomes for these individuals.

NK cell function is regulated by a family of receptors encoded by the killer cell immunoglobulin-like receptor (*KIR*) genes (18, 33). Within the *KIR* family, certain genes encode inhibitory receptors that recognize HLA class I ligands (i.e., HLA-Bw4 or HLA-C), whereas other *KIR* genes encode activating receptors which are not completely known. Studies on the role of KIRs in human immunodeficiency virus (HIV) disease have focused on the activating receptor encoded by the *KIR3DS1* allele. However, recent genetic association and functional studies of KIR and HIV disease have yielded widely disparate results on the role of *KIR3DS1* and its putative ligands, a subset of HLA class I-B alleles referred to as Bw4Ile80. The Bw4Ile80 cluster is a subset of HLA-B alleles that bear an isoleucine at position 80 in the α-1 helix, on the rim of the peptide-binding cleft. The inhibitory receptors encoded by *KIR3DL1* alleles, which are highly related in the extracellular domains to the activating receptor encoded by *KIR3DS1*, specifically recognize HLA-Bw4 ligands (5). Because of this similarity, KIR3DS1 has been assumed to also recognize Bw4Ile80 ligands. In 2002, Martin et al. reported that HIV-infected individuals in the Multicenter AIDS Cohort Study possessing the *KIR3DS1* allele demonstrated significantly delayed progression to AIDS, provided that the individuals also expressed a *Bw4Ile80* allele (20).

In 2005, Gaudieri et al. reported on the association of the entire *KIR* gene cluster and HLA class I in HIV disease progression in an Australian HIV cohort (8, 9). These authors observed a trend toward slowed $CD4^+$ T-cell percent loss among those who carried both *Bw4Ile80* and *KIR3DS1* (8). However, this trend was not statistically significant, and Gaudieri et al. simultaneously observed an acceleration of time to AIDS (1987 definition) among joint *KIR3DS1* and *Bw4Ile80* carriers. In 2006, Qi et al. published a follow-up report from the Multicenter AIDS Cohort Study cohort documenting an association between the coexpression of *KIR3DS1* and *Bw4Ile80* and enhanced protection against certain opportunistic infections in HIV-infected individuals (26), an effect partially attributed to very modest differences in viral load. In 2007, our group observed that *KIR3DS1* gene carriage was associated with higher $CD4^+$ T-cell counts and hence protec-

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tion against HIV type 1 (HIV-1) progression in early disease (4); however, we observed that this effect was not attributable to differences in the viral load and further was independent of *Bw4Ile80*. In other words, our analyses suggested that the *KIR3DS1* and *Bw4Ile80* genes were each associated with protection against HIV disease but via different mechanisms.

Until recently, it was not clear if KIR3DS1 was expressed on the surface of NK cells; however, two recent reports have conclusively established that KIR3DS1 is expressed on NK cells (6, 24) and that expression is dose dependent, with higher expression for homozygotes. These studies also demonstrated that KIR3DS1 recognizes neither HLA-Bw4 nor HLA-Bw6 ligands, at least when these major histocompatibility complex (MHC) class I molecules are expressed on Epstein-Barr virus-transformed B-lymphoblastoid cell lines. Similarly, an independent study by another group reported that KIR3DS1 fails to bind to soluble Bw4Ile80 tetrameric complexes (10). In contrast, Alter et al. have presented results from in vitro cytotoxicity assays suggesting that target cells possessing HLA-Bw4Ile80 are better targets for NK cells possessing *KIR3DS1* (1); however, no evidence was provided to confirm a physical interaction between the KIR3DS1 and HLA-Bw4 proteins.

Here, we present a study of the NK cell phenotype and function in 60 treatment-naïve, recently HIV-1-infected persons with defined HLA-B and *KIR3DS1*/*KIR3DL1* allotypes. We also measured the expression of CD38 on NK cells and $CD8^+$ T cells, a widely used marker of disease progression and virulence in HIV research and a marker of immune activation. The expression of CD38, as measured by flow cytometry, is known to be elevated on $CD8^+$ T cells in HIV disease, reaching steady-state levels in early HIV-1 infection (7), and predicts disease progression independently of the viral load (19). The individuals studied were selected from our recent genetic association study of KIR and HLA among 255 recently HIV-1-infected persons (4), in which *KIR3DS1* carriage alone was associated with higher $CD4^+$ T-cell counts, despite the absence of a difference in the viral loads. On the basis of these clinical findings, we performed this study to determine whether persons who carried the *KIR3DS1* gene had enhanced NK cell phenotypic and functional profiles and if these profiles were further enhanced by carriage of the putative KIR3DS1 ligands encoded by *HLA-Bw4Ile80* alleles. Flow cytometry-based detection of KIR3DS1 has been hampered by the absence of a monoclonal antibody that can bind to KIR3DS1 specifically and not cross-react with the related KIR3DL1 proteins (25). Hence, we used genotypic KIR assignments for our analyses rather than flow cytometry-based methods.

MATERIALS AND METHODS

Study overview. We selected 60 adults from the OPTIONS cohort study of early HIV-1 infection in San Francisco (3). All persons gave informed consent to participate in this study, and this study was approved by the University of California, San Francisco (UCSF), Committee on Human Research. Individuals were selected on the basis of having known HLA-A and -B types, *KIR3DS1* and *KIR3DL1* genotype assignments, a record of clinical parameters of HIV-1 infection prior to treatment, and banked, viably frozen peripheral blood mononuclear cells (PBMCs). The date of estimated HIV-1 infection was derived through an algorithm developed by F. M. Hecht and is based on the time of detection of HIV-1 RNA, the level and timing of the results of the detuned HIV-1 enzyme immunosorbent assay (14, 15), the timing of prior HIV-1 test results, self-reported HIV-1 exposures, and symptomatology consistent with acute HIV-1 infection.

Determination of *KIR3DS1* and *KIR3DL1* genotypes. We determined the *KIR3DS1* and *KIR3DL1* genotypes as previously described (4).

Cell staining and flow cytometric analysis. Cryopreserved PBMCs were thawed and washed with phosphate-buffered saline supplemented with 1% bovine serum albumin and 2 mM EDTA (FACS buffer). For staining, 5×10^5 cells were incubated with purified human immunoglobulin G (100 μg/ml) to block nonspecific binding. To define NK cells, we stained the cells with phycoerythrin-Texas Red-conjugated anti-CD3, Alexa700-conjugated anti-CD4, phycoerythrin-Cy7-conjugated anti-CD56, and Pacific Blue-conjugated anti-CD16. Anti-CD14 and anti-CD19 (both conjugated to allophycocyanin-Cy7) were used to collectively exclude monocytes and B cells. Freshly thawed or stimulated PBMCs were stained for cell surface antigens (CD3, CD4, CD8, CD14, CD19, CD56, and CD16; all antibodies were from Becton Dickinson, San Jose, CA) and stained with Amine Aqua (Invitrogen) to exclude dead cells. Cells were fixed in 2% paraformaldehyde and permeabilized with FACS-perm (Becton Dickinson, San Jose, CA), with the exception of CD3–phycoerythrin-Texas Red (Beckman Coulter, Miami, FL). Permeabilized cells were stained for intracellular gamma interferon (IFN-γ). Fluorescence minus one samples were prepared for each fluorochrome to facilitate gating (28). All cells were fixed with 2% paraformaldehyde and analyzed by flow cytometry with a four-laser LSR-II instrument (Becton Dickinson, San Jose, CA). Anti-mouse immunoglobulin G-coated beads were stained with each fluorochrome-conjugated mouse antibody separately and used for software-based compensation.

Cell culture and antigenic stimulation. Cryopreserved PBMCs stored by the UCSF/ARI AIDS Specimen Bank were thawed and used for measurements of NK cell frequency, number, and receptor expression. The thawed cells were washed with RPMI 1640 medium supplemented with 15% fetal bovine serum before staining or stimulation. NK cells and T-cell functions were assessed by cytokine flow cytometry. To measure NK cell function, PBMCs were cultured in medium alone or with MHC class I-deficient 721.221 cells (1:5 ratio). PBMCs cultured in medium alone were used for the measurement of endogenous NK cell function. Briefly, a 100-μl volume of thawed PBMCs was cultured at 5×10^5/ml in 96-well plates with the respective stimulant and 10 μg/ml fluorescein isothiocyanate-conjugated anti-CD107a antibody (Becton Dickinson, San Jose, CA) for 24 h; during the last 6 h of culture, monensin and brefeldin A were added to allow intracellular accumulation of cytokines. Data analysis was performed by using FlowJo flow cytometric analysis software (Tree Star, Ashland, OR). Statistical analysis was performed by using SAS System 9 for Windows XP (SAS Institute, Cary, NC) or GraphPad Prism statistical software (GraphPad Software, San Diego, CA). The Mann-Whitney U or unpaired *t* test with Welch's correction (which does not assume equal variances) was used for comparisons based on the results of Kolmogorov Smirnov normality tests. The Spearman rank test was used for correlation analyses.

RESULTS

Study sample description. Demographic and baseline characteristics of the patient population are shown in Table 1. The persons selected for this study did not differ from other members of the OPTIONS early-infection cohort with respect to age, gender, ethnicity, or KIR or HLA type (4). Forty-three percent of the cohort (26 persons) had at least one copy of *KIR3DS1*. Four patients were homozygous for *KIR3DS1*. Fifty percent, or 30 persons, possessed at least one copy of a HLA-*Bw4Ile80* gene, the putative ligand for KIR3DS1. Twenty percent of the cohort, or 12 persons, carried at least one copy of both *KIR3DS1* and *Bw4Ile80*. We detected no difference in the estimated length of infection at the time of this study by *KIR3DS1* carriage ($P = 0.25$), joint *KIR3DS1* and *Bw4Ile80* carriage ($P = 0.8$), *Bw4Ile80* carriage ($P = 0.4$), *B*58 supertype* carriage ($P = 0.6$), or joint *KIR3DS1* and *B*58 supertype* carriage ($P = 0.2$) (Mann-Whitney U test).

NK cell effector measurements. Staining and flow cytometry gating strategies for NK cell assignment and measurement of function are shown in Fig. 1. We observed that persons with at least one copy of the *KIR3DS1* gene had higher IFN-γ and CD107a expression on NK cells in the basal, unstimulated state than those who did not bear at least one copy (Fig. 2A, column

TABLE 1. KIR and HLA genetics and characteristics of treatment-naïve recently HIV-1-infected adults

Variable	Median (IQR[a]) or %	No. of patients
Age (yr)	35 (32, 39)	60
HIV-1 RNA (log_{10} copies/ml)	4.7 (3.8, 5.1)	60
$CD4^+$ T-cell counts/μl	552 (462, 748)	60
Estimated time (wk) infected[b]	13.1 (10.1, 19.1)	60
Male gender	97	58
Ethnicity		
Asian	8	5
Caucasian	68	41
African-American	8	5
Latino	15	9
Gene carriage		
KIR3DS1	43	26
Homozygous	7	4
KIR3DL1	93	56
Homozygous	57	34
HLA class I *Bw4*	67	40
Bw4Ile80	50	30
KIR3DS1 and *Bw4Ile80*	20	12
*HLA-B*57*	12	7
*HLA-B*58*	7	4
*B*58 supertype*	18.3	11
KIR3DS1 and *B*58 supertype*	5	3

[a] IQR, interquartile range (25th, 75th percentiles).
[b] Estimated time since HIV-1 infection (see Materials and Methods for reference).

1). We stimulated PBMCs from these individuals by coculture with the MHC class I-deficient 721.221 B-lymphoblastoid cell line (column 2, all panels, Fig. 2). 721.221 cells do not express any MHC class I proteins (HLA-A, HLA-B, HLA-C, HLA-E, or HLA-G) on their surface; therefore, they fail to express any known ligands for the inhibitory and activating KIR. Although these 721.221 stimulator cells lack all MHC class I proteins, we observed that NK cells from individuals with at least one copy of *KIR3DS1* had higher IFN-γ expression, and equivalent CD107a expression, compared with those who did not bear *KIR3DS1* (and are therefore *KIR3DL1* carriers) (Fig. 2A, column 2). KIR3DS1 homozygotes did not differ from KIR3DS1 heterozygotes by IFN-γ or CD107a measurements in the stimulated or unstimulated state (data not shown). As we have previously observed in the larger cohort of 255 patients from which these patients were drawn (4), we did not detect a difference in viral loads between the *KIR3DS1* carriers and those negative for this gene ($P = 0.62$). In the subsample, we observed no difference between the viral loads of those with joint carriage of *KIR3DS1* and *Bw4Ile80* ($P = 0.55$) and those who carried either one of the genes or neither, nor did we detect a difference in the viral loads of *KIR3DS1* carriers who did or did not possess *Bw4Ile80* ($P = 0.2$).

Discerning the role of Bw4Ile80 in *KIR3DS1*-associated NK cell function. We then compared IFN-γ and CD107a expression in NK cells from individuals with defined KIR and Bw4Ile80 haplotypes. As shown in Fig. 2B, we compared those who carried both *KIR3DS1* and *Bw4Ile80* to those carrying only one of these genes or neither gene. This was the same category definition used for comparisons in the genetic association studies reported by Martin et al. (20). We observed that carriers of this compound genotype, that is, those who carried both *KIR3DS1* and *Bw4Ile80*, had higher IFN-γ and CD107a expression levels than those who were not carriers of both genes (Fig. 2B).

Does possession of *Bw4Ile80* augment *KIR3DS1*-associated NK cell effector function? As shown in Fig. 2C, we performed one further comparison, in which we restricted the analysis to individuals carrying *KIR3DS1*. We sought to determine if those who bear both *KIR3DS1* and a *Bw4Ile80* allele had higher levels of IFN-γ or CD107a expression, in either the unstimulated or the stimulated state, than those who possess *KIR3DS1* but not *Bw4Ile80*. By making this comparison, we were testing to see if the presence of a putative activating ligand for KIR3DS1 is associated with greater effector activity in the unstimulated or stimulated state. This is a more direct test of KIR3DS1 dependence on the Bw4Ile80 ligand than that shown in Fig. 2B. When this comparison was made, we observed no difference in the levels of IFN-γ or CD107a expression by NK cells among *KIR3DS1* carriers who did or did not carry a *Bw4Ile80* allele (Fig. 2C). Taken together, our data suggest that *Bw4Ile80* carriage does not influence the functional profile of NK cells among persons who bear *KIR3DS1*.

Role of *HLA-B*57* and *-B*58* in *KIR3DS1*- or *KIR3DL1*-associated NK cell activity. In our study population, 11 persons carried either the *HLA-B*57* or the *HLA-B*58* allele (Table 1), which form the *B*58 supertype* (31). We used the *B*58 supertype*, rather than either allele alone, to increase the power of our statistical analysis and hence our ability to detect differences in NK cell activity, should they exist. *B*58 supertype* carriers are distinct from the individuals possessing other alleles within the *Bw4Ile80* cluster by virtue of their independent association with greatly slowed HIV disease progression. A recent report suggested that B*57 interacts differentially with specific KIR allotypes to confer added protection (22). We sought to determine if there was evidence of enhancement of joint carriage among *B*58 supertype* and *KIR3DS1* carriers. We compared joint *KIR3DS1* and *B*58 supertype* carriers to those who carried *KIR3DS1* but not a *B*58 supertype* allele. In an analysis limited by sample size (only three persons carried both *KIR3DS1* and *B*58 supertype*), we observed that joint carriers had higher NK CD107a levels in the unstimulated state (5.9% versus 3.5% [$P = 0.04$]) and higher CD107a and IFN-γ levels when cocultured with MHC class I-deficient 721.221 target cells (28.4% versus 16.2% [$P = 0.04$] and 8.4% versus 2.6% [$P = 0.007$], respectively). We observed no difference in IFN-γ levels in NK cells in the unstimulated state (0.56% versus 0.51% [$P = 0.9$]). *B*58 supertype* carriage did not account for the generally higher NK cell functional measurements among the *KIR3DS1* carriers.

CD38 expression on NK cells and $CD8^+$ T cells in early HIV-1 infection and the role of *KIR3DS1*. We examined the expression of CD38 on NK cells and $CD8^+$ T cells by measuring the median fluorescence intensity (MFI) by flow cytometry. T-cell levels of CD38 achieved in early infection are an effective proxy for the risk of disease progression (7). We evaluated whether the KIR type might influence CD38 expression levels on $CD8^+$ T cells and NK cells. The significance of CD38

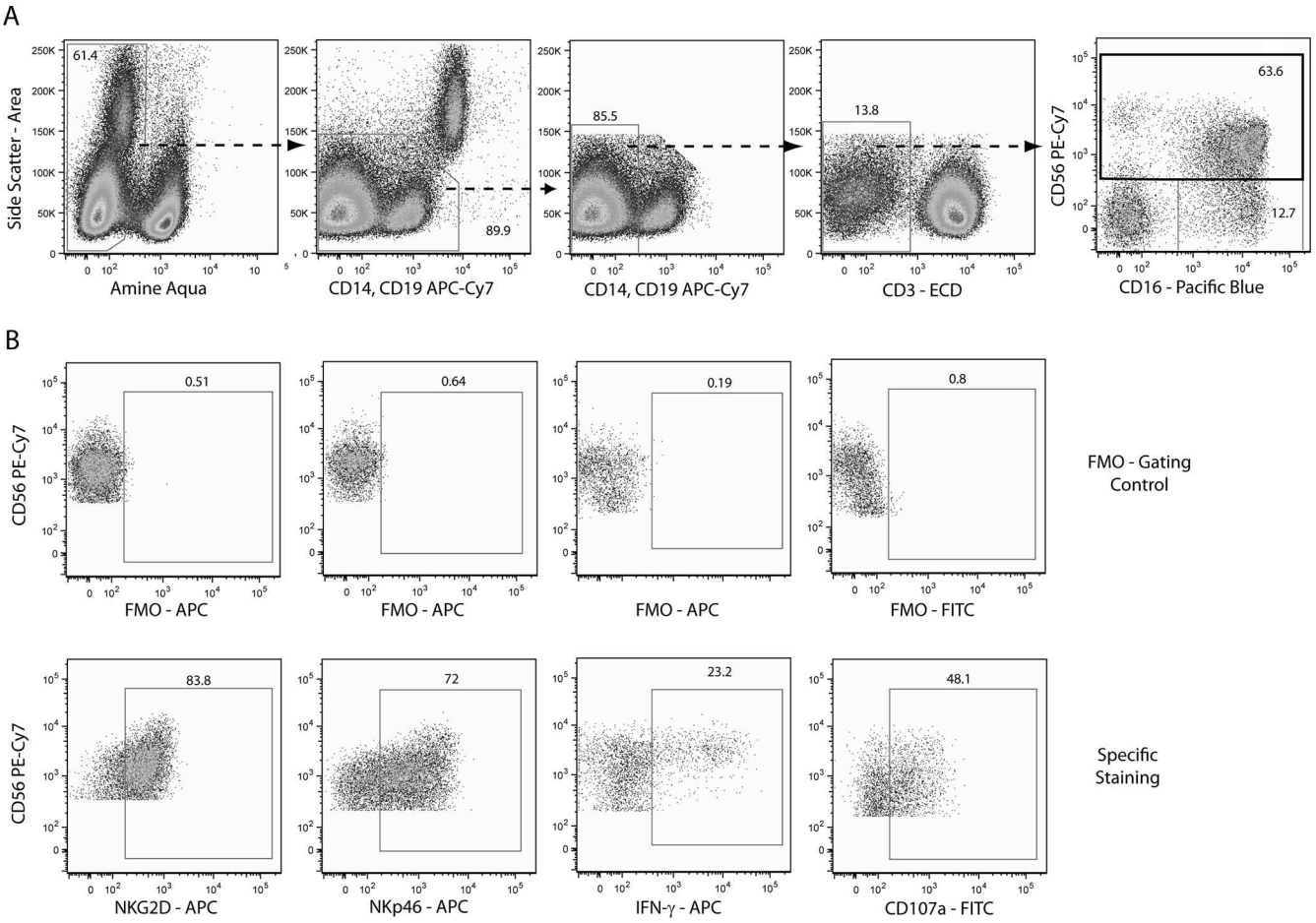

FIG. 1. Gating strategy to identify NK cell populations and functional measurements. (A) Dead cells were excluded by using amine-reactive dye, and monocytes and B cells were excluded on the basis of CD14 and CD19 gating, respectively. $CD4^+$ and $CD8^+$ T cells and $CD3^-$ cells were gated from the CD14- and CD19-negative lymphocyte population, and NK cells were derived from the $CD3^-$ gate on the basis of the expression of CD16 and CD56. NK cells were then subdivided into $CD56^{bright}$ and $CD56^{dim}$. (B) Cells positive for CD16 and CD56 were analyzed for surface expression of NKG2D, NKp46, and CD107a and intracellular expression of IFN-γ. FMO, fluorescence minus one; APC, allophycocyanin; ECD, phycoerythrin-Texas Red; FITC, fluorescein isothiocyanate.

up-regulation on NK cells in HIV disease is not clear. Between those who carried and those who did not carry *KIR3DS1*, the MFI of CD38 on NK and T cells was strongly associated (rho = 0.61 [$P = 0.001$] and rho = 0.59 [$P = 0.0003$], respectively [Spearman test]; data not shown). However, we observed that the level of CD38 expression (as reflected by the MFI) was lower on both NK and $CD8^+$ T cells among those who possessed *KIR3DS1* than among those who did not (Fig. 3A). There was no difference in the CD38 MFI on NK cells between those who were HIV-1 infected and carried *KIR3DS1* and those were not infected (and were of unknown KIR type). Among those who carried *KIR3DS1*, we observed (i) no relationship between CD38 MFI on NK cells and the HIV-1 load (Fig. 3C) and (ii) a weakening of the relationship between CD38 MFI expression on $CD8^+$ T cells and the HIV-1 load (Fig. 3C). Among those who do not carry *KIR3DS1*, there was a strong and direct correlation between the NK and $CD8^+$ T-cell CD38 MFI and the HIV-1 load (Fig. 3B).

We considered the possibility that the association of *KIR3DS1* and the CD38 MFI was due to a direct or indirect interaction with MHC class I ligands. We found that joint carriage of *KIR3DS1* and *Bw4Ile80* did not associate with differences in NK or $CD8^+$ T-cell CD38 MFI levels ($P = 0.69$ and $P = 0.32$, respectively). We did find carriage of *B*58 supertype* to be marginally associated with a lower $CD8^+$ T-cell CD38 MFI ($P = 0.09$) but not with the NK cell CD38 MFI ($P = 0.31$). We found carriage of *Bw4Ile80* cluster alleles not to be associated with differences in either the $CD8^+$ T-cell or the NK cell CD38 MFI ($P = 0.66$ and $P = 0.88$, respectively). Those who carried both *B*58 supertype* and *KIR3DS1* did not show a lower $CD8^+$ T-cell or NK cell CD38 MFI ($P = 0.39$ and $P = 0.86$, respectively [Mann-Whitney U test]).

DISCUSSION

In our study, we observed that recently HIV-1-infected persons who carried at least one copy of the *KIR3DS1* gene had higher NK cell effector activity in the unstimulated and stimulated state and had lower expression of CD38 (a marker previously associated with T-cell activation) on their NK and T

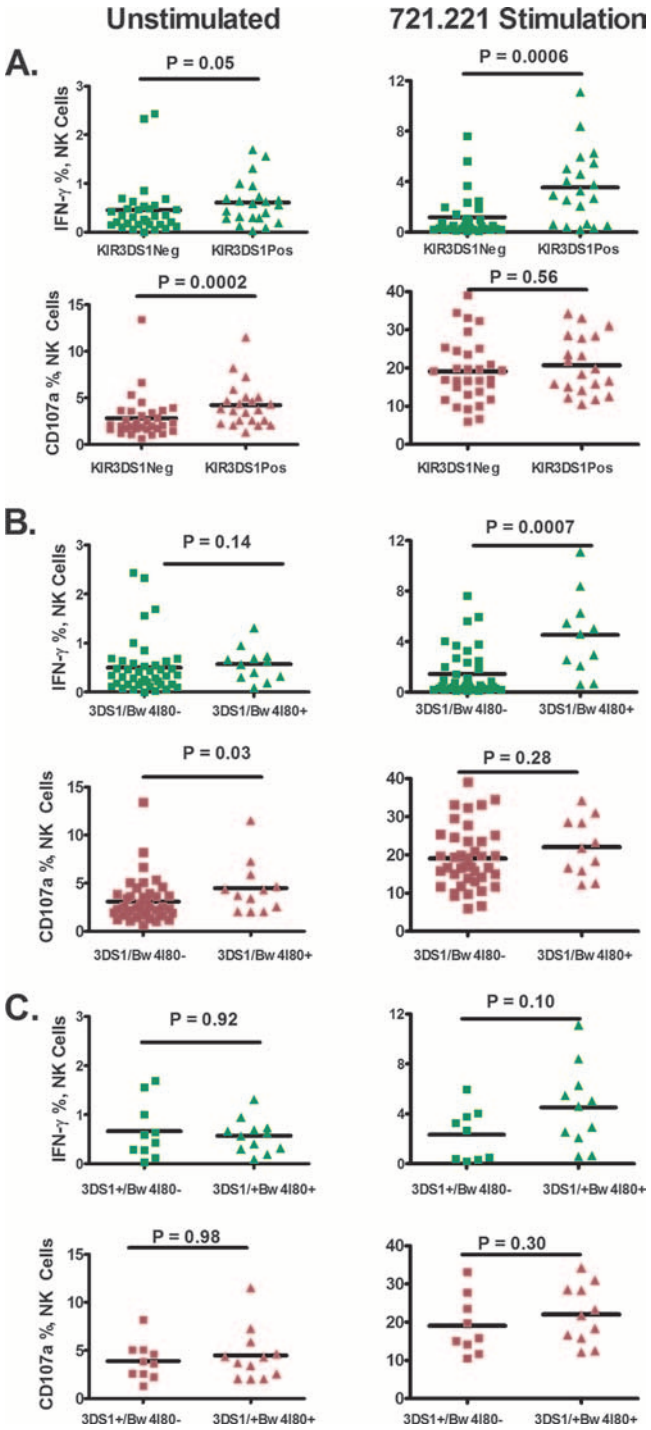

FIG. 2. NK cell functional activity by *KIR* and *Bw4* gene combinations. Columns 1 and 2 refer to IFN-γ (green) or CD107a (purple) measurements in unstimulated cells and cells stimulated by coculture with the MHC class I-deficient 721.221 cell line, respectively. (A) KIR3DS1Pos refers to those who carry at least one copy of *KIR3DS1*. Those who are *KIR3DS1* negative (KIR3DS1Neg) are homozygous for *KIR3DL1*. (B) 3DS1/Bw4I80+ refers to those who have the compound genotype, possessing both *KIR3DS1* and *Bw4Ile80*, whereas those who are 3DS1/Bw4I80 negative are those who have one or the other gene (but not both) or have neither. This is the comparison used in the earliest genetic association studies, and its results were interpreted to signify synergy between the two gene products. (C) Persons who are *KIR3DS1* carriers. The 3DS1+/Bw4I80− persons are those who carry *KIR3DS1* but not the *Bw4Ile80* gene, whereas the 3DS1/Bw4I80+ persons are those who have *KIR3DS1* and also carry *Bw4Ile80*. This third comparison allows one to directly test if the presence or absence of *Bw4Ile80* among *KIR3DS1* carriers is associated with differences in NK cell functional measurements. The Mann-Whitney U test was used for comparisons because of the nonnormal distribution of the underlying data.

cells compared with those who did not have *KIR3DS1*. We observed that the effect of *KIR3DS1* on NK cell function was independent of carriage of HLA-Bw4Ile80, the putative ligand for KIR3DS1.

KIR3DS1 carriage was associated with higher expression of IFN-γ and CD107a on NK cells in the unstimulated state. After stimulation by MHC class I-deficient 721.221 target cells and despite the lack of presentation of any ligand to the activating KIR3DS1 receptor, we observed that *KIR3DS1* carriers expressed more IFN-γ and equivalent levels of CD107a (Fig. 2A). The increase in functional activity among *KIR3DS1* carriers was not attributable to carriage of an *HLA-Bw4Ile80* allele by other cells in the culture. That is, among persons who carried *KIR3DS1*, the carriage of an *HLA-Bw4Ile80* allele was not associated with an additional increases in either basal or stimulated levels of IFN-γ or CD107a expression on NK cells (Fig. 2C). Hence, we have presented evidence that *KIR3DS1* correlates with greater NK cell effector function among persons in early HIV-1 infection. We cannot exclude the possibility that KIR3DS1 has ligands other than HLA-Bw4 that might be present in these cultures (2).

Although widely expressed on NK cells, KIRs are also expressed on T cells (32), which we observed at low frequencies (in the range of 2 to 4% of the T cells in these samples; data not shown) (6, 32). KIR expression on T cells may be up-regulated in certain chronic inflammatory conditions, such as arthritis (12). In HIV-1 disease, itself a chronic inflammatory condition, KIR might be acting to modulate T-cell signaling, which may have broad effects on these T cells (13), thereby influencing their activation state.

We observed that *KIR3DS1* carriage was associated with lower expression of CD38 on $CD8^+$ T cells and NK cells. This effect was not attributable to carriage of the *HLA-B*57* or *HLA-B*58* protective allele. The significance of CD38 expression on NK cells in HIV-1 disease is not known. In prior studies, CD38 expression on NK cells has been shown to be up-regulated after interleukin-2 exposure, and cross-linking of CD38 causes NK cells to release granzyme and cytokines (29). Therefore, it is not clear if higher CD38 expression on NK cells is predictive of poorer HIV disease outcomes but in our analyses does appear to correlate with a higher viral load. In HIV-1 disease, it is commonly observed that CD38 levels on $CD8^+$ T cells strongly correlate with HIV-1 viral loads (7). In our study, among those who carried *KIR3DS1*, we observed a weakening or loss of the expected strong associations between $CD8^+$ T-cell CD38 expression and the viral load. This association was preserved in those who did not carry *KIR3DS1* (Fig. 3B.). The viral load and expression of CD38 on T cells may be partially uncoupled among *KIR3DS1* carriers.

Elevated $CD8^+$ T-cell activation, as measured by the CD38 MFI, is known to be an independent predictor of poor disease

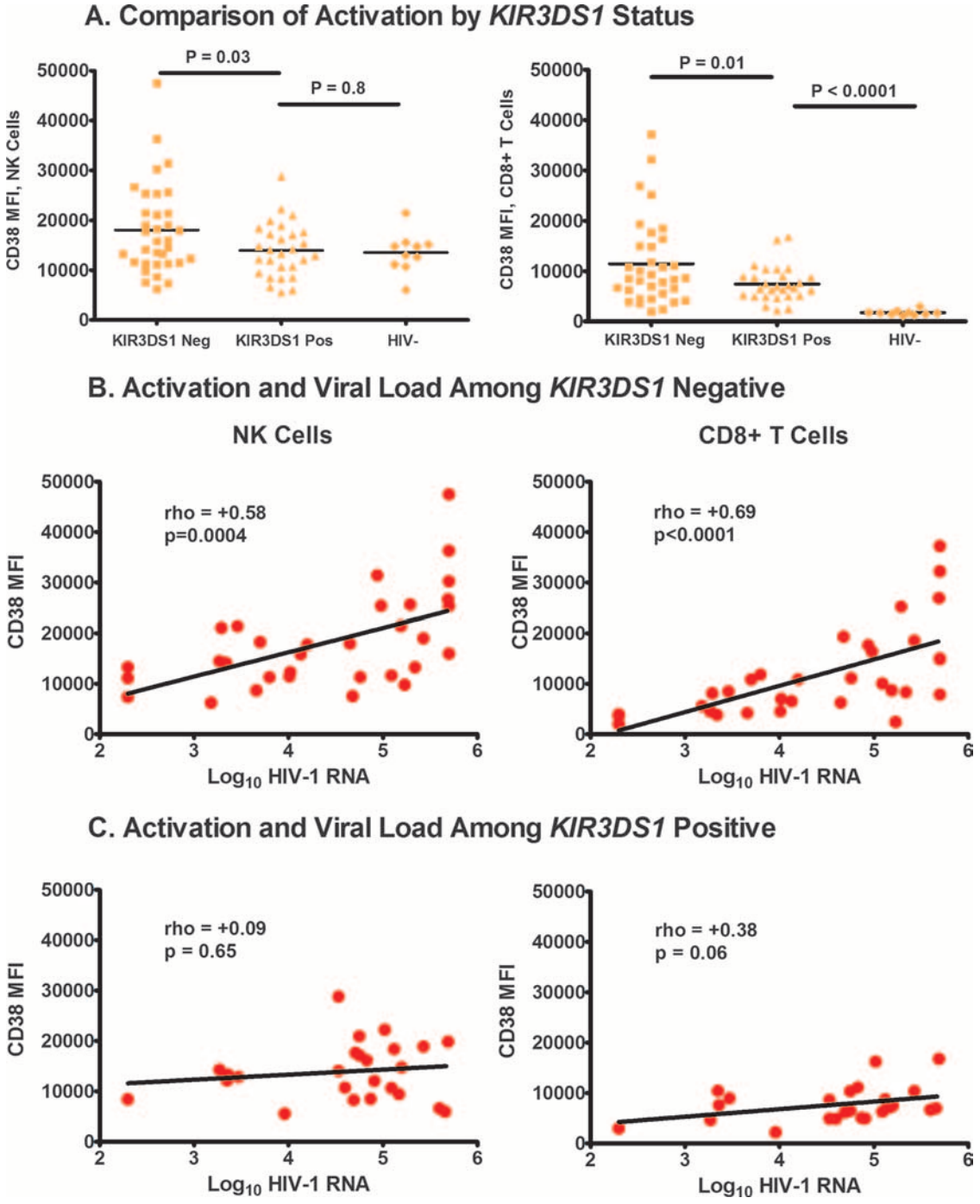

FIG. 3. (A) MFI for CD38 on NK and $CD8^+$ T cells, respectively, by *KIR3DS1* carriage. We used an unpaired *t* test with the Welch correction for these comparisons. (B) Relationship of CD38 expression on NK and $CD8^+$ T cells to the HIV-1 load among those who are not *KIR3DS1* carriers. The Spearman rank correlation test was used for the correlation analysis. (C) Relationship of CD38 expression on NK and $CD8^+$ T cells to the HIV-1 load among those who are *KIR3DS1* carriers. The Spearman rank correlation test was used for the correlation analysis.

outcomes in HIV disease (11, 19). Elevated T-cell activation levels, but not high-level viral replication, distinguish pathogenic from nonpathogenic primate lentiviral infections (17). In other words, a high viral load without accompanying elevated $CD8^+$ T-cell activation is not associated with progressive immunodeficiency. Hence, the difference in activation state and NK cell function we observed here are consistent with a prior clinical genetic epidemiology report by our group that *KIR3DS1* carriage is associated with elevated $CD4^+$ T-cell counts in early HIV-1 infection, despite little or no difference in the viral load (4). It has recently been observed that the HIV-1 load only partially explains the variation in $CD4^+$ T-cell decline among individuals, suggesting that other factors—viral or host derived—might modulate the relationship between the

viral load and the virulence of the infection (27). Our findings suggest that *KIR3DS1* carriage may be one of these factors.

Most of the persons in our study who carried *KIR3DS1* (Table 1) also carried a copy of *KIR3DL1*. Individual NK cells typically only express a single *KIR* allele from a given locus. However, because of a lack of specific serological reagents, we were unable to directly address the frequency of NK cells within the population that expressed KIR3DS1, KIR3DL1, or both receptors in these heterozygous individuals (25). In our studies, we measured the behavior of the entire NK cell pool from these persons; hence, the effects of *KIR3DS1* on NK cell function and disease outcome seen here might be diluted. In this study, we examined only four persons homozygous for *KIR3DS1* and thus were underpowered to characterize them in detail. The low frequency of *KIR3DS1* homozygosity in our study population of HIV-1-infected persons might indicate protection against HIV-1 transmission (23, 30) among exposed carriers. Absence of KIR inhibitory signals, and therefore the potential for higher NK cell activation, has been associated with protection against HIV-1 acquisition among highly exposed individuals in other studies (16).

In an analysis of limited statistical power ($n = 3$ versus $n = 18$), we observed some evidence that those carrying *B*58 supertype* (a *B*57* or *B*58* allele) and *KIR3DS1* had higher NK cell functional activity in both the unstimulated and stimulated states. This result may suggest that KIR3DS1 is preferentially ligated by these select members of the Bw4Ile80 cluster. Alternatively, these MHC class I alleles might have effects on NK cell activity through another mechanism, perhaps affecting another NK-activating receptor family or via an effect on dendritic cells. Of note, *KIR3DS1* carriage was associated with elevated NK cell functional measurements even among those who do not carry *B*58 supertype*, suggesting only a partial dependence on this allele. Persons who carry *KIR3DS1* and *B*58 supertype* likely represent around 3 to 5% of the general population of persons with HIV-1 infection (based on our data), making them a rare population. Although this analysis is intriguing, it must be regarded as preliminary, bearing examination in a larger cohort.

A recent KIR genetic association study addressing HIV outcomes has examined *KIR3DL1*004*, an inhibitory KIR variant that is not expressed on the cell surface (22). When present along with *HLA-B*57*, *KIR3DL1*004* carriage was associated with strong protection against HIV disease progression. That a KIR variant that does not reach the cell surface would be associated with dramatic protection is intriguing. The simplest explanation of these recent data is that inhibitory *KIR3DL1*004* is rarely or never ligated and therefore fails to restrain NK cell activity, leading to more NK effector activity in vivo. The emerging story for *KIR3DL1*004* may have characteristics in common with that for KIR3DS1, which, by our studies, does not appear to require its putative ligand (Bw4Ile80 cluster) to confer protection against HIV-1. Functional studies of KIR ligands that do not reach the NK cell surface may prove challenging (21) but are required to define the role of these intriguing variants.

In summary, we observed that recently HIV-1-infected adults who carried *KIR3DS1* had higher NK cell effector functions in both the unstimulated and stimulated states than those who did not carry *KIR3DS1*. This effect was not dependent on the carriage of a *Bw4Ile80* allele, the putative ligand for KIR3DS1. We further observed that persons who carried *KIR3DS1* had lower CD38 expression on NK and $CD8^+$ T cells, the latter of which is an important marker of disease progression in HIV disease. These effects were not attributable to joint carriage of *Bw4Ile80*. We previously observed that recently HIV-1-infected persons who carried *KIR3DS1* had higher $CD4^+$ T-cell counts despite the absence of a difference in the viral load. *KIR3DS1* may be mediating that effect on $CD4^+$ T-cell counts via partial uncoupling of T-cell activation and the viral load. How *KIR3DS1* carriage or expression might mediate this effect is not known but will be an important area of research.

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