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Natural Killer Cell Responses to HIV-1 Peptides are Associated With More Activating *KIR* Genes and *HLA-C* Genes of the *C1* Allotype

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Abstract

Background—What characterizes individuals whose natural killer (NK) cells are able to respond to HIV-1 peptides is not known.

Methods—The association between NK cell responses and *KIR* gene profiles and *HLA-B* and *HLA-C* alleles was investigated among 76 HIV-1-infected women in South Africa previously categorized as responders (n = 39) or nonresponders (n = 37) to HIV-1 peptide pools in a whole blood intracellular cytokine assay. Viral load was significantly lower and CD4 T-cell counts higher among responders compared with nonresponders ($P = 0.023$ and $P = 0.030$, respectively).

Results—Possession of one *HLA-C1* allele associated with increased magnitude of NK cell responses to Env ($P = 0.031$) and significantly decreased viral load ($P = 0.027$) compared with its absence. There was a trend to increased possession of *KIR2DL3*+*HLA-C1* in responders (71.8% vs 51.4%, $P = 0.098$) and decreased possession of *KIR2DL3/2DL3*+*C2C2* (2.6% vs 16.2%, $P = 0.053$). A total of 64.1% of responders versus 32.4% of nonresponders had 13 or more *KIR* genes ($P = 0.0067$). Notably, the 13-*KIR* gene containing the Bx21 genotype (has eight inhibitory and three activating genes *KIR2DS2*, *2DS4*, *2DS5*) showed substantially higher representation among the responders (28.2% vs 2.6%, $P = 0.001$). A significantly higher proportion of responders had both *KIR2DS2* and *KIR2DS5* compared with either gene alone (72.4% vs 37%; $P = 0.015$). At

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least one *HLA-C1* allele together with 13 or more *KIR* genes was associated with NK cell responsiveness (48.7% vs 13.5%; $P = 0.001$).

Conclusion—NK cell responses to HIV-1 peptides are more likely to occur among individuals with a genotype supporting a more activating NK cell phenotype and who possess at least one *HLA-C1* allele.

Keywords

HIV-specific NK cell responses; KIR; HLA-B; HLA-C

INTRODUCTION

Natural killer (NK) cells are highly versatile cells that contribute to both innate and adaptive immunity. NK cells under normal, noninflammatory conditions are strictly dominated by inhibitory signals, a mechanism that ensures that healthy cells are not inadvertently destroyed. Under altered conditions, through recognition of "missing self" (loss or downregulation of HLA Class I molecules), "induced self" through stress (upregulated host molecules), or through foreign recognition (allogeneic cells or pathogen infection), NK cells overcome inhibitory signals and become activated culminating in killing of target cells. These interactions between NK cells and target cells are all mediated by various activating and inhibitory receptors on NK cells and their corresponding ligands on target cells.[1,2] Among these receptors are killer-cell immunoglobulin-like receptors (KIRs) that bind specific human leukocyte antigen (HLA) Class I molecules that have, in several genetic studies, shown importance in relation to control of HIV-1 infection.[3–5]

HIV-1-infected individuals who are viremic display altered NK receptor ligand expression,[6] upregulation of inhibitory NK cell receptors,[7,8] and reduced expression of activating NK cell receptors.[7,9–11] These alterations would be expected to collectively predispose NK cells to a more inhibitory type of phenotype, raising the threshold that would need to be overcome for NK cells to contribute to control of HIV-1 infection through activation and subsequent elimination of virus-infected cells.

Our recent findings have described NK cell (non-T-cell/CD3-negative) responses to HIV-1 peptides among HIV-1-infected mothers and their infants that were associated with reduced maternal–infant HIV-1 transmission and associated with significantly lower viral loads and higher CD4 T-cell counts in the mothers.[12,13] Because exposure to HIV-1 peptides in the assay results in activation of NK cells (measured by intracellular detection of interferon-γ) in some individuals, we postulated that these "responders" would possess a more activating *KIR* gene profile or particular KIR–HLA combinations, that would explain the ability of their NK cells to overcome inhibitory signals and so be able to mount NK cell responses in the presence of HIV-1 peptides. In this study, we describe the *KIR* and *HLA-B* and *HLA-C* genes in relation to the detection of NK cell responses to HIV-1 peptides of the HIV-1-infected women from the mother–child cohort.[12,13] We show that the greater the *KIR* gene number, the Bx21 *KIR* genotype and *KIR* gene number in combination with an *HLA-C1* allele characterize those individuals with NK responses to HIV-1 peptides.

MATERIALS AND METHODS

Study Samples

Genomic DNA was extracted from whole blood of a total of 76 HIV-1-infected women using the QIAamp DNA Mini Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions. These 76 were when sufficient sample was available of 79

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HIV-1-infected women whose HIV-specific NK cell responses (CD3-negative) were previously described.[12,13] All were recruited at one of two sites in Johannesburg, South Africa, during the postpartum period as part of a study of maternal–infant HIV transmission.[12,13] Of these women, 39 had positive NK cell responses (forthwith termed "responders") to at least one HIV-1 peptide pool (Gag, Pol, Nef, Reg, and Env peptide pools tested) and 37 had no detectable NK cell responses (termed "nonresponders"); three of the original nonresponder group of 40 had no DNA sample available. Magnitudes of each individual's peptide pool(s) response are reported in Table 2 of reference 12 and graphically shown with corresponding magnitudes of patient CD4 and CD8 T-cell responses in the study by Tiemessen et al.[13] HIV-1 RNA levels (expressed as $\log_{10}$ units) were quantitated using the Roche Amplicor RNA Monitor assay (Roche Diagnostic Systems, Inc, Branchburg, NJ) with a lower detection limit of 400 HIV-1 RNA copies/mL. CD4 T-cell counts were determined using the commercially available FACSCount System from Becton Dickinson (San Jose, CA). The median viral load for the total group was 4.10 $\log_{10}$ (range, 2.6–5.69 log) (n = 76), and the median CD4 T-cell count was 436 cells/μL (range, 40–1655 cells/μL) (n = 57). Only one woman who was in the NK responder group received triple-drug HIV treatment. None of the others had received HIV treatment, although most had received single-dose nevirapine for the prevention of maternal–infant HIV transmission. For comparisons involving CD4 T-cell counts or viral load, exclusion of this sample did not alter any outcomes so was therefore included throughout.

This study was approved by the University of Wit-watersrand Committee for Research on Human Subjects and the Institutional Review Board of Columbia University and signed informed consent was obtained from all participants.

KIR GENOTYPING

KIR genotyping was performed using sequence-specific primer polymerase chain reaction (*Olerup* SSP *KIR* Genotyping kit; *Olerup* SSP AB, Stockholm, Sweden). Genomic DNA was genotyped for the presence or absence of the following *KIR* genes: *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3*, *KIR3DS1*, *KIR2DP1,* and *KIR3DP1*. Group B haplotypes possess one or more of the following genes: *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3, KIR2DS5,* and *KIR3DS1*. Group A haplotypes were defined by the absence of all Group B genes and the presence of nine genes: *KIR2DL1*, *KIR2DL3*, *KIR2DL4*, *KIR2DS4*, *KIR2DP1*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3,* and *KIR3DP1* (14th International HLA and Immunogenetics Workshop, 2005). The Group B haplotypes were collectively termed Bx, because they constitute a mixture of AB and BB haplotypes. *KIR* genotype profiles were assigned to the AA and Bx haplotype groups using the New Allele Frequency Database: http://www.allelefrequencies.net.[14]

HLA CLASS I GENOTYPING

HLA-B and *HLA-C* high-resolution genotyping was performed using a sequence-based typing strategy and the protocol previously described by Cereb et al[15] for heterozygous amplification of exon 2, intron 2, and exon 3 of the *HLA* loci. Nucleotide sequencing was performed on an ABI 3730 Genetic Analyzer using Big Dye Terminator Version 1.1 chemistry (Applied Biosystems, Foster City, CA). Allele assignment was performed using SeqScape Version 2.5 software (Applied Biosystems) and a library compiled from the 2.17.0 release of the IMGT/HLA Database.

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Statistical Analysis

Fisher exact tests were performed using *SISA*: Simple Interactive Statistical Analysis[16] and exact 95% confidence intervals of odds ratios of genotype frequency differences calculated. Two-sided tests were used and statistical significance was considered at $P < 0.05$. No adjustment was made for multiple comparisons. Mann-Whitney *U* tests were performed using SPSS Version 15.0 software (SPSS Inc, Chicago, IL).

RESULTS

KIR Genes and HLA-B and HLA-C Allotype Representation Among Natural Killer Responders and Nonresponders

KIR gene profiles and *HLA-B* and *HLA-C* alleles were determined for 76 HIV-1-infected women (39 responders and 37 nonresponders) and are described in Table 1 together with their viral loads, CD4 T-cell counts, and the HIV-1 peptide pool specificities originally determined for the positive NK cell responses.[12,13] Viral load was significantly lower and CD4 T-cell counts higher among the responders when compared with nonresponders (P = 0.023 and P = 0.030, respectively).

To date 14 distinct *KIR* genes and two pseudogenes have been described (Carrington and Norman, http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/mono_003/ch1d1.pdf and http://www.ebi.ac.uk/ipd/kir/). The extracellular region of KIR receptors binds with particular ligands (HLA Class I molecules); the cytoplasmic tail, which can be either short-tailed (S) or long-tailed (L), transduces the receptor-mediated signals. The L forms are usually inhibitory in function, whereas the S forms are stimulatory in function. Fourteen KIR genes and two pseudogenes were determined for each individual (Table 1). The most notable difference when comparing possession of individual KIR genes between responders and nonresponders was for KIR2DS5 (56.4% vs 37.8%, respectively; P = 0.11). Overall, more individuals who were responders possessed activating KIR genes (2DS1, 2DS2, 2DS5, 3DS1, although all independently; P > 0.05).

HLA-B alleles were further grouped as *HLA-Bw4* (*Bw4*) and *Bw6* allotypes based on five variable amino acids spanning residues 77–83 at the carboxyl-terminal end of the α_1 helix.[17,18] The *Bw4* allotype subset Bw4-80Ile contains an isoleucine at position 80 as opposed to the Bw4-80Thr subset that contains a threonine at the same position and serves as the better ligand for KIR3DL1[19–21] and is a putative ligand for KIR3DS1 based on epidemiologic data.[4] *HLA-C* alleles were grouped as Group 1 or C1 allotypes (asparagine at residue 80 and are known ligands for KIR2DL2, KIR2DL3, and KIR2DS2) or Group 2 or C2 allotypes (lysine at residue 80, known ligands for KIR2DL1 and KIR2DS1).[22] Data were analyzed between groups as presence of at least one allele of a particular allotype (*C1* or *C2*, *Bw4* or *Bw6*) and total allelic representation or allelic dose (*C1C1*, *C2C2*, *C1C2* and *Bw4/4*, *Bw6/6*, *Bw4/6*). There was no significant difference in representation of *HLA-Bw4* and *Bw6* allotypes or of the *Bw4-80Ile* allotype subset among *Bw4*-possessing individuals, or of *HLA-C1* and *HLA-C2*, between responders and nonresponders ($P > 0.05$). However, among women who had at least one *HLA-C1* allele (n = 57), the magnitude of their NK cell responses to Env was significantly increased (P = 0.031) and viral load was significantly decreased (P = 0.027) when compared with those without a *HLA-C1* allele (ie, *C2C2* homozygotes, n = 19) (Fig. 1).

Influence of *KIR* Genes and Corresponding HLA-B/C Ligands

KIR2DL2 and *KIR2DL3* segregate as alleles of the same locus and so allelic dose of these genes and their respective *HLA-C* allotypes as well as the compound effects of *KIR–HLA* combinations was analyzed in the context of the ability to develop HIV-specific NK cell

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responses (Table 2). The most notable difference between responders and nonresponders was the possession of *KIR2DL3+HLA-C1* (71.8% vs 51.4%, *P* = 0.098) and *KIR2DL3/2DL3+C2C2* (2.6% vs 16.2%, *P* = 0.053). These trends highlight the potential importance of possession of at least one *HLA-C1* allele and the presence of *KIR2DL3* in likelihood of NK cell responsiveness to HIV-1 peptides.

As for *KIR2DL2* and *KIR2DL3*, *KIR3DS1* and *KIR3DL1* segregate as alleles of the same locus,[23,24] but given that *KIR3DS1* is present in only a few individuals (5.2% in the total group) and as only one copy (*KIR3DS1/3DL1* heterozygotes), the majority of individuals are *KIR3DL1/3DL1* homozygotes and so effects of dose of either *KIR* gene could not be determined. In addition, combinations of *KIR3DL1+Bw4*, *KIR3DL1+Bw4-80Ile*, and *KIR3DL1-80Thr* yield the same findings as if the *HLA-B* allotype groupings are tested independently.

KIR Gene Numbers and Genotypes

As can be seen from the *KIR* gene profiles (Table 1), there exists variation in numbers of *KIR* genes (nine to 16) and in combinations of these genes in different individuals. Those who have higher numbers of genes have more activating KIRs than individuals with only nine genes who would have only one activating KIR. Most individuals possessed 13 *KIR* genes (39.5%) followed by nine genes (26.3%), 12 (18.4%), 11 (6.6%), 14 (3.9%), 15, and 16 (both 2.6%).

Individuals have six to eight inhibitory *KIR* genes and from one to six activating *KIR* genes. KIR2DL4, which shares structural and functional features with both inhibitory and activating receptors,[25–27] is categorized here as inhibitory. Equal proportion of responders and nonresponders had six inhibitory genes (25.6% vs 27%) and only one activating *KIR* gene (*KIR2DS4*); however, there was a shift in favor of more responders having eight inhibitory genes (56.4% vs 32.4%, *P* = 0.03) than nonresponders (Table 3). More responders had three activating genes (48.7% vs 29.75%) and more non-responders had two activating genes (27% vs 2.6%). Overall, significantly more responders had three or more activating genes than nonresponders (71.8% vs 45.9%, *P* = 0.035) (Table 3). This corresponded exactly with inhibitory:activating ratios (number of inhibitory genes ÷ number of activating genes) of 2.7 or less (less inhibition) and greater than 2.7 (greater inhibition).

Total *KIR* gene number distribution among responders and nonresponders (Fig. 2A) showed that a significantly higher proportion of responders possessed 13 *KIR* genes than nonresponders (53.8% vs 24.3%, *P* = 0.011). When analyzed as individuals harboring less than 13 or 13 or more *KIR* genes, 64.1% of responders versus 32.4% of nonresponders had higher *KIR* gene numbers (*P* = 0.0067), overall suggesting that NK cell responses are more likely to occur among individuals with more activating *KIR* genes.

Seventeen *KIR* genotypes were identified in this group of 76 HIV-1-infected women (Table 1; Fig. 2B). The most prevalent genotypes (greater than 5%) were AA1 (26.3%), Bx21 (15.8%), Bx5 (13.2%), Bx112 (9.2%), and Bx4, Bx20, and Bx71 (all 6.6%). Interestingly, the 13-*KIR* gene-containing Bx21 genotype showed substantially higher representation among the responders (28.2% vs 2.7%, *P* = 0.001), virtually accounting for the entire effect seen when comparing responder and nonresponders groups on the basis of *KIR* gene number alone. Bx21 contains eight inhibitory genes and three activating genes (*2DS2*, *2DS4*, *2DS5*). All genotypes in this population contained *KIR2DS4*, highlighting the potential importance of *KIR2DS2* and *KIR2DS5* co-occurrence in the development of HIV-specific NK cell responses.

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All *KIR* genotypes in our study group, with the exclusion of AA1 genotypes (26.3% of patients), possess either *KIR2DS2* and/or *KIR2DS5*; 40.8% possessed both genes, 26.3% had *KIR2DS2* alone, and 6.6% had *KIR2DS5* alone. A significantly higher proportion of responders had both *KIR2DS2* and *KIR2DS5* (Fig 2C), as opposed to either gene alone, compared with nonresponders (72.4% vs 37%; $P = 0.015$), further reinforcing the need for a more activating phenotype in likelihood of detection of HIV-specific NK cell responses.

Given the importance of possessing one copy of an *HLA-C1* allele, as evidenced by reduced viral load and increased NK cell response magnitude (Fig 1), we further established that a combination of at least one *HLA-C1* allele together with 13 or more *KIR* genes was associated with NK cell responsiveness (48.7% vs 13.5%; $P = 0.001$) (Fig. 2D). This equated to 86.5% of nonresponders possessing either lower (less than 13) *KIR* gene numbers or *HLA-C2C2* homozygosity.

DISCUSSION

An important role for NK cells in control of HIV-1 infection has been indicated by genetic association studies of KIR receptors that have as ligands specific HLA Class I molecules.[3–5] Furthermore, our recent work showed an association of NK cell responses to HIV-1 peptides (predominantly to Env and Reg peptide pools) with lower viral loads, higher CD4 T-cell counts, and stronger T-cell responses in HIV-1 infected women[13] and the association of these responses with reduced maternal–infant HIV-1 transmission.[12] Collectively, all these studies prompted us to begin to question the possible role that KIR and HLA Class I B and C molecules might play in the ability of patients' NK cells to overcome inhibitory signals sufficiently to mount responses to HIV-1 peptides. This study questions the relationships with NK cell responsiveness of the particular *KIR* gene repertoires and HLA of HIV-1-infected individuals that considers 1) presence or absence of a particular gene; 2) type (inhibitory or activating) and number of *KIR* genes; 3) *KIR* genotype; 4) representation of HLA Class I allotypes (C1, C2, Bw4, Bw4-80I, Bw6); and 5) specific KIR–HLA combinations. To this end, samples from 76 HIV-1-infected women were *KIR* and *HLA-C* and *HLA-B* genotyped and grouped as responders (a response to at least one peptide pool) and nonresponders.

Seventeen *KIR* genotypes were identified; to date, we have identified a total of 46 different *KIR* genotypes among 446 black South African mother and infant individuals.[28] The genotypes in our current study group encompassed all the higher prevalence *KIR* genotypes found in this larger group, viz AA1, Bx21, Bx5, Bx112, Bx4, Bx20, and Bx71. Looking at the genotypes based on total *KIR* gene number, it was apparent that significantly more NK responders possessed 13 or more genes and that this increase was attributed to more responders having eight as opposed to seven inhibitory genes and three or more activating genes. In general, higher *KIR* gene number is attributed to the presence of more activating genes. Of all the genotypes, it was the Bx21 genotype, which is the most highly represented of the Bx genotypes in our South African black population, that was most strongly associated with NK cell responses to HIV-1 peptides. This particular genotype contains eight inhibitory and three activating genes. Of the three activating genes, only the effects of *KIR2DS2* and *KIR2DS5* could be assessed because all genotypes contained *KIR2DS4*. Possession of both these genes, as opposed to only one or the other, was a characteristic of NK responders. Overall, these findings suggest that a more activating phenotype is associated with the presence of HIV-specific NK cell responses, consistent with the idea that under these conditions, the balance between inhibitory and activating signals of NK cells favors activation, which in turn contributes to more effective control of HIV-1 infection.

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Irrespective of what mechanism underlies NK cell responsiveness to HIV-1 peptides, an individual armed with *KIR* genes that allows for a greater opportunity for NK cell activation (more activating genes and in combination with at least one *HLA-C1* allele) is a requirement for response ability. Because all *HLA-C* alleles fall into either the *HLA-C1* or *HLA-C2* allotype subsets, the importance of HLA-C1 in reduction of viral load and increased magnitudes of HIV-1 peptide-specific NK cell responses points to the likely importance of its KIR partners KIR2DL2, KIR2DL3, and KIR2DS2. All individuals were either homozygous for *KIR2DL2* or *KIR2DL3* or are *KIR2DL2/KIR2DL3* heterozygotes because these are alleles of the same locus. Approximately 10% more responders than nonresponders possessed *KIR2DS2* (72% vs 62%) with responders having 7% more *KIR2DL3*. It was the combination of *KIR2DL3* plus *HLA-C1* that showed a trend to an increase in the responders, the importance of homozygosity of *KIR2DL3* in the absence of its ligand HLA-C1 (so *C2C2* homozygosity) being more highly represented in the non-responders further emphasizing the importance of this relationship in the responders ($P = 0.053$). It will be important to further study the effects of allelic variation at these *KIR* loci to establish if particular variants are more associated with different levels of KIR expression or altered binding affinities that might affect their interactions with HLA-C1 molecules. Importantly, we found these same KIR molecules to be the most significantly involved in maternal transmission of HIV-1 and in acquisition of HIV-1 in the infant.[28]

Another study of South African individuals showed that among those who had both *KIR2DL1* and *KIR2DS1* genes, the frequency of NK cells expressing one or both of these receptors tended to decrease with increasing viral load, a trend that was not seen in individuals who had *KIR2DL1* but not *KIR2DS1*.[11] These molecules are among the KIRs that bind HLA-C molecules; the affinity of these interactions is greatest for KIR2DL1-C2>KIR2DL2-C1>KIR2DL3-C1.[29] The affinity interactions of the corresponding activating receptors KIR2DS1-C2 and KIR2DS2-C1 tend to be less than their inhibitory counterparts. It has been previously suggested from genetic studies that KIR–HLA combinations associated with less inhibition might favor greater likelihood of NK cell activation as opposed to those with stronger affinity interactions, for example, the “weaker” interaction of KIR2DL3 and HLA-C1 has been associated with enhanced resolution of hepatitis C virus infection.[30] In African sex workers, it has been demonstrated that possession of inhibitory genes in the absence of genes for their cognate ligands was associated with reduced HIV-1 acquisition (KIR2DL2/2DL3 heterozygotes with no HLA-C1, KIR3DL1 homozygotes with no HLA-Bw4).[31] In addition, individuals with *KIR* genotypes having more activating *KIR* genes have also shown some protection.[31,32] Overall, the tendency toward weaker inhibition and so greater activation seems important in control of HIV-1 infection and protection from HIV-1 acquisition.

How can peptide-specific NK cell responses measured ex vivo in the whole blood assay be explained? Peptide–HLA Class I complexes have been shown to be recognized by activating KIR (KIR2DS1) receptors in cells infected with Epstein-Barr virus.[33] Furthermore, NK cells have been shown in vitro to kill their HIV-infected target cells in a receptor ligand-specific manner that involved activating KIR3DS1 and its putative ligand HLA-Bw4-80Iso.[34] It can therefore be envisaged that HIV-1 peptides delivered exogenously bind specifically to HLA Class I molecules on antigen-presenting cells and that these complexes are recognized by particular KIR receptors on NK cells. Clones of NK cells that express more than one or several activating receptors, all engaged with their cognate ligands would result in the integration of several signals that ultimately culminate in NK cell activation. Any one activating signal alone may prove insufficient for activation.[33,35] Peptide antagonism has recently been suggested as a possible mechanism for NK cell activation, and it was demonstrated that KIR-positive NK cells are more influenced by changes in peptide sequence than changes resulting from HLA Class I expression on target cells.[36] In the

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context of HIV-1-specific peptides in our assay, it would seem possible that some of these interactions with HIV-1 peptides could be antagonistic in nature, resulting in abrogating inhibitory KIR interactions with HLA Class I molecules and so overcoming the threshold for activation of NK cells. Because activating and inhibitory KIR interactions show similarities in sensitivity to alterations in peptide sequences,[33] it may be that a combination of binding of peptides to inhibitory KIR and to the corresponding activating KIR may together or independently result in an overall outcome of activation of NK cells, this governed by the extent to which the inhibitory-activating axis is altered. ADCC antibodies provide another means of NK cell activation by peptides in the whole blood assay,[37] the triggering of NK cells occurring through engagement of the CD16 activating receptor on NK cells. It stands to reason that in some patients, this could account for the entire response or a component of the response; in other individuals, other mechanisms may dominate.

Although the exact events underlying the specific nature of activated NK cell responses to particular HIV-1 peptides in whole blood assays remain to be elucidated, it is clear that both variation at the *KIR* locus and dose of particular *HLA-C* allotypes impact on the ability of NK cells to respond to HIV-1 peptides, a feature of importance in control of HIV-1 infection.

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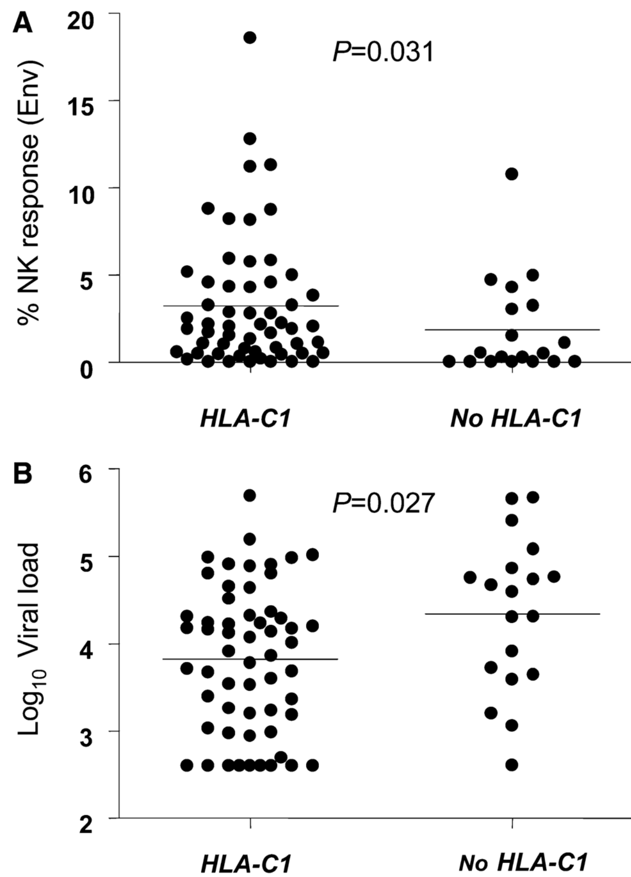

FIGURE 1.

Magnitudes of natural killer (NK) cell responses to Env (**A**) and viral loads (**B**) of individuals in the total group stratified according to the presence or absence of an *HLA-C1* allele. Responses represent the percent of CD3-negative (NK) cells that produce interferon-γ in response to the Env peptide pool after subtraction of background (described in the study by Tiemessen et al[13]). Possession of one *HLA-C1* allele groups together individuals who are *C1C1* homozygotes or *C1C2* heterozygotes (n = 57) in the total group; no *HLA-C1* indicates *C2C2* homozygotes (n = 19). Log_{10} viral load: HIV-1 RNA copies/mL.

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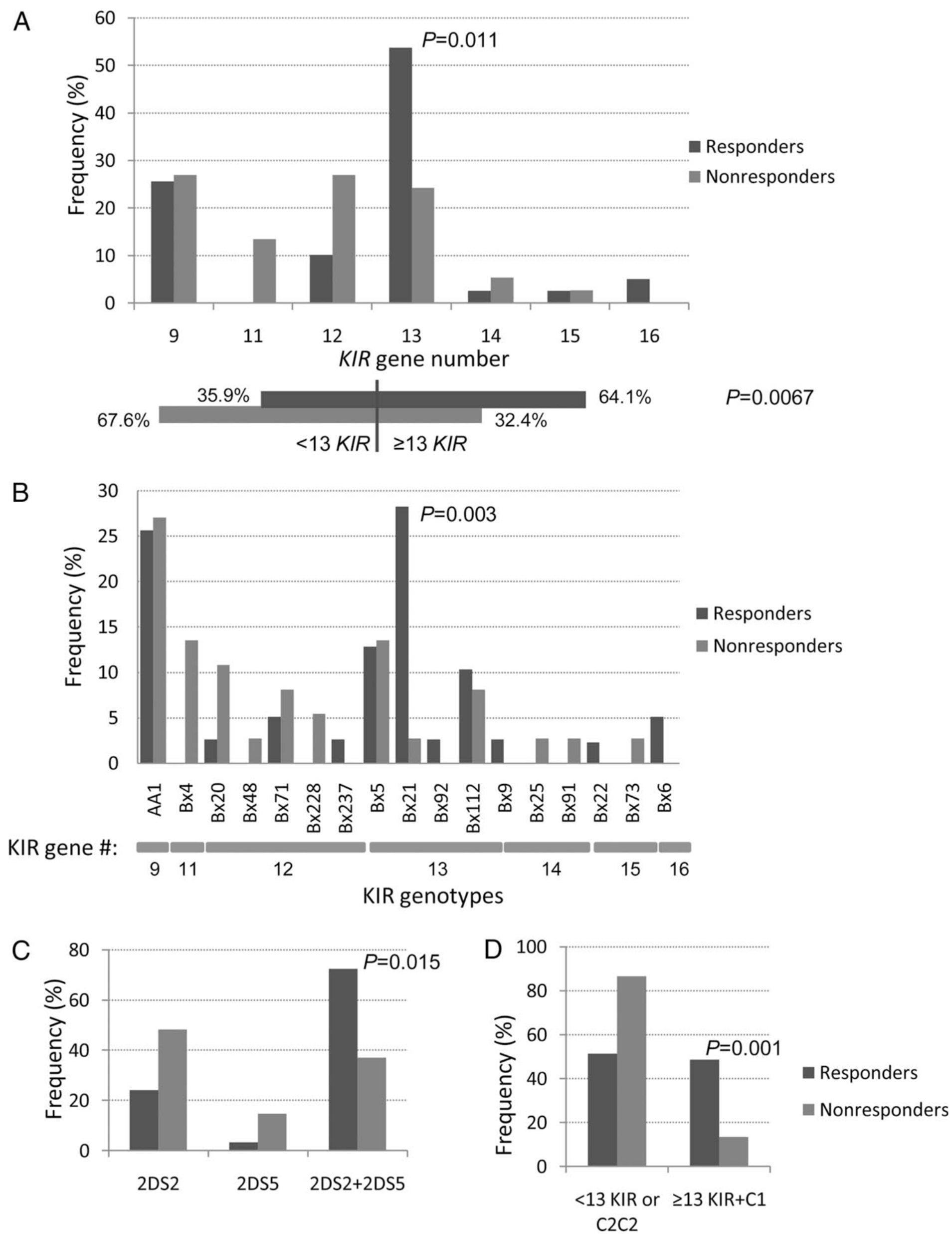

FIGURE 2.

Percentage representation of individual *KIR* gene numbers, *KIR* genotypes, and *KIR2DS2* and/or *KIR2DS5* genes among responders and nonresponders in the HIV-1-infected women. (**A**) Distribution of number of *KIR* genes (nine to 16) among responders and nonresponders. The top graph shows the representations for each *KIR* gene number; proportions of individuals stratified into less than 13 and 13 or greater *KIR* genes are shown below for both groups. (**B**) *KIR* gene haplotype (AA and Bx) representation among responders and nonresponders. *KIR* gene numbers corresponding to the particular *KIR* genotypes are indicated below the x-axis. (**C**) Frequencies of individuals possessing *KIR* genotypes that have *KIR2DS2* or *KIR2DS5* or both genes among responders and nonresponders. These were determined out of a total of 29 responders and 27 nonresponders (10 responders and 10 nonresponders were not included because they are AA1 genotypes, which do not possess either *2DS2* or *2DS5*). (**D**) Frequencies of individuals who possess 13 *KIR* genes or more

together with at least one *HLA-C1* allele among responders and non-responders. The remaining individuals would have either less than 13 *KIR* genes or be homozygous for the *HLA-C2* allele. *P* values for significant differences between groups are shown.

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TABLE 1

KIR and *HLA-B* and *HLA-C* Genotypes and Clinical Parameters of Patients Stratified According to the Presence or Absence of Natural Killer Cell Responses to HIV-1 Peptides

Patient ID	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3	2DS4	2DS5	2DP1	3DL1	3DL2	3DL3	3DS1	3DP1	KIR gene #	KIR genotype	HLA-B B allele	HLA-B B allele	HLA-B B allotype	HLA-C C allele	HLA-C C allele	HLA-C C allotype	CD4 cell count (cells/µl)	Viral load (RNA copies/ml)	Response specificity
Responders (n=39)																											
M001																	9	AA1	4403	4201	Bw4/6	0303	1701	C1C2	650	935	Env/Reg
M003																	13	Bx5	5802	3910	Bw4/6	1203	0602	C1C2	nd	8,120	Env/Reg
M004																	9	AA1	1503	0801	Bw6/6	0701	0210	C1C2	697	62,800	Env
M008																	13	Bx5	5301	1510	Bw4/6	0304	0401	C1C2	945	<400	Env/Reg
M010																	9	AA1	5802	4501	Bw4/6	1601	0602	C1C2	591	2,290	Env/Reg
M026																	13	Bx21	5801	1801	Bw4/6	0302	0210	C1C2	nd	14,900	Reg
M027																	9	AA1	5802	1303	Bw4/4	0602	0602	C2C2	40	56,900	Env
M029																	9	AA1	1510	0702	Bw6/6	0304	0702	C1C1	nd	1,510	Pol
M030																	9	AA1	4901	5101	Bw4/4	0701	0701	C1C1	1479	1,060	Env/Pol
M031																	13	Bx21	5703	4201	Bw4/6	0701	1701	C1C2	nd	22,700	Env
M035																	13	Bx112	5301	1510	Bw4/6	0401	0401	C2C2	nd	1,160	Env
M038																	9	AA1	5801	801	Bw4/6	0701	0701	C1C1	nd	44,500	Env
M039																	14	Bx9	5801	0801	Bw4/6	0302	0217	C1C2	nd	20,300	Env
M042																	9	AA1	4403	5802	Bw4/4	0303	0602	C1C2	254	14,500	Env
M047																	13	Bx112	5802	0801	Bw4/6	0205	0602	C2C2	575	20,600	Env
M048																	16	Bx6	1503	4201	Bw6/6	0210	1701	C2C2	567	4,400	Env/Reg
M052																	13	Bx21	5301	8101	Bw4/6	0804	0401	C1C2	nd	863	Env/Reg
M053																	13	Bx21	1401	1401	Bw6/6	0802	0804	C1C1	nd	964	Env/Reg
M069																	13	Bx81	4403	1503	Bw4/6	0210	0401	C2C2	450	20,100	Env
M071																	12	Bx71	1503	1510	Bw6/6	0210	0401	C2C2	436	38,800	Env
M155																	12	Bx71	4403	4201	Bw4/6	1601	0501	C1C2	402	7,320	Reg
M305																	12	Bx20	0801	1510	Bw6/6	0702	0804	C1C1	470	11,700	Env
M306																	9	AA1	0702	0702	Bw6/6	0702	0804	C1C1	138	4,810	Reg
M307																	13	Bx21	1510	4501	Bw6/6	0304	1601	C1C1	253	4,720	Env
M311																	13	Bx21	3910	4201	Bw6/6	1203	1701	C1C2	697	19,400	Env/Reg
M318																	13	Bx21	0702	4501	Bw6/6	0702	0602	C1C2	nd	32,200	Env
M330																	13	Bx21	0705	6701	Bw6/6	0702	1505	C1C2	692	1,600	Env
M425																	13	Bx112	4403	0702	Bw4/6	0702	0210	C1C2	nd	489	Env
M433																	13	Bx5	5801	0801	Bw4/6	0304	0602	C1C2	nd	5,980	Env/Reg
M563																	13	Bx21	4403	1510	Bw4/6	0304	0401	C1C2	713	13,700	Env/Reg
M589																	13	Bx5	5802	4501	Bw4/6	1601	0602	C1C2	280	21,000	Env
M624																	13	Bx21	4001	4201	Bw6/6	0304	1701	C1C2	370	<400	Env/Reg
M649																	13	Bx5	1801	4202	Bw6/6	0501	1701	C2C2	674	121,000	Reg
M654																	16	Bx6	5802	0801	Bw4/6	0701	0602	C1C2	330	1,700	Env
M655																	9	AA1	0702	1510	Bw6/6	0304	0702	C1C1	501	15,300	Env
M658																	13	Bx21	0801	5703	Bw4/6	0304	0701	C1C1	491	10,300	Reg
M671																	13	Bx112	5703	1503	Bw4/6	0401	0401	C2C2	1011	403	Env/Reg
M773																	15	Bx22	0702	1801	Bw6/6	0704	0401	C1C2	141	<400	Env
M791																	12	Bx237	1503	3910	Bw6/6	1203	0210	C1C2	819	<400	Env
Frequency (%):	100	74	80	100	74	13	72	36	100	56	97	100	100	100	8	100											
Nonresponders (n=37)																											
M002																	14	Bx25	4403	4403	Bw4/4	0701	0701	C1C1	410	17,000	
M006																	9	AA1	4403	4101	Bw4/6	0701	1701	C1C2	195	491,000	
M007																	9	AA1	5801	4501	Bw4/6	0701	1601	C1C1	nd	98,200	
M011																	11	Bx4	5802	3910	Bw4/6	1203	0602	C1C2	486	<400	
M012																	12	Bx20	5801	4201	Bw4/6	0701	1701	C1C2	596	3,990	
M017																	9	AA1	5801	0801	Bw4/6	0701	0701	C1C1	1655	2,430	
M018																	13	Bx5	5801	4201	Bw4/6	0701	1701	C1C2	nd	<400	
M019																	13	Bx5	5801	1503	Bw4/6	0701	0602	C1C2	253	63,800	
M020																	13	Bx5	0705	0801	Bw6/6	0302	0701	C1C1	nd	17,500	
M028																	13	Bx21	1401	4101	Bw6/6	0804	1701	C1C2	439	13,100	
M032																	15	Bx73	5801	5802	Bw4/4	0701	0602	C1C2	nd	154,000	
M033																	12	Bx20	1510	1801	Bw6/6	0704	0804	C1C1	nd	3,380	
M037																	11	Bx4	4403	1510	Bw4/6	0304	0701	C1C1	nd	78,900	
M041																	12	Bx228	0801	4201	Bw6/6	0701	1701	C1C2	1023	<400	
M043																	13	Bx112	1503	4202	Bw6/6	0210	1701	C2C2	81	460,000	
M124																	12	Bx71	4403	4403	Bw4/4	0701	0210	C1C2	253	103,000	
M151																	9	AA1	1510	4501	Bw6/6	0304	1601	C1C1	308	1,820	
M211																	12	Bx20	0801	1510	Bw6/6	0702	0401	C1C2	nd	15,940	
M246																	9	AA1	5802	1510	Bw4/6	0401	0602	C2C2	341	54,400	
M279																	12	Bx48	4201	4501	Bw6/6	0602	1701	C2C2	219	55,600	
M282																	9	AA1	4403	5301	Bw4/4	0401	0401	C2C2	170	466,000	
M310																	11	Bx4	0801	5802	Bw4/6	0205	0602	C2C2	86	1,590	
M321																	9	AA1	4403	1510	Bw4/6	0401	0401	C2C2	441	5,280	
M327																	13	Bx5	5801	8101	Bw4/6	0701	0401	C1C2	650	<400	
M331																	12	Bx71	5802	0801	Bw4/6	0701	0602	C1C2	647	16,700	
M415																	14	Bx91	5802	5802	Bw4/4	0602	1505	C2C2	295	258,000	
M542																	12	Bx71	4403	5802	Bw4/4	0401	0602	C2C2	391	3,870	
M575																	12	Bx228	5801	5802	Bw4/4	0802	0602	C1C2	299	<400	
M578																	9	AA1	4202	8101	Bw6/6	0401	1701	C2C2	483	73,200	
M623																	10	Bx4	5801	0801	Bw4/6	0701	0702	C1C1	419	3,490	
M625																	10	Bx4	5801	1503	Bw4/6	0602	1801	C2C2	270	46,300	
M626																	13	Bx5	5301	1503	Bw4/6	1601	0401	C1C2	313	96,000	
M639																	13	Bx112	0801	3910	Bw6/6	0304	1203	C1C1	288	43,700	
M646																	13	Bx112	0705	1503	Bw6/6	0702	0210	C1C2	277	80,500	
M650																	9	AA1	0702	1510	Bw6/6	1601	1505	C1C2	473	5,140	
M652																	9	AA1	5802	0801	Bw4/6	0205	0602	C2C2	458	8,160	
M774																	12	Bx20	5301	1510	Bw4/6	0304	0401	C1C2	357	78,200	
Frequency (%):	100	70	73	100	60	5	62	38	100	38	100	100	100	100	3	100											

nd: not determined; Env: envelope peptide pool, Reg: regulatory regions (Tat, Rev, Vif, Vpu, and Vpr) peptides combined

C1C1: two group-1 *HLA-C* alleles, C2C2: two group-2 *HLA-C* alleles, C1C2: heterozygote; Bw4/4: two *HLA-Bw6* alleles, Bw6/6: two *HLA-Bw6* alleles, Bw4/6: heterozygote 2DL, 3DL: inhibitory KIR; 2DS, 3DS: activating KIR; 2DP1, 3DP1: pseudogenes

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TABLE 2

Comparison of Frequencies of *KIR2DL2*, *KIR2DL3,* and *HLA-C* Allotypes and Combinations of *KIR-HLA-C* Between Responders and Nonresponders

	Responders (n = 39)	Nonresponders (n = 37)	Responders vs Nonresponders		
	Percent Representation		Odds Ratio	95% Confidence Interval	*P*
KIR alleles					
2DL2/2DL2	20.5	27	0.70	0.24–2.02	0.594
2DL2/2DL3	53.8	43.2	1.53	0.62–3.79	0.370
2DL3/2DL3	25.6	29.7	0.82	0.30–2.23	0.799
HLA-C alleles					
C1/C1	20.5	24.3	0.93	0.32–2.69	1.000
C1/C2	59	45.9	1.52	0.61–3.76	0.491
C2/C2	20.5	29.7	0.61	0.21–1.74	0.431
KIR-HLA combinations					
2DL1+C2	76.9	75.7	1.07	0.372–3.09	1.000
2DL2+C1	56.4	56.8	0.99	0.40–2.44	1.000
2DL3+C1	71.8	51.4	2.41	0.93–6.23	**0.098**
2DS1+C2	12.8	5.4	2.57	0.47–14.18	0.432
2DS2+C1	53.8	45.9	1.37	0.56–3.38	0.646
2DL1+C2C2	20.5	29.7	0.61	0.21–1.74	0.431
2DL2+C1C1	7.7	16.2	0.43	0.10–1.87	0.303
2DL3+C1C1	25.6	21.6	1.25	0.43–3.62	0.790
2DS1+C2C2	5.1	2.7	1.95	0.17–22.4	1.000
2DS2+C1C1	7.7	13.5	0.53	0.12–2.41	0.475
2DL2/2DL2+C1C1	0	2.7			0.487
2DL2/2DL2+C2C2	12.8	8.1	1.67	0.37–7.53	0.712
2DL2/2DL2+C1C2	7.7	16.2	0.43	0.10–1.87	0.303
2DL3/2DL3+C1C1	12.8	8.1	1.67	0.37–7.53	0.712
2DL3/2DL3+C2C2	2.6	16.2	0.14	0.02–1.19	**0.053**
2DL3/2DL3+C1C2	10.3	5.4	2.00	0.34–11.64	0.675
2DL2/2DL3+C1C1	10.3	13.5	0.73	0.18–2.96	0.733
2DL2/2DL3+C2C2	5.1	5.4	0.95	0.13–7.09	1.000

	Responders (n = 39)	Nonresponders (n = 37)	Responders vs Nonresponders		
	Percent Representation		**Odds Ratio**	**95% Confidence Interval**	***P***
2DL2/2DL3+C1C2	38.5	24.3	0.70	0.72–5.23	0.222

Bold *P* values indicate trends ($0.05 < P < 0.1$).

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TABLE 3

Comparison of Percent Representation of Higher Inhibitory and Activating *KIR* Gene Numbers and Inhibitory:Activating Gene Ratios Between Responders and Nonresponders

	Responders (n = 39)	Nonresponders (n = 37)	Responders vs Nonresponders		
	Percent Representation		Odds Ratio	95% Confidence Interval	*P*
***KIR* gene type and number**					
Eight inhibitory genes	56.4	32.4	2.7	1.06–6.87	0.030
Three or more activating genes	71.8	45.9	2.99	1.16–7.75	0.035
Ratio inhibitory:activating genes					
2.7 or less	71.8	45.9	2.99	1.16–7.75	0.035