NK Cell Tolerance of Self-Specific Activating Receptor KIR2DS1 in Individuals with Cognate HLA-C2 Ligand

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NK Cell Tolerance of Self-Specific Activating Receptor KIR2DS1 in Individuals with Cognate HLA-C2 Ligand

Gianfranco Pittari,* Xiao-Rong Liu,* Annamalai Selvakumar,* Zeguo Zhao,* Ernesto Merino,* Morgan Huse,* Joseph H. Chewning,†,‡ Katharine C. Hsu,†,‡ and Bo Dupont*†,§

NK cells are regulated by inhibiting and activating cell surface receptors. Most inhibitory receptors recognize MHC class I Ags and protect healthy cells from NK cell–mediated autoaggression. However, certain activating receptors, including the human activating killer cell Ig-like receptor (KIR) 2DS1, also recognize MHC class I. This fact raises the question of how NK cells expressing such activating receptors are tolerated to host tissues. We investigated whether the presence of HLA-C2, the cognate ligand for 2DS1, induces tolerance in 2DS1-expressing NK cells. Anti–HLA-C2 activity could be detected in vitro in some 2DS1 positive NK clones irrespective of the presence or absence of HLA-C2 ligand in the donor. The frequency of anti–HLA-C2 reactivity was high in donors homozygous for HLA-C1. Surprisingly, no significant difference was seen in the frequency of anti–HLA-C2 cytotoxicity in donors heterozygous for HLA-C2 and donors without HLA-C2 ligand. However, donors homozygous for HLA-C2, compared with all other donors, had significantly reduced frequency of anti–HLA-C2 reactive clones. The 2DS1 positive clones that express inhibitory KIR for self–HLA class I were commonly noncytotoxic, and anti–HLA-C2 cytotoxicity was nearly exclusively restricted to 2DS1 single positive clones lacking inhibitory KIR. 2DS1 single positive NK clones with anti–HLA-C2 reactivity were also present posttransplantation in HLA-C2 positive recipients of hematopoietic stem cell transplants from 2DS1 positive donors. These results demonstrate that many NK cells with anti–HLA-C2 reactivity are present in HLA-C1 homozygous and heterozygous donors with 2DS1. In contrast, 2DS1 positive clones from HLA-C2 homozygous donors are frequently tolerant to HLA-C2. The Journal of Immunology, 2013, 190: 4650–4660.

Protection of normal self tissues from immune aggression is tightly controlled. Autoaggressive T and B lymphocytes are mostly controlled through clonal deletion or anergy (1, 2). In contrast to T and B cells, NK cells develop tolerance to normal self-tissues largely by the “missing self–MHC class I” mechanism (3, 4). In this situation, inhibitory receptors with ligand specificity for self–MHC class I generate inhibitory signals upon interaction with cognate MHC ligand (5, 6). The NK repertoire, however, also contains activating receptors with ligand specificity for self-antigens. In mice, generalized expression of activating ligands results in reduced effector function and/or deletion of NK cells expressing cognate activating receptors, suggesting that NK cells receiving continuous activating receptor stimulation are either hyporesponsive or deleted (7–11). NK tolerance has also been reported in mixed allogeneic bone marrow chimeras (12). The human activating killer cell Ig-like receptor (KIR) 2DS1 (or, simply, 2DS1) recognizes HLA-C2 (C2, HLA–killer cell Ig-like receptor ligand group C2) (i.e., Asn77-Lys80 in the HLA-C H chain). 2DS1 is common among Caucasian populations, in which it ranges from 23% to 55%. The frequency of the natural ligand HLA-C2 is also high in the same populations, 54–66% (13). Because KIR and HLA segregate independently, 2DS1 is present in both HLA-C2 positive [HLA-C genotypes C2:C2 and C1:C2 (C1, HLA–killer cell Ig-like receptor ligand group C1)] and HLA-C2 negative individuals (HLA-C genotype C1:C1). 2DS1 positive (2DS1pos) NK cells have been detected in individuals with HLA-C2 (14–16). The frequency of peripheral blood NK cells expressing 2DS1 may exceed 20% (14). Recently, 2DS1 expression has been assessed on NK cells in peripheral blood from individuals with different HLA-C genotypes. 2DS1pos NK cells lacking inhibitory KIR receptors [2DS1 single positive (2DS1sp)] were identified in HLA-C2 homozygous donors. 2DS1sp NK cells from such individuals were not reduced in number but were found to be hyporesponsive when compared with 2DS1pos NK cells from HLA-C1 homozygous donors (16).

In this study, 2DS1pos NK clones were developed from donors with all three HLA-C genotypes—C1:C1, C1:C2, and C2:C2—for the purpose of determining the effect of the natural ligand, HLA-C2, on their frequency, phenotype, and tolerance to the self-ligand.
We report that 2DS1pos NK clones with anti–HLA-C2 reactivity can be obtained from individuals with any HLA-C genotype. The frequency of 2DS1pos clones with anti–HLA-C2 reactivity is equally high for donors with the HLA-C genotypes C1:C1 and C1:C2. In contrast, 2DS1pos clones from donors homozygous for HLA-C2 have significantly decreased frequency of anti–HLA-C2 reactivity, consistent with tolerance of 2DS1 to HLA-C2. We also find that the inhibiting receptor CD94/NKG2A is not a critical regulator of tolerance to HLA-C2 in HLA-C2 homozygous NK cells. Finally, we observe that 2DS1-mediated anti–HLA-C2 cytotoxicity in all donors almost exclusively is restricted to 2DS1pos clones.

Materials and Methods

NK cell donors

NK cells were obtained from seven individuals (five healthy donors and two transplant recipients). HLA class I genotyping was performed on genomic DNA by a combination of PCR amplification with sequence-specific primers and with sequence-specific oligonucleotide probes (17). KIR genotyping was performed by KIR sequence-specific primers (KIR Genotyping Kit; Invitrogen) and KIR haplotypes, and genotypes were assigned (18) (Table I). NK cells from healthy donors were negatively selected from freshly isolated PBMCs obtained from 30 ml peripheral blood, using a mixture of magnetically labeled mAbs specific for non-NK lineage Ags (Miltenyi Biotec) (19). For all experiments, postisolation NK cell purity was >90%. NK cells from transplant recipients were directly FACs sorted from bulk PBMCs (see NK cloning).

BaF/3 IL-15Ra/IL-15 transfectants

The pSFG retroviral vectors containing full-length cDNA of human IL-15Ra or IL-15 (kindly provided by Dr. Thomas A. Waldmann, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) were transfected into Phoenix E packaging cell line, to produce retroviral supernatants. BaF/3 cells were incubated with retroviral supernatants, for 6–8 h, in fibronectin-coated plates (Takara Biomedicals). Clones of IL-15Ra/IL-15 double-transfected pre–B-lymphocyte BaF/3 cells (BaF/3 IL-15Ra/IL-15) were obtained by limiting dilution, and stable expression of IL-15 and IL-15Ra was confirmed by monthly mAb staining. The cell line was maintained in RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mML-glutamine. The following feeders were added to the medium: 105 allogeneic EBV-B lymphoblastoid cell line (BLCL) (JY), 4 × 107 PBMCs obtained from three different donors, and 3 × 105 BaF/3 IL-15Ra/IL-15 cells. Feeders were γ irradiated (EBV-BLCL and PBMCs: 5.2 Gy; BaF/3 IL-15Ra/IL-15: 13.9 Gy). After sorting, plates were centrifuged at 500 rpm for 1 min and incubated in a 37°C, 5% CO2 humidified atmosphere. On day 5, 100 µl fresh medium and irradiated EBV-BLCL (105), PBMCs (4 × 105), and BaF/3 IL-15Ra/IL-15 (3 × 105) were added to each well. On day 10, 80 µl supernatant was removed and substituted with medium and irradiated feeders, as on day 5. On day 15, NK cell growth could be detected by a colorimetric change (purple to yellow) of the microculture supernatant. Proliferating NK clones were collected, transferred in 48-well plates, and supplemented with 800 µl medium and irradiated BaF/3 IL-15Ra/IL-15 (5 × 105). On day 22, 300 µl supernatant was removed from all wells and replaced with 500 µl medium and irradiated BaF/3 IL-15Ra/IL-15 (5 × 105). Between day 28 and day 32, NK cells were screened by flow cytometry to determine viability, clonality, and receptor expression. NK clones were harvested, functionally characterized, and cryopreserved for subsequent molecular studies.

Characterization of NK clones

KIR/NKG2A receptor expression. KIR and NKG2A expression was tested by flow cytometry. The mRNA copy numbers for individual KIR (see Quantitative PCR) were used for estimation of KIR surface expression when KIR receptors could not be individually recognized by monospecific mAbs. Normalized mRNA copy numbers for 2DL1, 2DS1, 2DL2–3, and 3DS1 were used to determine the lowest number associated with surface expression. Cell surface expression of 2DL1, 2DS1, 2DL2–3, and 3DS1 was compared to one of three groups: KIR expression present; KIR expression absent; and KIR surface expression not tested. Because the z27 mAb used to detect 3DS1 receptor also recognizes 3DL1 (20), the analysis for determining the relationship between 3DS1 cell surface expression and mRNA copy numbers was exclusively based on clones lacking 3DL1 expression.

FIGURE 1. Generation of NK clones from single NK cells with specific receptor repertoires. (A) Flow cytometric representation of NK subsets identified by HP-MA4 and 143211 mAbs. HP-MA4 recognizes NK cells expressing 2DS1, 2DL1, or both (subset 1+2, blue). Combined use of HP-MA4 and 143211 mAbs allows discrimination between 2DS1pos/2DL1neg (subset 1, red) and 2DS1neg/2DL1pos or 2DS1pos/2DL1pos (subset 2, green) NK cells. Resting NK cells obtained from a healthy donor are depicted. (B) P75 and P81: 2DS1pos/2DL1neg (HP-MA4pos/143211neg) NK clones. P74, P107, P99, and P73: 2DL1pos (HP-MA4pos/143211pos) NK clones. In 2DL1pos NK clones, 2DS1 expression is verified by real-time RT-qPCR.
pression (i.e., DX9<sup>+</sup>). The lowest KIR transcript number associated with detectable receptor surface expression was 40 copies for 2DL1, 13 copies for 2DS1, 23 copies for 2DL2-3, and 98 copies for 3DS1. These values were set as the minimal copy number of transcripts necessary for surface expression of each KIR. This procedure for identification of NK clones with KIR surface expression was applied to evaluate the effects of inhibitory KIR for self–HLA class I on anti–HLA-C2 reactivity by 2DS1<sup>+</sup> clones. In addition, it was used in the analysis of a possible effect of 3DS1 on anti–HLA-C2 cytototoxicity mediated by 2DS1.

Mean fluorescence intensity (MFI) values were used to determine expression levels of 2DS1 receptor.

**Cytotoxicity.** Cytotoxicity against EBV-BLCL was measured in standard 51Cr release assays performed in triplicate (or in duplicate for clones with limited cell number) for 4 h, at 37°C, at E:T ratio of 10:1 (3 × 10<sup>4</sup> target cells per well). Where indicated, effectors were tested in the presence of 10 μg/ml anti-human NKG2A, 4E (anti–HLA-B/C), or control anti-mouse F(ab<sub>2</sub>) fragment. EBV-BLCL target cells were obtained from the International Histocompatibility Working Group (IHWW; www.ihww.org/reference/index.html Consanguineous_Reference_Panel) or generated in our laboratory. EBV-BLCL possessed the following HLA class I genotypes: GA, A<sup>*02:01</sup>/A<sup>*03:01</sup>; B<sup>*40:01</sup>/B<sup>*44:02</sup>; C<sup>*07:01</sup>/C<sup>*07:02</sup>; C<sup>*15:02</sup>/C<sup>*15:03</sup>; C<sup>*15:01</sup>/C<sup>*15:02</sup>; A<sup>*02:01</sup>/A<sup>*03:01</sup>; F<sup>+</sup>/F<sup>+</sup>; and 1 mg/ml biotinylated HLA-E. For comparative studies in which one constituent was left out of the bilayer, the nonstimulatory biotinylated EB6 mAb (anti-2DL1/S1), 1 mg/ml biotinylated ICAM-I, or 0.1 mg/ml streptomycin up to 3 mo before being discarded. All EBV-BLCL were tested for expression of HLA-E, using PE-conjugated anti–HLA-E mAb (3D12; BioLegend).

51Cr release assays performed in triplicate (or in duplicate for clones with limited cell number) for 4 h, at 37°C, at E:T ratio of 10:1 (3 × 10<sup>4</sup> recipients). The values for nonspecific 51Cr release in 2DS1 pos clones were set to distinguish between nonspecific 51Cr release and specific cytotoxicity values. Frequencies of cytotoxic 2DS1 pos clones possessing different receptors or KIR and NKG2A was calculated as follows: (KIR or NKG2A copy number/GAPDH copy number) × 10<sup>3</sup>. Frequencies of clones were compared using the two-sided rank correlation coefficient. All statistical tests were performed using GraphPad Prism 5 (Mac Os X) (GraphPad). A p value ≤ 0.05 was considered significant.

**Quantitative PCR** Primers and probes. Sequences for 2DL1 primers, as well as 2DS1, 2DL2-3, 3DS1, and NKG2A primers and probes, were previously reported (22, 23). A 2DL1 probe and GAPDH primers were designed in our laboratory. Nanomolar oligonucleotide concentrations for real-time quantitative RT-PCR primers and probes (indicated below) were established using optimization matrix for both primers (range: 100–900 nM) and probes (range: 50–250 nM). Shorter probes with conjugated minor groove binder groups were prefered over standard DNA probes to increase reaction specificity. 2DS1: Forward (Fwd): 5′-TTCCTCATAGTGCCATGAR-3′ (5000), Reverse (Rev): 5′-AGGCGCCAGCAGAGAATG-3′ (500), 5′-6FAM-GGCTTCTATGAGAACACT-MGB-3′ (20), 2D1L Fwd: 5′-GGACGACCATGTGCGCTT-3′ (500), Rev: 5′-GGCTACTGGGAGCTGACAC-3′ (100), Probe: 5′-6FAM-CACATGAGGAGTTACCC-MGB-3′ (100); 2DS2: Fwd: 5′-TGGACAGAGGAGGAGATTG-3′ (300), Rev: 5′-CACGCTTCTCTGCACCA-3′ (300), Probe: 5′-6FAM-CTGACACCTTGAGAAGTGG-3′ (50). Using GAPDH as a reference gene, nanomolar oligonucleotide concentrations for real-time quantitative RT-PCR primers were ligated (pGEM-T Easy Vector; Promega) and transformed into Max Efficiency DH5α Competent Cells (Invitrogen). Recombinant plasmid DNA was extracted, the insert sequenced, and the concentration determined at 260 nm (Nanodrop 1000; Thermo Scientific).

**cDNA synthesis from NK clones.** cDNA for real-time RT-qPCR was extracted from cryopreserved NK clones using the MACS One-Step cDNA synthesis technology (Miltenyi Biotech). Briefly, poly(A) tails of mRNA in 4 α units were hybridized with oligo(dT) microbeads. Magnetically labeled mRNA retained in microcolumns was used as template for cDNA synthesis (1 h, 42°C). Prior to reverse transcription, RNase-free DNase I (Applied Biosystems) was added to mRNA (10 U, 2 min, room temperature), to completely remove traces of genomic DNA. RNase H from Escherichia coli (New England Biolabs) was added for in-column digestion of mRNA-bound cDNA (2 U, 30 min, 37°C). cDNA was stored at −20°C.

**PCR amplification of cDNA.** PCR amplifications used 2 μl NK clone cDNA in buffer solution in a 50-μl reaction mix containing FastStart Universal Probe Master (Roche Applied Science) and the primer/probe oligonucleotide described above. Quantification of housekeeping GAPDH was performed by TaqMan Gene Expression Assay for GAPDH (HS99999901_m1; Applied Biosystems). Reactions were performed in duplicate using an ABI 7300 PCR System (Applied Biosystems), under the following thermal cycling conditions: stage 1: 10 min, 95°C; stage 2: 25 cycles of [30 s, 94°C; 30 s, 60°C, 30 s, 72°C], and stage 3: 7 min, 72°C. PCR products were ligated (pGEM-T Easy Vector; Promega) and transformed into Max Efficiency DH5α Competent Cells (Invitrogen). Recombinant plasmid DNA was extracted, the insert sequenced, and the concentration determined at 260 nm (Nanodrop 1000; Thermo Scientific).

**Absolute quantification of NK clone transcripts.** Samples containing 10-fold serial dilutions (KIR and NKG2A: 3 × 10<sup>3</sup>–30; GAPDH: 3 × 10<sup>5</sup>–3 × 10<sup>6</sup>) of known gene copy numbers in recombinant plasmids were amplified in triplicate, along with each real-time RT-qPCR run. Five-point standard curves were generated to quantify each KIR and NKG2A transcript. Standard curves with a linearity (r<sup>2</sup>) of 0.985 and an efficiency ranging from 85 to 110% were considered acceptable. Threshold cycle values > 36 were considered nonspecific and discarded. Normalization of copy numbers for KIR and NKG2A was calculated as follows: [KIR or NKG2A copy number/GAPDH copy number] × 10<sup>3</sup>.

**Institutional review board approval** Informed written consent was obtained from all donors according to Memorial Sloan-Kettering Cancer Center Institutional Review Board Protocol (IRB# 95-054A for healthy donors and IRB# 09-141 for transplant recipients).
Results

IL-15 trans-presentation supports generation of 2DS1pos NK clones

2DS1pos clones have previously been obtained from donors lacking cognate HLA-C ligand (i.e., donors homozygous for the HLA-C1 ligand). In contrast, very few 2DS1pos clones were obtained from donors expressing HLA-C2 (19). Because IL-15 trans-presentation is the major growth and survival signal for NK cells (24–27), we investigated whether human NK cloning efficiency and clone survival could be enhanced by IL-15 trans-presentation in vitro (Fig. 1). Trans-presentation was achieved by coculture of FACS-sorted NK cells with murine Ba/I3 cells transfected with human IL-15Rs and human IL-15. This procedure supported clone development from all donors, irrespective of their HLA-C genotype. Each clone reached 0.25–4 × 10^6 cells, and the overall cloning efficiency was 35–40%. Clones were developed from seven donors, representing the three HLA-C genotypes: C1:C1, C1:C2, and C2:C2. HLA-KIR ligand groups and KIR genes for each NK donor are listed in Table I.

We analyzed 386 clones, which included the 2DS1SP phenotype and other 2DS1pos phenotypes (Table II). For example, 2DS1SP clones were obtained from donors with each HLA-C genotype (Table II, Columns A and B). Similarly, twenty-four 2DS1SP, which also expressed the inhibitory receptor CD94/NKG2A, were obtained (Table II, Column F). Accordingly, clones with a broad KIR repertoire, including 2DS1SP, can be obtained from donors with any HLA-C genotype, when IL-15 trans-presentation is the NK growth factor.

Frequency of 2DS1SP NK clones with anti–HLA-C2 cytotoxicity is decreased only in donors homozygous for HLA-C2

A total of 91 2DS1SP clones were isolated, of which 56 had anti–HLA-C2 cytotoxicity (Table III). Clones with anti–HLA-C2 cytotoxicity were obtained from any donor, regardless of the HLA-C genotype. Anti–HLA-C2 cytotoxicity was detected in 29 of 42 clones with the C1:C1 genotype (69%) and in 19 of 22 clones with the C1:C2 genotype (86%). In contrast, anti–HLA-C2 cytotoxicity was observed in only 8 of 27 clones with the C2:C2 genotype (30%) (C1:C1 versus C2:C2, p = 0.001, and C1:C2 versus C2:C2, p < 0.0001) (Fig. 2A, Table IIIA).

The frequency of anti–HLA-C2 cytotoxicity among 2DS1SP, C1:C2 clones was similar to that observed among C1:C1 clones (Fig. 2A, left and center). This finding demonstrates that clonal deletion or clonal anergy is not characteristic of 2DS1SP clones from donors heterozygous for HLA-C2. The 22 clones from C1:C2 heterozygous donors were tested on HLA-C2 heterozygous and homozygous target cells. The C1:C2 clones were significantly less frequently cytotoxic against target cells with the autologous C1:C2 genotype (p < 0.0001) (Fig. 2A, center, Fig. 2B; Table IIIB). Thus, 2DS1SP clones with anti–HLA-C2 reactivity derived from donors with the C1:C2 genotype are rarely cytotoxic to autologous targets. This decrease in frequency of anti–HLA-C2 cytotoxicity cannot be ascribed to the effect of inhibitory KIR expressed by the clones, because they all are 2DS1SP.

We finally determined the effect of inhibitory KIRs with ligand specificity for nonself–HLA-C class I on the function of 2DS1pos clones. Thirteen 2DS1pos, C2:C2 clones, which also expressed the inhibitory receptor 2DL3 with ligand specificity for HLA-C1, were obtained. Six of 13 (46%) clones had anti–HLA-C2 reactivity, which is not significantly different from the results obtained with 2DS1SP, C2:C2 clones (Fig. 2A, right, Fig. 2C; Table IIIIC). Therefore, 2DS1pos, HLA-C2 homozygous clones with nonself inhibitory KIR display anti–HLA-C2 reactivity comparable to that of 2DS1SP clones from the same donor.

2DS1SP NK clones with anti–HLA-C2 reactivity are present in recipients of 2DS1pos allogeneic hematopoietic stem cell transplantation

Allogeneic, myeloablative hematopoietic stem cell transplantation (HCT) provides a possibility for evaluating de novo development of donor-derived 2DS1pos NK cells in the presence of cognate HLA-C2 ligand. We investigated two cases in which the graft was obtained from 2DS1pos C1:C2 donors. 2DS1pos NK cells were identified post-HCT in both recipients (Fig. 2D). In the first case, ten 2DS1SP clones were obtained 100 d post-HCT from an HLA-C2 homozygous recipient. Four of the 2DS1SP clones displayed anti–HLA-C2 cytotoxicity (Fig. 2D, Case 1). In the second case, four 2DS1SP clones were obtained from an HLA-C1:C2 heterozygous recipient, 200 d post-HCT. Two clones displayed anti–HLA-C2 cytotoxicity (Fig. 2D, Case 2). Therefore, donor-derived 2DS1SP NK cells with ability to mediate anti–HLA-C2 cytotoxicity can be identified in an allogeneic host that expresses the cognate HLA-C2 ligand.

2DS1pos NK clones expressing at least one inhibitory KIR for self–HLA class I are tolerant

Among the inhibitory KIR with HLA class I ligand specificity, only 2DL1 and 3DL1 can be individually recognized by mAbs. The remaining inhibitory KIR 2DL2, 2DL3, and the activating receptor 2DS2 cannot be distinguished by monospecific Abs. Similarly, 2DS1 is not distinguishable from 2DL1, when both receptors are present on the same cell. KIR phenotyping was therefore supplemented with determination of mRNA copy numbers for each of the KIRs with ambiguous phenotypes. Absolute RT-qPCR quan-

Table I. Donor HLA class I and KIR

<table>
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<tr>
<th>Sample ID</th>
<th>HLA-KIR Ligand Group</th>
<th>KIR Genes</th>
<th>KIR Genotype</th>
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<td>Healthy volunteers</td>
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<tr>
<td>UDN0 001</td>
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<td>Bw4</td>
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<td>Bw4</td>
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*KIR haplotype numbers from Khakoo and Carrington (18).

*Unique donor number.

*Absence of KIR gene.
tification assays were performed for 2DL1 (166 clones), 2DS1 (285 clones), and 2DL2–3 (229 clones) KIR transcripts. The mRNA copy numbers for such KIRs were determined, and the minimal copy number associated with KIR surface expression was identified, as described in Materials and Methods and in Fig. 3. These minimal copy number values were used as reference to assign a KIR receptor phenotype to a total of 29 clones, whose surface minimal copy number values were used as reference to assign a phenotype. 2DL1 and 2DS1 expression in three clones over, we could identify 2DL1 and 2DS1 expression in three clones, and 2DL2–3 (229 clones) KIR transcripts. The mRNA

### Table II. NK cloning strategy

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<tr>
<th>Donor HLA-C Genotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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Total NK clones by sorting gate

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<tr>
<th>Phenotypic combination</th>
<th>2DS1&lt;sup&gt;b&lt;/sup&gt; or 2DL1&lt;sup&gt;b&lt;/sup&gt; or 2DS1/2DL1&lt;sup&gt;b&lt;/sup&gt; or 2DL2/2DL3/2DS1&lt;sup&gt;b&lt;/sup&gt;</th>
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<td></td>
<td>HP-MA4 pos, 143211 neg, CH-L neg, DX9 neg,</td>
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Table III. Cytotoxicity of 2DS1<sup>SP</sup> NK clones

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<th>Donor HLA-C Genotype</th>
<th>KIR Phenotype</th>
<th>Target HLA-C Genotype</th>
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<th>No, n (%)</th>
<th>% Lysis Median (Range)</th>
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<tr>
<td>C1:C1</td>
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<td>C2:C2</td>
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<td>34.3 (13.3–67.5)</td>
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<tr>
<td>C1:C2</td>
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<td>22</td>
<td>19 (86)</td>
<td>3 (14)</td>
<td>39.6 (13.3–62.6)</td>
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<tr>
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<td>8 (30)</td>
<td>19 (70)</td>
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<tr>
<td>C1:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;</td>
<td>C2:C2</td>
<td>22</td>
<td>19 (86)</td>
<td>3 (14)</td>
<td>39.6 (13.3–62.6)</td>
<td>1</td>
</tr>
<tr>
<td>C1:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;</td>
<td>C1:C2</td>
<td>22</td>
<td>3 (14)</td>
<td>19 (86)</td>
<td>24.3 (22.9–29.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;</td>
<td>C1:C2</td>
<td>22</td>
<td>8 (30)</td>
<td>19 (70)</td>
<td>24.9 (13.5–43.4)</td>
<td>1</td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;/L3&lt;sup&gt;pos&lt;/sup&gt;</td>
<td>C2:C2</td>
<td>13</td>
<td>6 (46)</td>
<td>7 (54)</td>
<td>17.7 (13.2–27.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Frequency of anti–HLA-C2 cytolytic clones in each group is compared.

<sup>b</sup>Magnitude of anti–HLA-C2 cytotoxic responses for each clone group is compared.

Effect of inhibitory receptor CD94/NKG2A on tolerance development of HLA-C2 homozygous 2DS1<sup>SP</sup> NK cells

The reduced frequency of anti–HLA-C2 reactivity observed in HLA-C2 homozygous, 2DS1<sup>SP</sup> clones is consistent with NK tolerance observed in mice transgenic for activating receptor ligands (7–11). In humans, the inhibitory receptor CD94/NKG2A could potentially counteract 2DS1 activation by the HLA-C2 ligand. We first determined if the 2DS1 receptor is signaling competent in 2DS1<sup>SP</sup>, HLA-C2 homozygous clones expressing CD94/NKG2A. EB6 mAb cross-linking of the 2DS1 receptor in the presence of ICAM-1 induces Ca<sup>2+</sup> flux (Materials and Methods). This activation signal is inhibited when HLA-E, the ligand for CD94/ NKG2A (32), is added (Fig. 4A). Similar results were obtained with three additional clones, demonstrating that the 2DS1 receptor is signaling competent. Next, we determined the correlation between NKG2A expression levels and anti–HLA-C2 reactivity of 2DS1<sup>SP</sup>/NKG2A<sup>pos</sup> clones. NKG2A mRNA transcript copy numbers correlated well with NKG2A receptor MFI in 2DS1<sup>SP</sup>/NKG2A<sup>pos</sup> clones from C1:C1 and C2:C2 donors (p = 0.002).
Cytotoxic 2DS1pos/2DL3pos target cells. Statistical analysis compares the frequency of anti–HLA-C2 clones (Fig. 4C). Therefore, expression of CD94/NKG2A inhibiting C2:C2 dimer 13.1% cutoff between clone cytotoxicity and noncytotoxicity. Using C1:C2 clones, shown here, and of 2DS1 SP clones were tested for cytotoxicity against C2:C2 EBV-BLCL (IHWG 9036) target cells. Case 1, HCT 1. Cytotoxicity of 2DL1 pos clones obtained from HCT recipients were tested against C2:C2 clones, shown in (A). C2:C2 2DS1SP clones were tested for cytotoxicity against C2:C2 target cells, shown in (A). Statistical analysis compares the frequency of anti–HLA-C2 cytotoxic C1:C2 clones detected using C2:C2 or C1:C2 target cells. C2:C2 2DS1SP/2DL3pos clones (n = 13) were tested for cytotoxicity against C2:C2 EBV-BLCL (IHW 9036) target cells. Statistical analysis compares the frequency of anti–HLA-C2 cytotoxic 2DS1pos/2DL3pos C2:C2 clones, shown here, and of 2DS1 SP C2:C2 clones, shown in (A), to the same C2:C2 target cells. (D) Donor-derived clones obtained from HCT recipients were tested against C2:C2 EBV-BLCL (IHW 9036) target cells. Case 1, HCT 1. Cytotoxicity of 2DL1pos clones (n = 6) and 2DS1 SP (n = 10) C1:C2 clones, obtained from a C2:C2 HCT recipient 100 d posttransplantation. Case 2, HCT 2. Cytotoxicity of 2DL1pos (n = 16) and 2DS1 SP (n = 4) C1:C2 clones, obtained from a C1:C2 HCT recipient 200 d posttransplantation. **p = 0.001, ***p < 0.0001.

(Fig. 4B). These data validated the inclusion of NKG2A mRNA copies for our correlation studies. We expected that high cytotoxicity would be observed in clones with low NKG2A mRNA copies. However, NKG2A mRNA copy numbers were not found to correlate with anti–HLA-C2 cytotoxicity in 2DS1SP, C2:C2 clones (Fig. 4C). Therefore, expression of CD94/NKG2A inhibitory receptor does not predict whether 2DS1SP, HLA-C2 homozygous clones mediate anti–HLA-C2 reactivity or whether they are tolerant to HLA-C2.

To directly test the inhibitory function of CD94/NKG2A on 2DS1SP/NKG2Apos, HLA-C2 homozygous clones, we determined the effect of anti-NKG2A F(ab’)_2 fragment on the cytotoxicity of 10 noncytolytic (Fig. 4D) and 14 cytolytic (Fig. 4E) clones. Anti–HLA-C2 cytotoxicity was determined using HLA-A*02:01 homozygous target (BLCL 9036), which expresses several HLA class I alleles with HLA-E binding leader peptides (33). HLA-E expression on this target was confirmed by mAb staining. Only one noncytolytic clone changed from noncytolytic to cytolytic when the CD94/NKG2A receptor was blocked with anti-NKG2A F(ab’)_2 (Fig. 4D). Furthermore, all 14 2DS1 SP clones with anti–HLA-C2 reactivity displayed enhanced cytotoxicity following blocking with anti-NKG2A F(ab’)_2 (Fig. 4E). Therefore, CD94/NKG2A provides only a modulatory, attenuating effect on 2DS1-mediated anti–HLA-C2 cytotoxicity, and is not a major factor controlling 2DS1 tolerance to HLA-C2 in HLA-C2 homozygous donors. These results also agree with a recent report of a single 2DS1pos/NKG2Apos clone in which 2DS1-mediated cytotoxicity was unaffected by the presence of CD94/NKG2A (34).
Eight 2DS1<sup>pos</sup> clones that also expressed the inhibitory receptor 3DL1 were obtained from a donor heterozygous for C1:C2 and homozygous for HLA-Bw4. The clones were tested against a panel of target cells homozygous for the 2DS1 activating ligand (i.e., C2:C2 homozygous) or lacking the activating ligand (i.e., C1:C1 homozygous). The presence of inhibitory ligand for 3DL1 (i.e., Bw4 homozygous) or absence of 3DL1 ligand (i.e., Bw6 homozygous) was similarly tested in different combinations of target cells (Fig. 5). Six of eight clones were inhibited by a target homozygous for HLA-C2:Bw4 (Fig. 5, left), consistent with the finding that clones with inhibitory KIR for self–HLA class I in most instances do not respond to HLA-C2 activation (Table IV). The same clones were tested on a target homozygous for HLA-C2 but lacking the HLA-Bw4 ligand for 3DL1 (i.e., HLA genotype C2:C2;Bw6:Bw6). All eight clones displayed anti–HLA-C2 cytotoxicity, demonstrating the combined activating effect of 2DS1 signaling and recognition of “missing self–HLA class I” (Fig. 5, center). Finally, the effect of lack of HLA-C2 ligand and absence of HLA-Bw4 on target cells (i.e., no stimulation of 2DS1 but recognition of “missing self–HLA class I”) is shown. Three clones were cytolytic, whereas five were inhibited (Fig. 5, right). Therefore, “missing self–HLA class I” recognition in the absence of 2DS1 activation provides a variable activation signal.

### Tolerance of 2DS1<sup>sp</sup> clones to cognate ligand in HLA-C2 homozygous donors is not dependent upon ligand-mediated downregulation of the 2DS1 receptor

Studies in transgenic mice have demonstrated ligand-mediated downregulation of the activating NK receptor (10, 11). A similar mechanism could be involved in 2DS1 tolerance to HLA-C2. MFI of 2DS1 expression was determined on the 8 C2:C2 2DS1<sup>sp</sup> clones displaying anti–HLA-C2 cytotoxicity and on the 19 C2:C2 2DS1<sup>sp</sup> clones lacking anti–HLA-C2 cytotoxicity to the HLA-C2 homozygous target. 2DS1 expression levels were similar in these HLA-C2 homozygous 2DS1<sup>sp</sup> clones, irrespective of their anti–HLA-C2 responsiveness (Fig. 6).

### Discussion

We demonstrate that 2DS1<sup>pos</sup> clones are readily obtained from normal donors, irrespective of their HLA-C genotype. The presence of both the activating 2DS1 receptor and its cognate ligand does not result in extensive deletion of such NK cells. Furthermore, 2DS1<sup>pos</sup> clones from HLA-C2 homozygous donors display anti–HLA-C2 reactivity in vitro similar to that in HLA-C1 homozygous donors, who do not carry the cognate ligand. Therefore, 3DS1 does not contribute to anti–HLA-C2 reactivity of 2DS1<sup>sp</sup> clones.

Because the genes encoding the two activating receptors 2DS1 and 3DS1 are in strong positive genetic linkage disequilibrium (35, 36), they frequently occur together. It is therefore potentially difficult to distinguish between a 3DS1- and a 2DS1-mediated effect. The minimal 3DS1 mRNA copy number needed for expression of 3DS1 receptor was 98 (Fig. 3; see Materials and Methods). This value was used as reference for determination of 3DS1 receptor expression. Ninety 2DS1<sup>sp</sup> clones from donors with different HLA-C genotypes were tested for 3DS1 expression. Sixty-five clones (72%) had the 2DS1<sup>sp</sup>/3DS1<sup>pos</sup> phenotype with 3DS1 mRNA copy numbers consistent with 3DS1 cell surface expression (median: 544; range: 102–2340). The remaining 25 clones had the 2DS1<sup>sp</sup>/3DS1<sup>neg</sup> phenotype. Their 3DS1 mRNA copies were either undetectable or present in numbers below the calculated minimal value needed for expression (median: 0; range: 0–79). The frequency of anti–HLA-C2 cytotoxicity was compared between 2DS1<sup>sp</sup>/3DS1<sup>pos</sup> and 2DS1<sup>sp</sup>/3DS1<sup>neg</sup> clones. The analysis was done separately for different HLA-C groups of donors because the HLA-C genotype affects the frequency of anti–HLA-C2 reactive 2DS1<sup>sp</sup> clones (Fig. 2). It is demonstrated in Table VI that the presence or absence of 3DS1 does not affect anti–HLA-C2 reactivity in 2DS1<sup>sp</sup> clones. This finding was observed in clones from HLA-C1 positive (i.e., C1:C1 and C1:C2) and clones from HLA-C2 homozygous donors. Therefore, 3DS1 does not contribute to anti–HLA-C2 cytotoxicity by 2DS1<sup>sp</sup> clones.

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### Table IV. 2DS1<sup>pos</sup> NK clones with one or more inhibitory KIR for self–HLA class I

<table>
<thead>
<tr>
<th>Donor HLA-C Genotype</th>
<th>Effector Cell KIR Phenotype</th>
<th>Target Cell HLA-KIR Genotypes</th>
<th>Cytolytic</th>
<th>n</th>
<th>Yes, n (%)</th>
<th>No, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1:C2</td>
<td>2DS1/2DL1</td>
<td>C1:C2;Bw4</td>
<td>32</td>
<td>31 (97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1:C2</td>
<td>2DS1/2DL1/2DL3</td>
<td>C1:C2;Bw4</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1:C2</td>
<td>2DS1/2DL1/2DL3/3DL1</td>
<td>C1:C2;Bw4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1</td>
<td>C1:C2;Bw4</td>
<td>14</td>
<td>14 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1/2DL3</td>
<td>C1:C2;Bw4</td>
<td>11</td>
<td>11 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1/2DL3/3DL1</td>
<td>C1:C2;Bw4</td>
<td>14</td>
<td>14 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1</td>
<td>C2:C2;Bw4</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1/2DL3</td>
<td>C2:C2;Bw4</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1/2DL1/2DL3/3DL1</td>
<td>C2:C2;Bw4</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>46</td>
<td>46 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table V. 2DS1<sup>pos</sup> C1:C1 NK clones coexpressing inhibitory KIR for self–HLA class I

<table>
<thead>
<tr>
<th>Donor HLA-C Genotype</th>
<th>KIR Phenotype</th>
<th>Target HLA-C Genotype</th>
<th>Cytolytic</th>
<th>n</th>
<th>Yes, n (%)</th>
<th>No, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1:C1</td>
<td>2DS1 and 2DL2/3</td>
<td>C2:C2;Bw4</td>
<td>46</td>
<td>42 (91)</td>
<td>4 (9)</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C1:C1</td>
<td>2DS1 and 3DL1</td>
<td>C2:C2;Bw4</td>
<td>2</td>
<td>2 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Frequency of cytolytic clones to targets with different HLA-C genotype is compared.
EB6, Anti-KIR2DL1/S1; HLA-E, hNKG2A ligand. This analysis was performed on NKG2Apos clones. This analysis was performed on EB6+ICAM-I+HLA-E; black line, EB6+HLA-E. (C) Correlation between NKG2A mRNA copy numbers and NKG2A receptor MFI in 2DS1SP/NKG2Apos clones. The degree of cytotoxicity was compared. **p < 0.01.

FIGURE 5. 3DL1 interaction with cognate HLA-Bw4 can override 2DS1 activation. 2DS1SP/3DL1pos clones (n = 8) were obtained from a 2DS1 healthy donor with the HLA-C1:C2:Bw4 genotype and were tested for cytotoxicity against EBV-BLCL target cells with different HLA-C and -B genotype. Left, Cytotoxicity against C2:C2;Bw4 EBV-BLCL (IHWG 9036) target cells. Center, Cytotoxicity against C2:C2;Bw6 EBV-BLCL (DD) target cells. Right, Cytotoxicity against C1:C1;Bw6 EBV-BLCL (NK) target cells. Data were generated in two independent experiments. Assays were performed in duplicate, with E:T ratio 10:1. The dotted line represents the 13.1% cutoff between clone cytotoxicity and noncytotoxicity. The degree of cytotoxic response between different groups is compared. ***p < 0.01.

donors heterozygous for HLA-C2 do not express sufficient ligand to induce 2DS1 tolerance. In contrast, 2DS1pos clones from HLA-C2 homozygous donors have significantly reduced frequency of anti–HLA-C2 reactive clones. These results demonstrate that NK cells with an activating KIR specific for a self–major histocompatibility Ag are not all deleted from the repertoire, but are rendered tolerant when sufficient density of the ligand is expressed. NK cell tolerance has been reported in mouse models of NK cells that express activating receptors for self-antigens (7–11), but the differential effect of tolerance induction by homonymous versus heteroerogenous expression of the activating ligand has not previously been reported. NK tolerance was also observed in mice with mixed allelic bone marrow chimerism. NK cells in these mice expressed the activating Ly49D receptor, and one strain also expressed the putative MHC class I ligand H2-D\(^{D}\) (12, 37, 38). These reports, as well as the current study, demonstrate self-tolerance of activating MHC class I specific receptors in the absence of known inhibitory receptors to self–MHC class I.

Nearly all 2DS1pos clones with anti–HLA-C2 reactivity are 2DS1SP or 2DS1pos with irrelevant, nonself inhibitory KIR. These clones would be expected to display hyporesponsiveness owing to lack of NK licensing (39, 40). It is therefore surprising that 2DS1pos clones from HLA-C1 homozygous and heterozygous donors display potent anti–HLA-C2 responses, because they lack inhibitory receptors for self–MHC class I. Our findings are, however, consistent with the recently reported phenomenon termed “functional NK plasticity” described in mouse models. In these models, mature NK cells were transferred between wild-type mice and MHC class I–deficient hosts. These mature NK cells displayed functional plasticity and adapted to the MHC environment of the host (41, 42). Our studies confirm and extend these findings by demonstrating NK plasticity of developing NK cells in both the syngeneic and the allogeneic HLA-C2 positive host.

It is surprising that 2DS1pos clones from donors heterozygous for HLA-C2 do not display any evidence of reduced in vitro responses to HLA-C2. Determination of HLA-C2 Ag binding to 2DS1 in vitro demonstrates very weak binding affinity (43, 44). Because HLA Ags are codominantly expressed, the gene products from both HLA-C alleles will be displayed. HLA-C2 homozygous individuals will therefore express twice the amount of HLA-C2 ligand as will HLA-C2 heterozygous donors. Our study indicates that the amount of HLA-C2 ligand expressed by HLA-C2 homozygous host cells is sufficient to induce tolerance in 2DS1pos NK cells, whereas the amount expressed by HLA-C2 heterozygous donors is
HLA-C2 invariably suppressed 2DS1 activity. The effect of LILRB1 on inhibitory KIR with ligand specificity for self–HLA class I almost never occurs very rarely. In contrast, cells in donors homozygous for HLA-C2 receptor in IL-15–primed NK clones could obtain a similar low frequency of anti–HLA-C2 reactivity, which is high in HLA-C1 homozygous donors. Therefore, 2DS1 activation and ligand-induced tolerance. We did explore insufficient for activation of 2DS1. We also demonstrate lack of ligand-induced receptor downmodulation of 2DS1 on NK cells from HLA-C2 homozygous donors. In contrast, the Ly49H receptor is downregulated in mice expressing the m157 viral ligand (10, 11). Collectively, these results support that 2DS1 interactions with HLA-C2 are weak, which agrees with results from binding affinity studies (43, 44).

The aim of the current study was to define the genetic basis for 2DS1 activation and ligand-induced tolerance. We did explore some possible mechanisms for tolerance to self in 2DS1pos NK cells in donors homozygous for HLA-C2. Inhibition of 2DS1 activation by CD94/NKG2A only occurs very rarely. In contrast, inhibitory KIR with ligand specificity for self–HLA class I almost invariably suppressed 2DS1 activity. The effect of LILRB1 on HLA-C2 homozygous 2DS1pos clones was also tested. Such clones frequently did not display anti–HLA-C2 reactivity. However, we also demonstrate that the HLA-C genotype influences the frequency of anti–HLA-C2 reactivity, which is high in HLA-C1 homozygous and low in HLA-C2 homozygous donors. Therefore, our results suggest only a possible contribution of LILRB1 receptor to tolerance development and maintenance.

It has recently been proposed that NK activation is controlled by the localization of activating receptors in the NK plasma membrane. The presence or absence of inhibitory receptors with ligand specificity for self–HLA class I is, in these studies, suggested to regulate the activating receptor (45). It is possible that the 2DS1 receptor in IL-15–primed NK clones could obtain a similar localization in the plasma membrane, facilitating 2DS1 activation. Another possible mechanism for mediating self-tolerance to the HLA-C2 ligand in HLA-C2 homozygous donors is cis interactions between the 2DS1 receptor and the HLA-C2 ligand on the individual NK cell (46). The present study does not address this issue. Ongoing studies with functional human NK cells in HLA class I transgenic mice may provide new insight on this issue (X.-R. Liu, Z. Zhao, L.D. Shultz, D.L. Greiner, and B. Dupont, unpublished observations).

NK alloreactivity is known to affect hematopoietic stem cell engraftment. Rejection of murine parental bone marrow grafts by F1 hybrid NK cells is regulated by missing self–MHC class I recognition, in combination with signals from activating receptor–ligand interactions. In some mouse strains, the activating NKG2D and its ligands are dominating, whereas in other strains the activating Ly49D receptor in the presence of H2-Dd mediates graft rejection (38, 47). Allogeneic NK cells also participate in protecting HCT recipients against leukemia relapse, and this effect is primarily observed in patients with acute myeloid leukemia (AML). The initial clinical studies involved HLA-haploidentical transplants for which the recipients lacked HLA class I ligands for inhibitory KIRs present in the donor (48). Donor-derived NK allostirivation was interpreted as being caused by “missing self–HLA class I ligand” in the recipient. Another mechanism for development of alloreactive NK cells is HLA-C2–mediated activation of 2DS1pos NK cells from HLA-C1 homozygous individuals (19, 49). We have recently demonstrated protection from relapse of AML following HCT from 2DS1 donors with the HLA-C genotypes C1:C1 and C1:C2; this benefit is absent if the HCT donor has the C2:C2 genotype. The present study of 2DS1pos clones with anti–HLA-C2 reactivity derived from donors with different HLA-C genotypes provides a mechanistic interpretation of these clinical observations: 2DS1pos donors with the C1:C1 and C1:C2 genotypes have similar ability to generate a large number of anti–HLA-C2 clones, but HLA-C2 homozygous donors have significantly reduced frequency of such clones. These results support a model in which rejection of developing leukemic cells in many cases may be mediated by NKG2D activation of NK cells by NKG2D ligands (50, 51). Such NKG2D responses frequently require additional stimulatory signals. Amplifying, stimulatory signals might be provided by “missing self–HLA class I ligand,” as observed in HLA-haploidentical HCT (48) or by donor-derived 2DS1pos NK cells activated by HLA-C2 Ags in the recipient (36).

The present report addresses the functional effects of interactions between the activating receptor, 2DS1, and its ligand, HLA-C2. However, 2DS1 and the gene for another activating receptor, 3DS1, frequently exist together owing to strong positive genetic linkage disequilibrium (35). Clinical genetic association studies of hematopoietic transplantation in AML have demonstrated different functional associations for the two genes in transplantation outcome. Specifically, 2DS1, but not 3DS1, was found to be associated with protection against posttransplantation leukemia relapse, whereas 3DS1 was associated with improved survival (36).

Table VI. Impact of 3DS1 expression on the anti–HLA-C2 cytotoxicity of 2DS1pos clones

<table>
<thead>
<tr>
<th>Donor HLA-C Genotype</th>
<th>KIR Phenotype</th>
<th>Target HLA-C Genotype</th>
<th>n (%)</th>
<th>Yes, n (%)</th>
<th>No, n (%)</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1:C1, C1:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;/3DS1&lt;sup&gt;pos&lt;/sup&gt;</td>
<td>C2:C2;Bw4</td>
<td>45 (70)</td>
<td>33 (73)</td>
<td>12 (27)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;/3DS1&lt;sup&gt;pos&lt;/sup&gt;</td>
<td>C1:C1</td>
<td>19 (30)</td>
<td>15 (79)</td>
<td>4 (21)</td>
<td></td>
</tr>
<tr>
<td>C2:C2</td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;/3DS1&lt;sup&gt;pos&lt;/sup&gt;</td>
<td>C2:C2;Bw4</td>
<td>20 (77)</td>
<td>7 (35)</td>
<td>13 (65)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>2DS1&lt;sup&gt;SP&lt;/sup&gt;/3DS1&lt;sup&gt;pos&lt;/sup&gt;</td>
<td>C2:C1;Bw4</td>
<td>6 (23)</td>
<td>1 (17)</td>
<td>5 (83)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>C1:C1 clones identical to those described in Fig. 2 and Table III. One C2:C2 clone was not included in this analysis owing to lack of cDNA for 3DS1 RT-qPCR amplification.

<sup>b</sup>Frequency of anti–HLA-C2 cytolytic clones in each group is compared.
In agreement with these clinical findings, we demonstrate in this article that the anti–HLA-C2 reactivity of 2DS1 clones is independent of the presence or absence of 3DS1 expression. It is currently not known how 3DS1 affects NK function, but studies of AIDS patients with HIV-1 indicate that the 3DS1 receptor might bind HLA-B (Bw4-801) in HIV-1-infected cells and target NK cells toward infected cells (52). Collectively, these studies and ours suggest that important NK effector functions are mediated by activating receptors with ligand specificity for HLA class I.

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References


Disclosures

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