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

Geraldine M. O'Connor, Kieran J. Guinan, Rodat T. Cunningham, Derek Middleton, Peter Parham and Clair M. Gardiner


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Natural 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) 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. 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 Z27 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.

NK cells recognize and kill HLA Bw4 – 80Ile HIV-1-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.

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Abbreviations used in this paper: KIR, killer cell Ig-like receptor; ECD, extracellular domain; LILR, leukocyte Ig-like receptor; F, forward; R, reverse.

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

Geraldine M. O’Connor,* Kieran J. Guinan,* Rodat T. Cunningham, † Derek Middleton, † Peter Parham, ‡ and Clair M. Gardiner2*
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, 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 (Invitrogen 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 Ab (Beckman Coulter), and anti-CD3PerCP Ab, anti-leukocyte Ig-like receptor (LILR)B1 Ab (Beckman Coulter), anti-CD94 Ab (DakoCytomation), and anti-NKG2A Ab (BD Biosciences). LILR1-negative clones were preferentially chosen for analysis.

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), CACCTGAGCTCTACAA and reverse (R), CACTGAGTACCTAATCACAG) to ensure quality of the cDNA; and 3SD1RT (F, GGCACCCAGCAACCCCA and R, AACGCCGACGATCCAGGA) for the presence of 3DS1 mRNA using TaqDNA polymerase (Invitrogen Life Technologies).

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 hexamer primers using ImProm-II Reverse Transcription System (Promega). Full-length 3DS1 was amplified with primers that contained NotI and EcoRI restriction sites for cloning into TOPo cloning kit (Invitrogen Life Technologies) and sequenced. An error-free clone was digested and subcloned into the expression vector pCDDEFII. Leader-FLAG (F, 5’-ecagaggggcaacagcagccagcagctctg-3’) and FLAG-D0 (F, GGCACCCAGCAGCAACCCCA and R, AACGCCGACGATCCAGGA) were generated by recombinant PCR. Recombinant PCR was used to generate a leader-FLAG-D0 construct. This construct and the full-length 3DS1 construct were digested with NotI and EcoHI and the leader-FLAG-D0 sequence was ligated into the 3DS1 construct.

Generation of KIR3DL1EC/D3ξ-expressing cell lines

Chimeric constructs consisting of the ECD of different 3DL1 linked with the intracellular CD3ξ chain were generated by recombinant PCR using a similar strategy to that described previously (19). Briefly, error-free clones of 3DL1*001, 3DL1*002, and 3DS1 were used as templates for first round PCR, which generated a product containing a ζ-chain overlap. Similarly, first-round PCR from a ζ-chain-containing plasmid generated a ζ-chain with a 3DL1 overlap. Second-round PCR created a chimeric construct encoding 3DL1 in ECD and CD3ξ in cytoplasmic domain. PCR products were cloned into a GFP-expressing plasmid such as pCDDEFII. Jurkat cells were then transfected by electroporation and cultured in G418 (Sigma-Aldrich) at

FIGURE 1. Variability in HLA-Bw4 affects recognition by 3DL1*001. A, 3DL1*001/1IECD Jurkat cells were transfected with a NFAT-luciferase reporter plasmid. Cells were then stimulated with the 721.221 cell line and a panel of its HLA transfectants at a stimulator:responder ratio of 1:5. Firefly luciferase activity was measured after 18 h as a readout of NFAT activation and is expressed relative to unstimulated control. CD3/CD28 Dynabeads (3 beads/cell) were used as a positive control. Data shown are representative of four independent experiments and error bars represent SDs. B, 3DL1*001-positive clones were used as effectors in a 51Cr release cytotoxicity assay against a panel of 721.221 transfectant targets. Cells were incubated with anti-KIR3DL1 Ab DX0 at 10 μg/ml. Data shown are representative of three independent experiments and error bars represent SD.
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/CD3ζ, or NFAT-luciferase construct) was transfected into 10^7 Jurkat cells in 250 μl of cytomix electroporation buffer (120 mM KCl, 0.15 mM CaCl$_2$, 10 mM K$_2$HPO$_4$/KH$_2$PO$_4$, 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 x 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% CO$_2$, 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 (MgCO$_3$)$_2$Mg(OH)$_2$.5H$_2$O, 2.67 mM MgSO$_4$, 0.1 M EDTA, 33.5 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.

Table I. Polymorphism present in Bw4/Bw6 epitopes of HLA-B alleles

<table>
<thead>
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<th>Allele</th>
<th>Serotype</th>
<th>Amino Acid Residue Number</th>
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<td></td>
<td>77</td>
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<tr>
<td>HLA-B*0702</td>
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<td>S</td>
</tr>
<tr>
<td>HLA-B*2705</td>
<td>Bw4</td>
<td>D</td>
</tr>
<tr>
<td>HLA-B*3801</td>
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<tr>
<td>HLA-B*1513</td>
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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, inhibitory wells. Cells were incubated for 18 h at 37°C with 5% CO$_2$, 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 (MgCO$_3$)$_2$Mg(OH)$_2$.5H$_2$O, 2.67 mM MgSO$_4$, 0.1 M EDTA, 33.5 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**

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,

**FIGURE 2.** Primary NK cells from a donor expressing 3DL1*001 are differentially inhibited by HLA-B allotypes. Purified NK cells were incubated with a panel of 721.221 transfectants at a stimulator:responder ratio of 1:1 (CD69) or 1:5 (CD107a). Cells were coincubated at 37°C for 6 h (CD107a) or 18 h (CD69), and then stained with anti-KIR3DL1PE (Z27) and anti-CD56PECy5. Data for HLA-B*5101 is shown as a representative plot in A. B and C, Data for CD69 and CD107a, respectively.
Hirarch of recognition was similar to that seen in Fig. 1.versed in the presence of the anti-3DL1 Ab DX9 (Fig. 1).3DL1*001-positive clones, and this inhibition of killing was re-

Cells expressing HLA-Bw4 allotypes were protected from lysis by

led to the generation of a NFAT signal as expected (Fig. 1). 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 epitopes 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 at a stimulator: responder ratio of 1:5. Firefly luciferase activity was measured after 18 h as a readout of NFAT activation and is expressed relative to un-
stimulated control. CD3/CD28 Dynabeads (3 beads/cell) were used as a
positive control. Data shown are the average of four independent ex-
periments. Error bars, SEM.

KIR3DL1*001

Relative 1 Lactase Activity

KIR3DL1*002

Relative 1 Lactase Activity

FIGURE 3. KIR3DL1 polymorphism affects HLA-B recognition. 3DL1*001ECD and 3DL1*002ECD Jurkat cell lines were transfected
with NFAT-luciferase reporter plasmid. Cells were then stimulated with
721.221 cells and a panel of its HLA transfectants at a stimulator:

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 identifi-

3DS1, a short-tailed allele of 3DL1 that is predicted to be activa-
tory, is highly homologous to other 3DL1 alleles extracellularly.
Although 3DS1 mRNA is transcribed, 3DS1 does not bind to the

Anti-3DL1 mAb DX9 and Z27. Although the two Abs had
similar reactions with 3DL1, their reaction with 3DS1 was differ-
ent (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 percent-
age of NK cells expressed 3DS1 when donors were homozygous
for this allele when compared with 3DL1 (Fig. 4B). 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 on the cell surface where it may be func-
tionally 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 differ-
ent (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 percent-
age 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 3 construct. These transfected cells
bound Z27 to a level that clearly distinguished them from untrans-
fected 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 stain-
ing (Fig. 4D) of the Jurkat transfectant show cell surface expres-
sion of the KIR3DS1ECD/CD3 3 construct, suggesting that intra-
cellular retention of the construct is not responsible for the lack of

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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 parent-
al or Bw6-positive cell line as expected. Although both 3DL1
allotypes 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
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. 5B). 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 alleles 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 allele was more pronounced. Khakoo et al. (19) previously reported a limited interaction between 3DL1*002 and
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 were 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.

Leu is present in 3DS1. In general, the 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 DX9 staining of 3DL1. This is in contrast to 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.

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 to activatory 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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