KIR2DS1-mediated activation overrides NKG2A-mediated inhibition in HLA-C C2-negative individuals

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Abstract

NK cell cytotoxicity is controlled through a balance of both activating and inhibitory signals. The HLA specificity of alloreactive NK cells has been previously shown to be controlled by inhibitory killer immunoglobulin-like receptors (KIRs). Alloreactive NK cells lyse targets that lack the HLA ligand for their inhibitory KIR. We have characterized in detail an alloreactive NK clone in which the specificity is controlled by an activating receptor, KIR2DS1. Only target cells expressing the HLA-C group 2 (C2) epitope were lysed by this clone and homozygous C2 targets were lysed more strongly than heterozygous C1/C2 targets. Anti-CD158a (KIR2DS1) blocked lysis of targets confirming KIR2DS1 was responsible. Although this NK clone expressed NKG2A, an inhibitory receptor whose ligand is HLA-E, targets with ligands for both KIR2DS1 and NKG2A were lysed by this clone indicating that the KIR2DS1-mediated activation signal overrides the NKG2A-mediated inhibitory signal. KIR2DS1 activated NK clones in polyclonally expanded NK cultures from a donor that lacked the C2 epitope accounted for ~1% of all NK cells. This study highlights a potential role for NK cells controlled by activating KIR in mediating NK alloreactivity.

Introduction

NK cell cytotoxicity is controlled by a balance of both activating and inhibitory signals. To prevent autoreactivity, NK cells do not become competent for cytotoxicity (armed or licenced) unless they express at least one inhibitory receptor recognizing self-ligands (1, 2). As the ligands for some NK inhibitory receptors are allelic epitopes present on class I HLA molecules, NK cell clones that depend on such receptors for inhibition exhibit alloreactivity. It is thought that the inhibitory signals mediated by these receptors under normal conditions are dominant over signals generated by activating receptors (3, 4).

The killer immunoglobulin-like receptors (KIRs) recognize allelic epitopes present on HLA-A, HLA-B or HLA-C molecules. HLA-C alleles can be divided into two groups, HLA-C group 1 (C1) and HLA-C group 2 (C2), based on their amino acid sequences in the α1 helix. C1-group alleles have the amino acid serine (Ser) at residue 77 and asparagine (Asn) at residue 80, whereas C2-group alleles have the amino acid Asn at residue 77 and lysine (Lys) at residue 80 (5). The inhibitory receptor KIR2DL1 recognizes C2-group alleles while KIR2DL2 and KIR2DL3 (CD158b) recognize C1-group alleles. Although the amino acid sequences of activating KIR (KIR2DS1, KIR2DS2 and KIR3DS1) suggest that they might bind to similar ligands as their inhibitory counterparts (KIR2DL1, KIR2DL2/3 and KIR3DL1), it has been difficult to show that activating receptors bind to these ligands.

Alloreactive NK clones from C1 homozygous individuals lyse C2 homozygous EBV-transformed B lymphoblastoid cell lines (BLCLs). These clones have been shown to be alloreactive due to expression of the inhibitory receptor KIR2DL2 or KIR2DL3 (CD158b) for which the C2 homozygous targets cannot supply the C1 self-ligand that is they recognize ‘missing self’ (6). However, it has been shown that some NK clones that lyse C2-expressing targets lack CD158b and react with the anti-CD158a antibody which recognizes KIR2DL1 or KIR2DS1. Moreover, activation of these NK clones by C2 homozygous targets was abolished by the addition of anti-CD158a, suggesting that these NK clones mediate alloreactivity by recognition of C2 ligands by the activating receptor KIR2DS1 (6). Despite this early data, there has been little additional evidence of activation of NK cells through KIR2DS1 recognition of the C2 ligand. Stewart et al. (7) used KIR2DL1 and KIR2DS1 tetramers to determine whether these receptors bound to C2. KIR2DL1 tetramers bound to C2-positive uninfected B cells and EBV-infected...
B cells. In contrast, KIR2DS1 tetramers were found to bind only to C2-positive B cells after EBV infection. It was postulated that this difference in KIR2DS1 binding was due to increased HLA-C density on the surface of EBV-infected cells.

In 2007, Chewning et al. (8) provided evidence to support the earlier work of Moretta et al. that KIR2DS1 binding could be responsible for NK cell lysis of C2-positive BLCL target cells showing that such lysis could be blocked by anti-CD158a antibody.

Here, we provide further evidence supporting the findings of Chewning et al. that KIR2DS1-positive NK clones are activated by recognition of C2 on BLCL targets. In addition, we show that the activation signal generated by engagement of KIR2DS1 overrides the inhibitory signal generated by engagement of CD94/NKG2A in these clones. Furthermore, we report that C2 homozygous targets undergo greater cell lysis than C1/C2 heterozygous targets consistent with the idea that activation through KIR2DS1 is dependent on class I density at the cell surface. We also demonstrate that KIR2DS1-activated cells can lyse PHA blasts in addition to EBV-transformed cells from the same individual, indicating that non-transformed cells express enough HLA-C to activate NK cells through KIR2DS1 highlighting a potential role for these NK clones in mediating NK alloreactivity.

**Methods**

**EBV cell lines**

EBV-transformed BLCLs were either 10th International HLA-compatibility Workshop cells or generated by the Department of Clinical Immunology, Royal Perth Hospital (Table 1). The 721.221 class I-negative cell line was a gift from J. McCluskey (University of Melbourne, Australia). The RPMI-8866 cell line was obtained from the American Type Cell Collection (Manassas, VA, USA). All cells were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) with 10% heat-inactivated FCS (ThermoTrace, Melbourne, Australia) at 37°C in 5% CO2.

**NK cells**

Blood was obtained from laboratory volunteers and blood donors from the Australian Red Cross Blood Service with informed consent. HLA-A, -B and -C typing of all donors was performed by DNA sequencing (Table 2). NK cells were purified from blood by ficoll density gradient centrifugation with RosetteSep (StemCell Technologies, Vancouver, Canada). Purified NK cells were plated out at limiting dilutions into 96-well round-bottom plates in NK medium [RPMI-1640, 10% FCS, 0.1 mM MEM, non-essential amino acids and 1 mM sodium pyruvate (Invitrogen)]. Irradiated, allogeneic, ficoll separated PBMC feeder cells (pooled from 10 donors) were added to each well at a concentration of 1 x 10^6 cells per 100 µl and then cultured at 37°C in 5% CO2. On day 1, 100 µl of medium was removed and replaced with fresh NK medium containing 400 IU ml⁻¹ IL-2 (Chiron, Emeryville, CA, USA). On day 4, 100 µl of medium was removed and replaced with irradiated PBMC feeder cells added to each well at a concentration of 8 x 10^6 cells per 100 µl. On day 11, 100 µl of medium was removed and replaced with 100 µl fresh NK medium containing 200 IU ml⁻¹ IL-2. On day 14 onwards cell growth was monitored by light microscopy and cells were split if necessary and fed with 1 x 10^6 per ml irradiated RPMI-8866 cells every 3-4 days. NK clones were screened for NK receptor expression and alloreactivity by flow cytometry and ⁵¹Cr release, respectively.

Ficoll separated purified polyclonal NK cells were expanded by culturing with irradiated allogeneic feeder cells at a 1:10 concentration for 12 days with 200 IU ml⁻¹ IL-2 replacing with fresh NK medium every 2–3 days. Before use in the CD107a assay, these NK cells were cultured with irradiated RPMI-8866 at a 1:10 concentration for 7 days with 200 IU ml⁻¹ IL-2 replacing the medium containing every 2–3 days.

**Generation of PHA blasts**

PHA blasts were generated from ficoll-purified PBMC; 10-µg ml⁻¹ PHA (Sigma–Aldrich, St Louis, MO, USA) was added to 1 x 10^6 cells ml⁻¹ and cultured for 4 days. Cells were then diluted to 2 x 10^5 cells ml⁻¹ in RPMI-1640 containing 10% FCS and 20 IU ml⁻¹ IL-2 and grown for an additional 3 days at which point they were stored at −80°C in 1 ml of 90% FCS and 10% dimethyl sulfoxide (BDH Chemicals, Poole, UK). Before use as cytotoxicity targets, PHA blasts were cultured for 48 h with 20 IU ml⁻¹ IL-2.

**Table 1.** Class I HLA typing and NK epitopes of BLCL target cell panel

<table>
<thead>
<tr>
<th>Cell identifier</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>NK epitopes</th>
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Flow cytometry

KIR receptor expression was identified on each NK clone using PE-conjugated antibodies to NKG2A/B, CD158a, CD158b (Beckman Coulter, Fullerton, CA, USA) and NKB1 (BD Biosciences, Franklin Lakes, NJ, USA). KIR3DL2 expression was determined using Q66 mAb (gift from D. Pende, University of Genoa, Italy) and anti-human PE-conjugated IgG (Beckman Coulter). Flow cytometry was performed on a Coulter EPICS XL-MCL™ flow cytometer and the BD Biosciences FACSCanto™ flow cytometer. Class I HLA expression on BLCL and PHA blasts was determined using the pan class I mAb W6/32 (a gift from J. McCluskey, University of Melbourne, Australia). FITC-conjugated anti-human PE-conjugated antibodies to NKG2A/B, CD158a, KIR3DL2 and NKB1 were added and the cells were incubated for a further 5 h. NK cells were then stained with anti-CD56–peridinin chlorophyll protein (eBiosciences, San Diego, CA, USA) at concentrations of 10 µg ml−1 for 20 min at 37°C. Assays were performed in triplicate and standard mean error was calculated for each target.

CD107a cytotoxicity assay

NK cell expression of CD107a was used to measure NK cytotoxicity by cultured polyclonal NK cells. Culture polyclonal NK cells were pre-incubated with anti-CD158a (BD Biosciences, Franklin Lakes, NJ, USA). KIR3DL2 expression was identified on each NK clone using PE-conjugated antibodies to NKG2A/B, CD158a, NKB1 and P10 also expressed CD158k (KIR3DL2).

Clones C9 and E11 also expressed NKG2A and clones C9 and P10) was CD158b negative, suggesting that another receptor must explain its alloreactivity. All three CD158b-negative targets. Of these, three were CD158b positive but one (clone C9) did not, we considered the possibility that NK clones were expressing KIR2DL1, a KIR2DL1-specific PCR reaction was performed using primers as described (10). Full-length KIR2DS1 cDNA was amplified from NK clone C9 using the following primers: KIR2DS1F, 5’TGTAAAAACGGCGCCAGATATGTCCGTCTAG ATGCGTCGT3’ and KIR2DS1R, CAGGAAAACA GTATGACC GTGAAAACA CAGTGTACCAA.

Thermocycling conditions were 95°C for 5 min, 5 cycles of 97°C for 20 s, 65°C for 45 s, 72°C for 90 s, 25 cycles of 95°C for 20 s, 61°C for 45 s, 72°C for 90 s and a final extension of 72°C for 10 min. Both KIR2DS1 PCR products were sequenced using BigDye Terminator technology (Applied Biosystems, Foster City, CA, USA) and analysed using SeqScape (Applied Biosystems). KIR genotyping of each donor (Table 2) was performed using a multiplex PCR-Sequence Specific Priming developed by Sun et al. (11).

Results

Alloreactive NK clones expressing CD158a lyse C2-expressing targets

As expected, we found that individuals who express the C1 epitope have alloreactive NK clones expressing KIR2DL2 or KIR2DL3 (CD158b) which are inhibited by BLCL targets expressing C1 and lyse targets which lack C1. Of the 122 NK clones tested from a C1 homozygous donor (Donor 1), 28 NK clones lysed C1-negative (C2 homozygous) targets. 26 of these 28 clones expressed CD158b. However, two clones (clone C9 and E11) were CD158b negative (Fig. 1), suggesting that another receptor must explain their alloreactivity. Similarly, 4 of 100 NK clones from another C1 homozygous donor (Donor 2) lysed C1-negative (C2 homozygous) targets. Of these, three were CD158b positive but one (clone P10) was CD158b negative, suggesting that another receptor must explain its alloreactivity. All three CD158b-negative NK clones expressed CD158a (KIR2DL1 or KIR2DS1). Clones C9 and E11 also expressed NKG2A and clones C9 and P10 also expressed CD158k (KIR3DL2).

The allospecificity of clone C9 cannot be explained by KIR3DL2

It has been reported that KIR3DL2 recognizes HLA-A*0301 and HLA-A*1101 but not HLA-A*0201 (12,13). As the C1 homozygous target (C2 negative) used for screening NK clones also expressed HLA-A3 and the C2 homozygous target (C1 negative) did not, we considered the possibility that the lack of cytotoxicity against the C1 homozygous target

<table>
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<th>Donor</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>NK epitopes</th>
<th>KIR2DL1</th>
<th>KIR2DL2</th>
<th>KIR2DL3</th>
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<td>C*07</td>
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was due to HLA-A3-mediated inhibition through KIR3DL2. NK clone C9 was therefore tested against six HLA-A3 homozygous and six HLA-A2 homozygous (i.e. HLA-A3 negative) BLCL targets (Fig. 2). All these targets lacked the C2 epitope. While clone C9 lysed the HLA class I-negative cell line, 721.221, it was inhibited by all A*0301 and A*0201 homozygous cell lines, indicating that KIR3DL2-mediated inhibition was unlikely to explain this clone’s allospecificity.

The allospecificity of clone C9 is determined by the presence of the C2 epitope in a dose-dependent manner

To further characterize the allospecificity of clone C9, we tested a more comprehensive panel of BLCL including C1 homozygous, C2 homozygous and C1/C2 heterozygous targets (Table 1). Clone C9 lysed all five C2-positive targets and none of the C2-negative targets (Fig. 3). Interestingly, cytotoxicity was stronger against C2 homozygous targets than the C1/C2 heterozygous targets.

Clone C9 expresses KIR2DS1*

We postulated that the alloreactivity of clone C9 was conferred by the activating receptor KIR2DS1 (CD158a) interacting with the C2 epitope. In order to confirm that clone C9 transcribes a functional KIR2DS1 gene, cDNA was prepared from clone C9 and KIR2DS1 was amplified using KIR2DS1 sequence-specific primers (10) (Fig. 4) and primers that would amplify the full-length KIR2DS1 cDNA (data not shown). The sequence-specific primers detected KIR2DS1 transcript. The full-length KIR2DS1 PCR product was also amplified and sequenced. The sequence was identical to KIR2DS1*002. Since CD158a stains both KIR2DS1 and its inhibitory counterpart KIR2DL1, we also tested the mRNA using KIR2DL1-specific primers. Clone C9 did not transcribe KIR2DL1 although a KIR2DL1 transcript could be amplified from a positive control (data not shown).

Activation by C2-positive targets is mediated by KIR2DS1 and overrides NKG2A-mediated inhibition

In order to prove that lysis of C2-positive targets by the CD158b-negative and CD158a-positive clone C9 was mediated by KIR2DS1, we determined the effect on cytotoxicity of blocking KIR2DS1 using anti-CD158a mAb. The addition of anti-CD158a completely abolished lysis of C2-positive targets and had no effect on the lysis of C2-negative targets confirming the role of KIR2DS1 (Fig. 5). Clone C9 also expressed the NK inhibitory receptor NKG2A. As all the BLCL target cells used in these experiments express HLA class I alleles that provide the necessary leader peptides for expression of HLA-E, NKG2A on clone C9 might be expected to bind to its ligand HLA-E and inhibit lysis of the
target cell. To determine whether NKG2A was functional in clone C9, we used mAb z199 to block any NKG2A-mediated inhibition. As shown in Fig. 5, the addition of anti-NKG2A had no effect on lysis of the class I-deficient control (721.221 cell line) but moderately enhanced lysis of 6 out of 7 target cells, indicating that NKG2A was functional. This data indicate that in the absence of blocking mAbs, KIR2DS1-mediated activation overrides NKG2A-mediated inhibition. Interestingly, blocking of NKG2A did not result in enhanced lysis of one of the C1 homozygous target cells (IHW 9034). Blocking with the pan-HLA class I mAb, W6/32 resulted in lysis of this target (data not shown), suggesting that the ligand inhibiting NK cell lysis of this target cell is an HLA class I molecule but not HLA-E.
KIR2DS1-activated NK clones represent 1% of all NK cells in C1 homozygous individuals

To determine the frequency of NK clones that are activated by KIR2DS1 and whether this varies among individuals of different HLA-C and KIR genotype, we determined CD107a expression on CD158a-positive NK cells when incubated with C2-positive BLCL targets. Polyclonal NK cells from a C1 homozygous, KIR2DS1-positive donor (Donor 3), a C1 homozygous, KIR2DS1-negative donor (Donor 4) and a C1/C2 heterozygous, KIR2DS1-positive donor (Donor 5) were incubated with C1 homozygous and C2 homozygous BLCL targets and the percentage of CD107a-positive cells determined. C2-positive targets were predicted to induce CD107a expression only on CD158a-positive NK cells from subjects who have the KIR2DS1 gene and in whom C2 is not a self-epitope (i.e. C1 homozygous donors). As shown in Fig. 6A, C2-positive (either homozygous or heterozygous) targets induced CD107a expression on CD158a-positive NK cells only from the C1 homozygous, KIR2DS1-positive donor. Representative cytofluorograms are shown in Fig. 6 (B1–6). Figure 6 (B1–3) shows that a C1 homozygous target cell induced very little CD107a expression on CD158a-positive cells from a C1 homozygous, KIR2DS1-positive donor (1.9% of CD158a-positive cells Fig. 6, B2), whereas CD107a expression was induced on 33.8% of CD158a-positive cells by a C2 homozygous target (Fig. 6, B3). These represent some 0.8% of all NK cells. Neither C1 homozygous nor C2 homozygous targets induced CD107a expression on CD158a-positive cells from a C1 homozygous KIR2DS1-negative donor (Fig. 6, B4–6). We performed similar

Fig. 6. NK cell activation through KIR2DS1 (CD158a) only occurs in C2 negative, KIR2DS1-positive individuals. (A) NK cells from three donors, Donor 3 (C1+C2– KIR2DS1+: solid bars), Donor 4 (C1+C2– KIR2DS1–: grey bars) and Donor 5 (C1+C2+ KIR2DS1+: open bars) were incubated with 721.221 cell line (positive control), two C2 homozygous cell lines (IHW9019 and IHW9084), two C1/C2 heterozygous cell lines (Q945288T and R97349692S) and two C1 homozygous cell lines (IHW9034 and IHW9085) and CD107a expression was quantified on the CD158a-positive population. All three NK donors lyse 721.221 cells, but only the C1 homozygous, KIR2DS1-positive donor lyases C2 homozygous BLCLs and to a lesser extent C1/C2 heterozygous BLCLs. NK cells from all donors are inhibited by the C1 homozygous (C2 negative) BLCL targets. (B) Representative flow cytometric plots for the C1 homozygous, KIR2DS1-positive (Fig. 6, B1–3) donor and the C1 homozygous, KIR2DS1-negative (Fig. 6, B4–6) donor with the percentage of cells in each quadrant shown. After incubation with target cells, CD158b-negative cells were identified (B1 and B4). CD107a and CD158a staining of the CD158b-negative NK cells (selected from B1 or B4) are shown after incubation with a C1 homozygous target (B2 and B5) or C2 homozygous target (B3 and B6). CD158a-positive cells from the C1 homozygous, KIR2DS1-positive donor show CD107a staining (8.2% of the CD158a-positive cells) only after incubation with the C2 homozygous target (B3) but not the C1 homozygous target (0.7% of the CD158a-positive cells). The CD158a-positive cells from the C1 homozygous, KIR2DS1-negative donor do not show CD107a staining when incubated with C1 or C2 homozygous targets (0.1 and 0.2% of CD158a cells, respectively).
experiments using C2 homozygous, KIR2DS2-positive individuals and HLA-Bw6 homozygous, KIR3DS1-positive individuals to determine if NK cells could be activated by the C1 or Bw4 epitopes through KIR2DS1 or KIR3DS1, respectively. We were unable to detect NK cell activation in such experiments (data not shown).

**NK cells activated by C2 kill EBV non-infected targets**

As it has been shown that KIR2DS1 binds to EBV-infected cells, but not to uninfected cells (7), we tested the ability of KIR2DS1-activated NK cells to kill PHA blasts and EBV BLCL from the same individual. Polyclonally expanded NK cells from a C1 homozygous, KIR2DS1-positive individual (Donor 3) were incubated with C1 homozygous, C2 homozygous and C1/C2 heterozygous PHA blasts and BLCL targets, and CD107a expression measured on CD158a-positive cells. CD107a expression was induced on CD158a-positive NK cells from Donor 3 by both C2-positive BLCL and PHA blasts (Fig. 7), although in all cases, C2-positive BLCL induced CD107a in a higher percentage of NK cells than PHA blasts from the same individual. As expected, CD107a expression was not induced on CD158a-positive NK cells from a KIR2DS1-positive C1/C2 heterozygous donor (Donor 5) by either C2-positive BLCL or PHA target cells (Fig. 7). To determine whether the difference in the ability of BLCL and PHA blasts to induce CD107a expression on NK cells might reflect differences in the level of class I expression, BLCL and PHA blasts were stained using the class I HLA-specific mAb W6/32. The mean fluorescence intensity of class I HLA expression was 20–50% higher on BLCL than PHA blasts (data not shown).

**Discussion**

We have demonstrated in this study that the specificity of alloreactive NK cells can be determined by the activating receptor KIR2DS1. These results confirm the results of other studies providing evidence that NK cells can be activated by KIR2DS1 recognition of the C2 epitope (6–8). The current data also confirms the finding of Chewning et al. that only C1 homozygous (i.e. C2 negative) KIR2DS1-positive individuals are capable of generating such alloreactive NK clones dependent on KIR2DS1. This is intuitively logical as KIR2DS1-activated clones would be autoreactive in individuals whose HLA-C alleles include the C2 epitope. Thus, C1 homozygous individuals produce alloreactive NK clones depending on KIR2DS1. This is intuitively logical as KIR2DS1-activated clones would be autoreactive in individuals whose HLA-C alleles include the C2 epitope. Thus, in addition to detecting missing self, NK cells are capable of detecting ‘non-self’.

It is generally believed that under normal conditions, inhibitory signals are dominant over activating signals in controlling NK cell activation. NK cells are only ‘licenced’ for cytotoxicity if they express an inhibitory receptor for ‘self’. The majority of KIR2DS1-expressing NK clones characterized in this study, and in the studies by Moretta et al. (6) and Chewning et al. (8), co-expressed NKG2A suggesting...
that KIR2DS1-dependent alloreactive NK cells are licensed by NKG2A interaction with self. Our data suggest that the interaction of activating receptors for non-self-ligands, such as NK cells from a KIR2DS1 positive, C2-negative individual interacting with C2, can override NKG2A-mediated inhibition. It has previously been reported that under cellular stress the activating receptor NKG2D can override inhibitory signals to allow NK cells to eliminate tumour or infected cells (14) but our data are the first evidence of an activating KIR behaving in a similar manner. Why the inhibitory signal generated through NKG2A does not suppress the activation signal generated through KIR2DS1 is unclear. It has been speculated that the ability of inhibitory signals to dominate over activating signals is due to the low affinity with which activating receptors bind to their ligand compared with their inhibitory counterparts (15,16). The kinetics of NKG2A binding to HLA-E are similar to those for inhibitory KIR binding to HLA-C (15) so it seems unlikely that our current data showing the inability of NKG2A to override KIR2DS1 can be explained by low affinity binding of NKG2A to HLA-E.

This study only examined one allele of KIR2DS1 in detail, KIR2DS1*002, which is expressed by clone C9 and we do not know the KIR2DS1 alleles expressed by the other donors. Four alleles of KIR2DS1 have been reported (17). KIR2DS1*001 and KIR2DS1*004 differ from KIR2DS1*002 at positions 70 and 90 of the first immunoglobulin domain, respectively. KIR2DS1*003 differs from KIR2DS1*002 only in the leader sequence. Differences in the immunoglobulin domains may result in different binding affinities. Therefore, we cannot be sure that KIR2DS1*001 and KIR2DS1*004 would behave like KIR2DS1*002, whereas it seems likely that KIR2DS1*003 would have similar specificity to KIR2DS1*002 in its interaction with its C2 ligand.

Stewart et al. (7) demonstrated that KIR2DS1 tetramers only bound to C2 on EBV-infected cells compared with normal B cells and B cells infected with members of the human herpesvirus family and postulated that this was due to an increase in class I HLA expression induced by EBV infection. In the current study, lysis of C1/C2 heterozygous BLCL was weaker than for C2 homozygous BLCL, suggesting that increased target cell density of HLA-C enhances KIR2DS1-mediated activation. We also found that BLCL were more susceptible to lysis than PHA blasts from the same person and that this correlated with lower class I HLA expression. Nevertheless, KIR2DS1-activated NK cells were able to lyse PHA-activated T cells (non-EBV infected) from both C2 homozygous and heterozygous individuals despite lower class I expression than on BLCL from the same individuals.

It is unclear as to whether other activating KIR (KIR2DS2, KIR2DS3, KIR2DS4, KIR3DS1 and KIR3DS1) can mediate NK alloreactivity. While we and others (6-8, 18, 19) have been unable to demonstrate that NK cell activation mediated through KIR2DS2 interacting with C1, or through KIR3DS1 interacting with Bw4 (20), it is possible that this may be due to the fact that these epitopes are simply not the cognate ligands for these receptors. Therefore, further work is required to resolve this issue.

It is not surprising that KIR2DS1-dependent NK clones which only arise in C1 homozygous individuals have been largely overlooked. When considering potential donors for haploidentical stem cell transplant (HSCT), C1 homozygous donors are rarely considered because all haploidentical recipients would also express C1, thereby preventing any inhibitory receptor-mediated NK alloreactivity by such donors. Leukaemia patients with all three ligands (C1, C2 and Bw4) for inhibitory KIR are thought to be unable to benefit from NK-mismatched HSCT, as all inhibitory KIR on haploidential donor NK cells would be inhibited by HLA ligands on the recipient’s cells. The data described in this report suggest that it may be possible for patients with all three epitopes to benefit from the use of alloreactive NK cells. NK cells from a C1 homozygous sibling donor sharing one haplotype with a C1/C2 heterozygous recipient may provide an anti-leukemic effect [and reduced graft versus host disease (GvHD)] through KIR2DS1-activated NK clones. Such NK cells may also be evident in HSCT using an unrelated donor where the donor has KIR2DS1 and the recipient has C2. Indeed, there is evidence to suggest that C1/C2 heterozygous bone marrow transplant recipients who receive a transplant from a KIR2DS1-positive donor have a lower risk of developing acute GvHD compared with the same transplant in a C1 homozygous recipient (21). These findings have potentially important implications in the selection of HSCT donors.

Acknowledgements

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BLCL</td>
<td>B lymphoblastoid cell line</td>
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<tr>
<td>C1</td>
<td>HLA-C group 1</td>
</tr>
<tr>
<td>C2</td>
<td>HLA-C group 2</td>
</tr>
<tr>
<td>GvHD</td>
<td>graft versus host disease</td>
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<tr>
<td>HSCT</td>
<td>haploidential stem cell transplant</td>
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<tr>
<td>KIR</td>
<td>killer immunoglobulin-like receptor</td>
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References

12 Pende, D. 1996. The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors which is characterised by three Ig-like domains and is expressed as a 140 kD disulphide-linked dimer. J. Exp. Med. 184:505.