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Functional Polymorphism of the KIR3DL1/S1 Receptor on Human NK Cells¹

Geraldine M. O’Connor,* Kieran J. Guinan,* Rodat T. Cunningham, † Derek Middleton, † Peter Parham,‡ and Clair M. Gardiner²*

NK cells express both inhibitory and activatory receptors that allow them to recognize target cells through HLA class I Ag expression. KIR3DL1 is a receptor that recognizes the HLA-Bw4 public epitope of HLA-B alleles. We demonstrate that polymorphism within the KIR3DL1 receptor has functional consequences in terms of NK cell recognition of target. Inhibitory alleles of KIR3DL1 differ in their ability to recognize HLA-Bw4 ligand, and a consistent hierarchy of ligand reactivity can be defined. KIR3DS1, which segregates as an allele of KIR3DL1, has a short cytoplasmic tail characteristic of activatory receptors. Because it is very similar to KIR3DL1 in the extracellular domains, it has been assumed that KIR3DS1 will recognize a HLA-Bw4 ligand. In this study, we demonstrate that KIR3DS1 is expressed as a protein at the cell surface of NK cells, where it is recognized by the 2Z7 Ab. Using this Ab, we found that KIR3DS1 is expressed on a higher percentage of NK cells in KIR3DS1 homozygous compared with heterozygous donors. In contrast to the inhibitory KIR3DL1 allotypes, KIR3DS1 did not recognize HLA-Bw4 on EBV-transformed cell lines. The Journal of Immunology, 2007, 178: 235–241.

N atural killer cells are cytotoxic lymphocytes of the innate immune system that are particularly important in the control of viral infections (1, 2). NK cells express a range of activatory and inhibitory cell surface receptors, and their responses are controlled by the overall balance of positive and negative signals received (3, 4). Cell activation can result from either increased activatory or decreased inhibitory signaling inputs. An important source of inhibitory signals comes from HLA-specific receptors, including members of the killer cell Ig-like receptor (KIR)³ family and the CD94/NKG2a heterodimer (5, 6). When HLA expression is altered during viral infection or during transformation, this inhibitory signal is disrupted; NK cells thus become activated and can mediate cytotoxicity against the target cell.

HLA class I genes are highly polymorphic, and most of the variability resides in the Ag-binding groove comprised of the α1 and α2 domains of the H chain (6, 7). This variability affects the peptide that can bind and therefore influences recognition of Ag by the immune system (8). To cope with diversity inherent in HLA, NK cell receptors target relatively conserved epitopes within HLA. HLA-B alleles are characterized by having either a Bw4 or a Bw6 epitope at residues 77–84 of the α1 H chain (9). Although there is no known receptor for Bw6, approximately one-third of HLA-B alleles have the Bw4 epitope that is recognized by the KIR3DL1 (3DL1) receptor on NK cells (10). Although highly conserved, there is some limited variability within the Bw4 epitope and at least four different amino acid sequences are known (11). This diversity has the potential to influence NK cell recognition through 3DL1.

KIR genes are characterized by diversity at many levels including polygeny, polymorphism, and expression characteristics (5). This is a reflection of their recent and rapid evolution (12). We hypothesized that given the strong pressure on KIR to diversify, polymorphism present in individual genes would have functional consequences within the human immune system. As our prototype gene we investigated 3DL1, which we have previously shown to be highly polymorphic (13). Most of the alleles of the 3DL1 locus encode for inhibitory receptors, of which 3DL1*001 and 3DL1*002 are the most common (13). We therefore chose these receptors for investigation along with the distinctive KIR3SD1 (3DS1) allele.

3DS1 is present in ~38% of the population (14). It is highly homologous to 3DL1 in its extracellular domains (ECD) but resembles activatory KIR in the intracellular portion of the molecule. Activatory KIR have no inherent signaling capacity because they have a truncated cytoplasmic tail (5). However, upon receptor ligation, they can recruit positive signaling adaptor molecules that result in NK cell activation (15–17). No serological reagents for 3DS1 have been described to date, and this has hampered biochemical and functional characterization of this receptor. 3DS1 is of particular interest because it has been reported that, in combination with HLA-Bw4 molecules with isoleucine at position 80 (Bw4–80Ile), its expression is associated with delayed progression to AIDS (17). This association was only seen in the presence of both 3DS1 and Bw4–80Ile, suggesting that in these patients, these receptors interact functionally. One attractive hypothesis is that 3DS1+ NK cells recognize and kill HLA Bw4–80Ile HIV-infected targets, allowing control of infection. Due to the very high degree of similarity to 3DL1 extracellularly, 3DS1 is expected to have similar ligand specificity, but neither this nor definitive cell surface expression have been demonstrated.
In this study, we demonstrate that polymorphism within 3DL1 influences recognition by the HLA-Bw4 epitope and, in turn, polymorphism within HLA-B contributes to altered recognition of 3DL1. In addition, we demonstrate that 3DS1 is expressed at the cell surface, where it is recognized by the Z27 Ab. However, despite a significant genetic association with delayed disease progression in HIV-infected patients (17), we did not detect any functional interaction between 3DS1 and HLA-Bw4.

Materials and Methods

Cell culture

The T cell line Jurkat, the MHC class I-deficient EBV-transformed B cell line 721.221, and its transfectants 721.221B*0702, 721.221B*2705, 721.221B*3801, 721.221B*5101, and 721.221B*5801 (10) were maintained in RPMI 1640 medium (In vitro Life Technologies) supplemented with 10% FCS (PAA Laboratories). Transfectants with similar levels of protein expression were used in experiments.

Generation of NK cell cultures

Blood samples were drawn from normal healthy individuals from whom written consent was obtained. PBMCs were isolated using Lymphoprep (Axis- Shield) gradient. DNA was isolated using Wizard Genomic DNA isolation kit (Promega), and 3DL1 allelic typing analysis was performed as described previously (13). NK cells were isolated by magnetic bead isolation using NK Isolation kit II (Miltenyi Biotec) according to the manufacturer’s instructions. NK cells were stained with anti-CD56FITC Ab (BD Biosciences), anti-3DL1PE (Z27) and anti-CD56PECy5 (BD Biosciences). CD69 samples were stained with anti-CD69 Ab (BD Biosciences). Anti-3DL1PE (Z27) and anti-CD69PECy5 (BD Biosciences). CD69 samples were stained with anti-CD69 Ab (BD Biosciences). anti-NKG2A Ab (Beckman Coulter). LILRB1-negative clones were preferred for analysis. Functional interaction between 3DS1 and HLA-Bw4.

RT-PCR

Total RNA was extracted from Z27-negative and Z27-dim-sorted NK cell populations with Tri Reagent (Molecular Research Centre). cDNA was generated with random hexamers using ImProm-II Reverse Transcription System (Promega). PCR was conducted on cDNA samples with the following primers: 2DL4RT (forward (F), CTGTCCTCGAGCTCTACAA and reverse (R), CACTGTAGACTCTAATACACAG) to ensure quality of the cDNA; and 3DS1RT (F, GGCCGCCACGCAACCCCA and R, AAGGGCACGCATCATGGA) for the presence of 3DS1 mRNA using TaqDNA polymerase (In Vitro Life Technologies).

CD107a/CD69 activation

Purified NK cells were plated at 1 × 10⁶ cell/ml and stimulated with 721.221 class I cells at a stimulator:responder ratio of 1:5 (CD107a) or 1:1 (CD69). Anti-CD107a FITC Ab and 10 μM monensin (Sigma-Aldrich) were added at time of stimulation for the CD107a experiment. Cells were incubated at 37°C for 6 h (CD107a) or 18 h (CD69), and then stained with anti-CD107a (Z27) and anti-CD69PEcy5 (BD Biosciences). CD69 samples were stained with anti-CD69 Ab (BD Biosciences).

51Cr release cytotoxicity assay

Target cell lines (721.221 and transfectants) were labeled with 50 μCi of Na⁵¹CrO₄ for 1 h at 37°C. Cells were then washed twice in complete medium and incubated with effector cells (NK cell clones, NK cell polyclonal cultures) at an E:T ratio of 6:1. After 4 h at 37°C, a sample of medium and incubated with effector cells (NK cell clones, NK cell poly-erentially chosen for analysis.

Anti-NKG2A Ab (Beckman Coulter). LILRB1-negative clones were pref-

man Coulter), anti-CD94 Ab (DakoCytomation), and leukocyte Ig-like receptor (LILR)B1 Ab (Beckman Coulter), anti-CD3PerCP Ab, anti-leukocyte Ig-like receptor (LILR)A1 Ab (BD Biosciences) and anti-CD56-positive, CD3-negative, 3DL1-positive cells were sorted on a BD FACS Aria (BD Biosciences). Polyclonal cultures were maintained in CellGro medium (CellGenix) supplemented with 10% FCS (PAA Laboratories). Transfectants with similar levels of protein expression were used in experiments.

Generation of KIR3DS1 FLAG

Total RNA was isolated from PBMCs of a 3DS1-positive individual using Tri Reagent (Molecular Research Center). cDNA was generated with random hexamers using ImProm-II Reverse Transcription System (Promega). Full-length 3DS1 was amplified with primers that contained NotI and EcoRI restriction sites for subcloning (F, 5’-cgaatctgagcagacagaccgtgtgtgtgtgtgtggtgtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtggtg
2 mg/ml. After selection, GFP-expressing cells were sorted to generate stable cell lines expressing different allelic variants of 3DL1. These variants were named KIR3DL1*001ECD, KIR3DL1*002ECD, and KIR3DS1ECD for 3DL1*001, 3DL1*002, and 3DS1 alleles, respectively. They expressed protein at similar levels as measured by flow cytometry.

**Transfection**

Transfection of Jurkat cells was performed using a Bio-Rad Gene Pulser II electroporator. A total of 15 μg of plasmid DNA (KIR3DS1FLAG, KIR3DL1/CD3, or NFAT-luciferase construct) was transfected into 10^7 Jurkat cells in 250 μl of cytomix electroporation buffer (120 mM KCl, 0.15 mM CaCl2, 10 mM K2HPO4/KH2PO4, 25 mM HEPES, 2 mM EGTA, 2 mM ATP, and 5 mM glutathione (pH 7.6)) in a 2-mm gap cuvette. The cuvette was then subjected to two pulses, each 240 V and 100 μF, 30 s apart.

**Reporter assay**

A total of 10^7 KIR3DL1ECD/CD3ζ Jurkat cells was transfected with 15 μg of NFAT-luciferase plasmid. Transfected Jurkat cells were plated at a concentration of 1 × 10^5 cells/ml, and stimulator cells (721.221 cell line and its transfectants) were added to give a stimulator:responder ratio of 1:5. CD3/CD28 Dynabeads (Dynal Biotech) (3 beads/cell) were added to positive wells. Cells were incubated for 18 h at 37°C with 5% CO2, after which they were harvested, and the cells were lysed for 15 min on a shaker at room temperature with 50 μl of 1× Passive Lysis Buffer (Promega). Firefly luciferase activity was assayed by the addition of 40 μl of luciferase assay mix (20 mM tricine, 0.26 mM (MgCO3)4Mg(OH)2.5H2O, 2.67 mM MgSO4, 0.1 M EDTA, 33.3 mM DTT, 270 μM CoA, 470 mM luciferin, 530 μM ATP) to the sample; luminescence was read using the Reporter microplate luminometer (Turner Designs) and expressed relative to unstimulated control.

**Results**

Polymorphism of HLA-Bw4 alleles affects recognition by 3DL1*001

3DL1 recognizes the subgroup of HLA-B molecules that express the Bw4 serological epitope. We wished to investigate whether natural variability within the Bw4 epitope of HLA-B alleles affects recognition by 3DL1. To address this question, genetic constructs were generated that consisted of the ECD of different alleles of 3DL1 linked to the CD3ζ-chain intracellularly. These were transfected into the Jurkat T cell line, which we know to be KIR negative, to create a panel of Jurkat cells expressing single 3DL1 alleles in isolation. 3DL1*001 (KIR3DL1*001ECD), 3DL1*002 (KIR3DL1*002ECD), and 3DS1 (KIR3DS1ECD) expressing cells were identified by GFP staining and cell sorted to generate stably transfected cell lines. Ligation of 3DL1 on the cell surface leads to positive signaling through NFAT, which can then be measured by a standard reporter assay. To identify receptor specificity, we used a panel of stimulator cells (721.221 cell line) transfected with individual HLA alleles. This reductionist system allows interactions between 3DL1 and HLA to be studied in isolation from the complexities of multiple receptor expression. Incubation of KIR3DL1*001ECD-transfected Jurkat cell line with stimulator 721.221 cell lines expressing Bw4, but not Bw6 HLA-B allotypes,
led to the generation of a NFAT signal as expected (Fig. 1A). Clear and consistent differences in the strength of signal generated from different HLA-B allotypes were observed, even when the amino acid sequence of the HLA-Bw4 epitope were similar (see Table I). Interaction of 3DL1*001 with B*5101 consistently produced a stronger inhibitory signal than any other HLA-B allotype tested, whereas B*2705 generated only a very weak signal.

3DL1*001 specificity was also examined in NK cell clones. IL-2-activated NK clones that were positive for 3DL1*001 were used as effector cells against our panel of 721.221 transfectants in a 51Cr release assay as described previously (20). As expected, cells expressing HLA-Bw4 allotypes were protected from lysis by 3DL1*001-positive clones, and this inhibition of killing was reversed in the presence of the anti-3DL1 Ab DX9 (Fig. 1B). The hierarchy of recognition was similar to that seen in Fig. 1A with B*5101 giving the greatest level of protection, followed by B*3801, B*5801, and B*2705.

Primary, bulk populations of peripheral blood NK cells were purified from two 3DL1*001 homozygous donors and incubated with the panel of 721.221 transfectants, after which activation (CD69) and degranulation (CD107a) markers were examined. Double staining with the anti-3DL1 Ab Z27 allowed for identification of the 3DL1-positive NK cells. Incubation of NK cells with the 721.221 parental cell line led to a dramatic increase in CD69 expression in both KIR-positive and KIR-negative NK cells (Fig. 2). Quantification of the reduction in the percentage of CD69 and CD107a-positive 3DL1*001-positive NK cells produced a hierarchy that is consistent with that obtained from the Jurkat transfectant and clonal cytotoxicity assays. B*5101 is consistently the strongest ligand for 3DL1*001 followed by B*3801 and B*5801. Although B*3801 appears to be significantly stronger than B*5801 in both the Jurkat and clone assays, the difference is less clear with freshly isolated NK cells. B*2705 is also capable of inhibiting CD69 and particularly CD107a expression in this assay. Thus, using a number of approaches, it is clear that HLA-B Bw4 variability impacts on 3DL1 recognition.

**KIR3DL1 polymorphism affects HLA-Bw4 recognition**

We next examined whether differences between the 3DL1*001 and 3DL1*002 allotypes affected their interaction with the panel of HLA-B allotypes. Using the Jurkat assay, both 3DL1 alleles showed recognition of HLA-Bw4 allotypes but not of the parental or Bw6-positive cell line as expected. Although both 3DL1 alleles showed a preference for B*5101, their interactions with B*2705 and B*5801 were markedly different. Although B*2705 appears to be a weak ligand for 3DL1*001, with only a marginal increase over the Bw6 alleleotype HLA-B*0702, it is a much stronger ligand for 3DL1*002 (Fig. 3). The reverse is true for HLA-B*5801. Thus, polymorphism present in the 3DL1 receptor affects NK cell recognition of HLA-Bw4.

**KIR3DS1 is expressed at the NK cell surface**

3DS1, a short-tailed allele of 3DL1 that is predicted to be activating, is highly homologous to other 3DL alleles extracellularly. Although 3DS1 mRNA is transcribed, 3DS1 does not bind to the anti-3DL1 Ab DX9 and cell surface expression of the 3DS1 protein has not been demonstrated. To address this question, we generated a full-length 3DS1 construct that contained a FLAG epitope at the N terminus by inserting the FLAG sequence between the leader sequence and the first coding exon. This construct was then transfected into the Jurkat cell line and stained extracellularly using an anti-FLAG Ab. As shown in Fig. 4A, cells that were transfected with the FLAG-tagged 3DS1 construct stained with the anti-FLAG Ab whereas the mock-transfected cells did not, indicating that 3DS1 is expressed on the cell surface where it may be functionally active.

NK cells from 60 3DL1-typed donors were analyzed for binding to the anti-3DL1 mAb DX9 and Z27. Although the two Abs had similar reactions with 3DL1, their reaction with 3DS1 was different (Fig. 4B). Whereas DX9 was clearly negative, Z27 gave a weak positive reaction that defined a subpopulation of Z27+ NK cells present only in donors who were heterozygous or homozygous for 3DS1. Interestingly, we found that a significantly higher percentage of NK cells expressed 3DS1 when donors were homozygous for this allele when compared with 3DS1 heterozygotes (p < 0.0001; Fig. 4C). A Z27 dim subpopulation of CD8, but not CD4, T cells was also detected in 3DS1-positive donors (data not shown). These results indicated that 3DS1 is expressed at cell surfaces. To investigate this question further, we transfected Jurkat cells with the 3DS1ECD/CD3 ϶ construct. These transfected cells bound Z27 to a level that clearly distinguished them from untransfected cells (Fig. 4D). In summary, these results demonstrate that 3DS1 is expressed at cell surfaces and can be recognized by an anti-3DL1 Ab.

**KIR3DS1 does not recognize HLA-B on EBV-transformed B cells**

To investigate the interaction of cell surface 3DS1 with HLA-B, we used the KIR3DS1ECD Jurkat cell line in the reporter assay as described above. We first demonstrated that our 3DS1 chimera was capable of transducing signal by cross-linking the receptor with the Z27 Ab (Fig. 5A, left panel). However, no interaction between 3DS1 and any of the HLA-B alleles tested was detected (Fig. 5A). Confocal microscopy imaging (data not shown) and Z27 Ab staining (Fig. 4D) of the Jurkat transfectant show cell surface expression of the KIR3DS1ECD/CD3 ϶ construct, suggesting that intracellular retention of the construct is not responsible for the lack of...
interaction seen. Our finding that the Z27 dim population represented 3DS1-positive cells allowed us to generate a polyclonal culture of 3DS1-positive NK cells by sorting and expanding Z27 dim NK cells. RT-PCR analysis confirmed 3DS1 expression in the Z27 dim but not in Z27-negative population (Fig. 5

Polyclonal cultures of 3DS1-positive NK cells were used as effectors against a panel of 721.221 transfectants in a cytotoxicity assay. If 3DS1 is activated by HLA-B allotypes, we would expect that 721.221 cells transfected with HLA-B alleles would be killed to a greater degree than HLA-negative cells by 3DS1-positive cultures and that this effect could be reversed with Z27 Ab blocking. Although some differences were seen between 3DS1-positive and -negative cultures, these were only very slight and, more importantly, blocking of 3DS1 with the Z27 Ab did not alter killing of any of the target cell lines examined (Fig. 5C). These data agree with the Jurkat transfectant data and further support that 3DS1-positive NK cells are not activated by HLA-B.

Discussion

KIR have been identified in recent years as an important family of receptors expressed by NK cells, which allow them to respond to presence and levels of HLA class I Ag on target cells during infection or transformation events (16). 3DL1 is a polymorphic KIR that interacts with the HLA-Bw4 public epitope (10). We hypothesized that polymorphism present in both 3DL1 and HLA-B would have functional consequences and indeed this proved to be the case. As has been reported previously (21, 22), we have observed that polymorphism within HLA-Bw4 alleles affected recognition by 3DL1. Using a number of experimental approaches, a consistent hierarchy of HLA-Bw4 reactivity was identified for the 3DL1*001 allotype. B*5101 gave the strongest response in all assays, whereas B*2705 was generally the weakest. Because B*2705 is characterized by having a different Bw4 amino acid sequence compared with the other Bw4 alleles tested, this may in part contribute to the results seen (see Table I). In addition, different HLA alleles have different peptide binding specificities that are influenced in part, but not exclusively, by the Bw4 epitope present. Divergent peptide repertoires presented by alleles with similar Bw4 epitopes will also contribute to the patterns of reactivity observed. The data presented in this study demonstrate that 3DL1 is influenced by different Bw4 epitopes and that this results in altered functional responses. Similarly, we found that polymorphism present within 3DL1 affected functional responses of NK cells because different allotypes were inhibited to a different degree by a single HLA-B allotype. Although B*2705 was recognized by both 3DL1*001 and 3DL1*002 allotypes, inhibition through the 3DL1*002 allotype was more pronounced. Khakoo et al. (19) previously reported a limited interaction between 3DL1*002 and
FIGURE 5. KIR3DS1 does not recognize HLA-Bw4. A, KIR3DS1ECD construct is functional because cross-linking with the Z27 Ab at 2 μg/ml transduced a positive signal (left panel). KIR3DS1/CD3 Jurkats were stimulated with 721.221 cells and a panel of its HLA transfectants at a stimulator: responder ratio of 1:5 (right panel). Firefly luciferase activity was measured after 18 h as a readout of NFAT activation and is expressed relative to unstimulated control. Data shown are the average of four independent experiments and error bars show the SEM. B, 3DS1 mRNA expression is restricted to cells reactive with the Z27 Ab. cDNA was generated from sorted Z27-negative and Z27 dim staining NK cell populations. RT-PCR was used to measure expression of KIR2DL4 (positive control for quality of cDNA) and KIR3DS1. C, KIR3DS1-positive cells from a homozygous donor were sorted, expanded, and used as effectors against a panel of 721.221 HLA transfectants in a 4-h 51Cr release cytotoxicity assay. Cytotoxicity is presented as percentage killing of the 721.221 parental cell line. Data shown are representative of three independent donors and error bars show SD.

B*2705. Our results contrast with this and support the recent report by Carr et al. (23) in which 3DL1*002 was inhibited by B*2705. The study by Carr et al. (23) also found functional differences between 3DL1 alleles; in contrast to 3DL1*002 and similar to our findings for 3DL1*001, the 3DL1*007 allele was not inhibited by B*2705. Similar to Yawata et al. (24), the B*5801 HLA-Bw4 allele was informative in demonstrating functional polymorphisms of 3DL1. It provided a relatively strong ligand for the *001 allele in contrast to *002, which it barely triggered. Although both 3DL1*001 and *002 alleles were activated by B*3801 and B*5801, the signal activated through 3DL1*001 was much stronger, confirming it as a sensitive KIR receptor (24). The most informative differences between our 3DL1 alleles was apparent when we examined data for both B*2705 and B*5801 HLA types. Thus, 3DL1 alleles contribute to a qualitative spectrum of NK cell functional responses in terms of specific HLA-Bw4 recognition. This functional polymorphism expands the pool of pathogens to which NK cells can respond, and individual people will differ in their abilities to respond to pathogen depending on their combination of KIR genotype, phenotype, and HLA type.

3DL1 is highly polymorphic, and we have previously demonstrated that heterogeneity in flow cytometry staining patterns reflected allelic diversity at the 3DL1 locus (13). Particular alleles of 3DL1 (3DL1*001 and *002) had a bright peak when stained with the DX9 Ab, whereas others (3DL1*005) had a dim staining profile. Individuals who were heterozygous for combinations of these patterns had a characteristic bimodal pattern of staining. There were two allotypes identified in our study that did not react with the DX9 Ab: 3DL1*004 and 3DS1. It was subsequently demonstrated that 3DL1*004 was found to be poorly expressed at the cell surface, which explained its staining pattern (25). In this study, we have definitively demonstrated that 3DS1 protein is expressed at the NK cell surface. It is not recognized by DX9 and in support of the suggestion in Ref. 26, we have defined Z27 as an Ab that recognizes 3DS1 with a characteristic very dim staining profile by flow cytometry. The low intensity of Z27 Ab staining may be due to a lower Ab affinity for 3DS1 relative to other alleles of 3DL1 or possibly a lower level of cell surface expression. Although Z27 and DX9 Abs have very similar staining profiles of 3DL1, their exact epitopes differ (19). Tyr200 has been defined as an amino acid important for DX9 but not Z27 recognition of 3DL1. This residue is common to all 3DL1/S1 alleles and is therefore not directly involved in Z27 discrimination of 3DS1. It is possible that amino acid residue 199, in close proximity, may contribute to differential Ab recognition because Pro is present in all 3DL1 alleles whereas Leu is present in 3DS1. In general, the intensity of Z27 Ab staining is consistently higher than that seen with DX9 staining of 3DL1. Using the Z27 Ab to stain NK cells of a panel of normal donors, we have found that individuals homozygous for 3DS1 express this receptor on a higher proportion of NK cells compared with 3DL1 heterozygotes. Although this may have an as yet unidentified functional significance, it may simply reflect a gene dose effect for inhibitory alleles of 3DL1, as recently observed by Yawata et al. (24).

Although 3DS1 appears to segregate as an allele of 3DL1, it differs from other inhibitory 3DL1 alleles in a number of respects. Most striking, in terms of its structure, is its similarity in the transmembrane and cytoplasmic tail regions toactivatory KIR receptors (13). 3DS1 is very similar to 3DL1 in its extracellular region, prompting the assumption that it would also recognize HLA-Bw4 ligand. We have shown that 3DS1, either on polyclonal NK cell cultures or present on the Jurkat transfectants, did not recognize any HLA-Bw4 allotype tested. Although 3DS1 is very similar to
3DL1 extracellularly, the patterns of amino acid substitutions differ from other alleles. Although inhibitory alleles of 3DL1 tend to share polymorphic substitutions in a “patchwork pattern” of substitutions, 3DS1 is unusual because it has six unique amino acid substitutions in its ECD (13). Our data suggest that these changes may result in differences in ligand specificity compared with other 3DL1 alleles.

It is also possible that peptide may play a role in restricting recognition of HLA-Bw4 by 3DS1. Presentation of peptide Ag by HLA class I to TCR-restricted T lymphocytes has been well characterized. However, data are beginning to emerge to support a possible role for the presentation of peptide to NK cells. Under normal conditions, NK cells recognize HLA class I Ag through inhibitory receptors on the cell surface. During a viral infection, removal of the inhibitory signal through down-regulation or absence of HLA class I Ag, provides a mechanism of NK cell activation (16). In addition, the presence of foreign peptide presented by HLA class I Ag can perturb inhibitory receptor ligation on the NK cell, which can result in its activation. This has previously been demonstrated in a gene therapy setting where T cells transduced with a retroviral vector became susceptible to autologous NK cell lysis (27). T cells presented foreign peptide on a HLA-Bw4 background, perturbing 3DL1 inhibitory signals to autologous NK cells that caused recognition and cytotoxicity of the T cells.

In terms of activatory KIR, it is not surprising that 3DS1 does not recognize HLA-Bw4 because NK cells should not be activated during normal homeostasis. However, we predict that 3DS1 will recognize HLA-Bw4 in a peptide-specific manner. A precedent for this comes from KIR3DL2, where the interaction between KIR3DL2 and HLA-A3 or HLA-A11 has been shown to be highly peptide specific; HLA-A3 or HLA-A11 tetramers folded in the presence of an EBV, but not self or HIV peptides, allowing binding (28). It is possible that there is a similar specificity with 3DS1 (although because we tested 3DS1 using EBV-transformed cell lines, it is unlikely to be EBV specific) with the presentation of specific, perhaps infection-associated, peptides by HLA-Bw4 required for recognition. Recognition of HLA-specific peptide by 3DS1 may provide the molecular basis for the genetic association between coexpression of 3DS1 and HLA-Bw4, and long-term non-progression in HIV (17). Our understanding of the functional specificities of KIR is in its relative infancy compared with our knowledge of their genetics. Our findings in this study expand our knowledge of the functional consequences of KIR allelic diversity and adds another level of complexity that must be considered when investigating interactions between KIR and their HLA ligands.

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Disclosures

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