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The Association of MHC Class I Proteins with the 2B4 Receptor Inhibits Self-Killing of Human NK Cells

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The killing activity of NK cells is carried out by several activating NK receptors, which includes NKp46, NKp44, NKp30, NKp80, NKG2D, and 2B4. The ligands of these receptors are either self-derived, pathogen-derived, stress-induced ligands or tumor ligands. Importantly, none of these killer ligands are expressed on NK cells and thus self-killing of NK cells is prevented. A notable exception with this regard, is the ligand of the 2B4 receptor. This unusual receptor can exert both activating and inhibiting signals; however, in human NK cells, it serves mainly as an activating receptor. The ligand of 2B4 is CD48 and in contrast to the ligands of all the other NK activating receptors, CD48 is also present on NK cells. Thus, NK cells might be at risk for self-killing that is mediated via the 2B4-CD48 interaction. In this study, we identify a novel mechanism that prevents this self-killing as we show that the association of the MHC class I proteins with the 2B4 receptor, both present on NK cells, results in the attenuation of the 2B4-mediated self-killing of NK cells. The Journal of Immunology, 2010, 184: 2761–2768.

Natural killer cells are cytotoxic lymphocytes that belong to the innate immune system. They were initially described as cells able to kill virally infected and tumor cells (1), although it is known today that they possess several additional functions such as production of growth factors during pregnancy (2) and even being APCs (3). The killing of NK cells is regulated by surface receptors that either induce or inhibit the cytotoxic response. NK inhibition is mediated mainly via the recognition of MHC class I molecules by NK inhibitory receptors, such as the killer-Ig-like receptors (KIRs), CD94/NKG2A, and the leukocyte Ig-like receptors (LIRs). Recently, we have shown that TIGIT could also inhibit NK cells by binding to PVRL-2 and PVR (4). MHC class I proteins are also needed for proper NK cell education (5, 6) and in the absence of MHC class I proteins the killing activity of NK cells is impaired.

The killing of NK cells is extracted by a limited number of activating and coactivating NK receptors, including the natural cytotoxicity receptors NKp46, NKp44, NKp30 (known as the NCRs), as well as by NKp80, DNAM, CD96, and the NKG2D receptor (7, 8). Another family of killer receptors that is involved in the killing mediated by NK cells is the CD2 family (9, 10). The CD150 subfamily, within the CD2 family, includes several dual-functional receptors such as CD150, CD84, CD229, CD244 (2B4), NTB-A, and CS1 (11) that can exert both activating and inhibitory signals, depending on the adaptor proteins, which they interact with (12). An example for such a receptor is 2B4, the subject of this research, which contains within its cytoplasmic tail four signaling ITSM (immunoreceptor tyrosine-based switch motif) (13).

The ligands for the activating NK receptors that have been identified so far include stress-induced ligands for NKG2D (14), viral ligands for NKp44, NKp46, and NKp30 (15–17), a soluble ligand for NKp30 (18), a tumor ligand for NKp30 (19), and self-ligands such as AICL and PVR/PVRL-2 for the NKp80 (20) and DNAM/CD96 (21) receptors, respectively. Finally, the CD48 protein, has been identified as a ligand for 2B4 (22) and CD2 (10). In humans, 2B4 almost exclusively functions as an activating receptor and its inhibitory properties are mostly evident in genetic deficiencies in which the signaling lymphocyte activation molecule-associated protein (SAP) is absent. In such a deficiency named X-linked lymphoproliferative disease (XLP) (23), different phosphatases such as Src homology region 2 domain-containing phosphatase 1 (SHP-1), SHP-2, and SHIP are recruited, instead of SAP, and 2B4 becomes inhibitory (24).

Among all the killer receptor ligands, CD48 is the only protein expressed on NK cells (25). Therefore, we wondered why NK cells do not kill each other via the 2B4-CD48 interactions, and hypothesized that NK cells probably possess a mechanism that prevent this 2B4-mediated self-killing.

Materials and Methods

Cell, transfectants, mab, and proteins

The cell lines used in this work were the MHC class I-negative human B lymphoblastoid cell line 721.221, various MHC class I transfectants, the human NK cell line YTS eco, and the Fe receptor positive murine mastocytoma cell line P815. Primary human NK cells were isolated from PBLs by using a human NK cell isolation kit and an auto-MACS instrument (Miltenyi Biotec, Auburn, CA).

For the generation of YTS cells expressing the chimeric protein containing the extracellular portion of NKp44 fused to the transmembrane and cytoplasmic tail portion of 2B4, plasmid constructs were prepared in EcoRI-
The percentage of specific lysis is shown for an E:T ratio of 5:1. Shown is one representative experiment of 10 performed.

BBMH1 pBABE vector. Transfection with the encoding plasmid for the chimeric NKp44/2B4 protein or for the chimeric NKp44/Cw7 was performed in three PCR steps: first, amplification of the extracellular domain of NKp44 containing 3 aa from the beginning of the transmembrane domain of 2B4 or HLA-Cw7, using the same 5’ primer for both proteins GGAATTCCGGCCACCATGGGCGCTGGCGAGCCCTACAC (including the EcoRI restriction site) and 3’ primer for 2B4 CACCAAAGGGCAATGGGGCGCTGGCGAGG or 3’ primer for HLA-Cw7 RATGGCCCATGGCGAGCCCTACAC. Second, amplification includes 3 aa derived from the end of the extracellular domain of NKp44 with the transmembrane domain and the tail of of 2B4 receptor or HLA-Cw7, for the 2B4 using 5’ primer CACATTGGCCATGGGCGCTGGCGAGG and for the HLA-Cw7 using 5’ primer CACATTGGCCATGGGCGCTGGCGAGG. The 3’ primer for both proteins CCGGATCCGCTAAGATTTAACATCAATGGGGGCTGCAGGG or 3’ primer for HLA-Cw7 CAATGGGGGCTGCAGGG. Third, amplification includes 3 aa derived from the beginning of the transmembrane domain of 2B4 or HLA-Cw7, using the same 5’ primer of the first PCR and the 3’ primer of the second PCR. All transfectants were periodically monitored for expression by staining with the appropriate mAb.

The mAbs used in this work are C17 mAb (Beckman Coulter, Fullerton, CA) directed against 2B4; anti-CD48 mAb; GB7 (IgG1), W632, and mem147 (IgG1) directed against MHC class I molecules; anti-BBM1 directed against HLA-B and -C free heavy chains. The CellQuest software program was used for data acquisition and analysis.

Cytotoxic assays

The cytotoxic activity of YTS cells was assessed in 5 h [35S]-Met release assays, as previously described (26). In the redirected lysis experiment target p815 cells were preincubated for 1 h, on ice with 0.1 µg/well of the indicated Abs. Percent-specific lysis was calculated as 100% × [cpm (experimental well) – cpm (spontaneous release)/cpm (maximum release – cpm (spontaneous release))]. Maximum release was determined by adding 0.1 M NaOH. The spontaneous release was <25% of maximal release. For the statistical analysis, we used t-test.

Class I MHC tetramers-SA-PE that are used in this work are composed of a complex of four HLA-A2 MHC class I molecules, each bound to the specific peptide and conjugated with a fluorescent protein (Beckman Coulter).

**FIGURE 1.** Identification of a mAb that inhibits the 2B4-mediated killing. A, Staining of NK cells or YTS eco cells with the GB7 mAb (gray histogram). Background (BG) is the staining of the same cells with secondary FITC-conjugated goat anti-mouse Abs (black histogram). B, GB7 inhibits the 2B4-mediated killing. YTS eco, or NK cells were tested in a redirected killing assay against P815 target cells coated with an anti-2B4 Ab (black bars) or with an anti-2B4 mAb combined with the GB7 mAb (gray bars). *p ≤ 0.002; **p ≤ 0.001. C, The GB7-mediated inhibition is specific to 2B4. Redirected killing assay mediated by NK cells was performed using anti-CD16, NKp30, NKp44, and NKp46, and NK2D Abs incubated with P815 cells in the presence (gray bars) or absence (black bar) of the GB7 mAb. Med is for medium only. D, Titration of GB7 mAb. Redirected killing assay mediated by YTS eco cells (upper) or primary bulk NK cells (lower) was performed using different mAb concentrations (0.004–0.5 µg/well) of GB7 mAb or anti-CD99 mAb with and without 0.1 µg/well of the 2B4 mAb. The percentage of specific lysis is shown for an E:T ratio of 5:1. Shown is one representative experiment of 10 performed. **p ≤ 0.001.
Protein band sequencing

The peptide mixtures was either solid phase extracted with C18 resin filled tip (ZipTip Milipore, Billerica, MA) and nanosprayed into the Q-TOF2 system in 50% CH3CN 1% CHOOH solution or injected to 0.75 Ul C18 column on a capillary HPLC system (CapLC Waters, Milford, MA) coupled to the MS. Mass spectrometry was carried out with Q-TOF2 (Micromass, Manchester, England) using nanospray attachment (27). Data analysis was performed using the biolynx package (Micromass), and database searches were performed with the Mascot package (Matrix Science, London, England).

FIGURE 2. GB7 mAb recognizes MHC class I proteins. A, Sequencing the protein band recognized by GB7. Immunoprecipitation was performed using the GB7 mAb and analyzed on SDS-PAGE gels. The protein bands were sent for sequencing and the identified peptides are shown. B, The GB7 mAb recognizes HLA-A, -G, and -B, but not -C proteins. Staining of different 721.221 transfectants (indicated in the x-axis) was performed either with the GB7 mAb (black bar) or with W6/32 mAb (gray bar).

FIGURE 3. Inhibition of the 2B4-mediated killing by various anti-MHC class I mAbs. A, FACS staining of YTS eco and NK cells. Staining (indicated by arrows, gray histogram) was performed with the following Abs: GB7, W632, mem147 (all directed against MHC class I), and the BBM1-anti-β-2-microglobulin. BG is the staining of the cells with secondary FITC-conjugated goat anti-mouse Abs (black histogram). B, Redirected killing assay performed with bulk NK cell cultures (black bar) and YTS eco cells (gray bars) by using P815 cells, precoated either with the anti-2B4 mAb alone or with the 2B4 mAb, together with the different anti MHC class I mAbs (indicated in the x-axis). The percentage of specific lysis is shown for a E:T ratio of 5:1. Shown is one representative experiment of five performed. *p ≤ 0.002; **p ≤ 0.001.
shRNA

The shRNA constructs directed against 2B4 (Open Biosystems, Huntsville, AL) were designed to include a hairpin of 21-bp sense and antisense stem and a 6-bp loop. Each hairpin sequence was cloned into lentiviral vector (plKO.1). This vector allows for production of viral particles using lentiviral packaging cell lines (293-T). Two days after transfection, the supernatants containing the viruses were collected and filtered. These viruses were used to transduce YTS eco cells in the presence of polybrene (5 μg/ml).

Immunoprecipitation

YTS eco cells (15 × 10⁶–20 × 10⁶/ml) or YTS/NKp44-2B4 were washed four times with cold PBS containing 1 mM MgCl₂ and 0.1 mM CaCl₂. For immunoprecipitation with GB7, the cells were biotinylated with EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) for 30 min at 4°C and washed four times with cold PBS. For immunoprecipitation with anti-2B4 (C17), or anti-CD99 (12E7), or anti-β-2-microglobulin (BBM1), the cells were first incubated with 0.1 μM of vanadate for 10 min at 37°C, washed four times with RPMI 1640.

After washing, cells were lysed with lysis buffer (PBS containing 150 mM NaCl, 50 mM TRIS [pH 7.6], 0.5% Nonidet P-40, 9 mM iodoacetamide, 5 mM EDTA, 1 mM PMSF, 1:100 aprotinin). Cell lysates were then immunoprecipitated for 3 h at 4°C with 20 μg of the necessary Ab followed by Protein-G plus beads (Pierce). The immunoprecipitates were washed with lysis buffer, and the proteins were eluted by using the IgG elution buffer (Pierce).

All the samples were loaded on SDS-PAGE and then transfer to nitrocellulose membrane. The blotted proteins were visualized by antistreptavidin HRP or by anti-free H chain (HC10) or anti-NKp44 or anti-2B4, followed by anti-mouse HRP (DakoCytomation, Carpinteria, CA), using ECL detection (Biological Industries, Bet-haemek, Israel). A sample from the cell lysate was taken before immunoprecipitation for the detection of protein amount.

Results

Inhibition of the 2B4-mediated killing of NK cells by a novel mAb

Because both 2B4 and its ligand CD48 are expressed on NK cells, we reasoned that a mechanism aiming at preventing the 2B4-CD48 self-killing should exist. As the 2B4 killing machinery is intact in YTS cells and YTS also express CD48 and because we have previously generated mAbs against YTS to identify the function of CD100 (28), we screened 200 hybridomas that recognized YTS and NK cells (example is shown in Fig. 1A) for their ability to inhibit the 2B4-mediated killing of YTS and NK cells. In these assays, we triggered 2B4 either on NK cells or on YTS cells by using an anti-2B4 mAb and included also the 200 hybridomas that we have generated. As can be seen in Fig. 1B, one of all hybridomas tested named GB7 inhibited the 2B4 mediated killing of both YTS and bulk NK cell cultures. The other hybridomas tested had no effect (data not shown). We next tested whether the killing extracted by NK receptors, other than 2B4, such as CD16, NKp44, NKp46, and NKG2D will also be inhibited by GB7 mAb and observed that the GB7-mediated inhibition is restricted to 2B4 only (Fig. 1C). The effect is specific because the titration of the 2B4 mAb resulted in a gradually reduced inhibition in both YTS eco and primary bulk NK cell cultures (Fig. 1D).

MHC class I proteins inhibit the 2B4-mediated killing

To identify the protein recognized by the GB7 mAb, we performed immunoprecipitation experiments using YTS cells and the GB7 mAb. We obtained two bands in the size of ~45 kDa and a slightly larger band of around 75 kDa (Fig. 2A). All three bands were sent for sequencing and, surprisingly, all peptides derived from all bands corresponded to peptides derived from MHC class I proteins (Fig. 2A). To further confirm that indeed GB7 recognizes MHC class I molecules, we stained different 721.221 transfectants expressing various MHC class I proteins with the GB7 mAb and observed that GB7 recognizes HLA-G, HLA-A, HLA-B, but not HLA-C molecules (Fig. 2B). As expected, the control mAb W632, a pan MHC class I mAb, recognized all MHC class I proteins (Fig. 2B).

To further demonstrate that indeed MHC class I molecules are capable of inhibiting the 2B4-mediated killing, we used several anti-MHC class I mAbs and performed redirected killing experiments. As expected, all four mAbs (GB7, W6/32, mem147, and BBM1) recognized MHC class I proteins on YTS and NK cells (Fig. 3A). Strikingly and in agreement with the inhibition of 2B4-mediated killing observed with GB7 mAb, all anti-MHC class I mAb inhibited the 2B4-mediated killing of both primary NK and YTS cells (Fig. 3B).

Engagement of MHC class I proteins by their appropriate inhibitory receptors inhibits self-killing of YTS cells

The above results show that cross-linking of MHC class I proteins on NK cells inhibits the 2B4-mediated killing. Under physiological conditions, such cross-linking could result from the cis or trans interactions formed between the NK inhibitory receptors and their cognate MHC class I proteins that are expressed on the NK cells. To test whether indeed cross-linking of the MHC class I proteins
by the inhibitory receptors would inhibit the 2B4-mediated YTS self-killing, we initially expressed in YTS cells the LIR1 inhibitory receptor that recognizes a large group of MHC class I proteins (29, 30). LIR1-positive YTS transfectants were obtained, however, they stopped proliferating and died within a short period (data not shown). We assumed that the reason for this phenomenon is because the LIR1 interaction with MHC class I proteins on YTS cells results in an inhibitory signal that prevented YTS proliferation. This observation might explain why the YTS tumor cell line, which is derived from a cancer patient (31), lacks the expression of all known inhibitory receptors. We next thought to express in YTS cells a KIR receptor that will interact with a specific MHC class I protein and for that purpose we determined the MHC haplotype of YTS cells to be HLA-A-26, A-31, Cw-01, Cw-08, B-48, B-55. We previously successfully expressed the KIR2DL1 receptor in YTS cells (32) and we think that this efficient expression was due to the absence of the appropriate MHC class I ligand for KIR2DL1. YTS cells express HLA-Cw-08 that is a ligand for KIR2DL3 and therefore we expressed in YTS cells a wild-type KIR2DL3 receptor and a mutated KIR2DL3 protein, in which the two tyrosine residues in its ITIM motif were mutated to phenylalanin (KIR2DL3[2YF]) (Fig. 4A). In agreement with our hypothesis, expression of KIR2DL3 was observed on the cell surface; however, the transfectant grew for about 3 wk and then stopped proliferating and died (data not shown). In contrast, the mutated KIR2DL3 protein was stably expressed on YTS cells for a long period.

To verify that indeed the ITIM mutations in YTS-KIR2DL3 (2YF) render this receptor nonfunctional, we performed a redirected killing assay with P815 cells coated with anti-KIR2DL3 mAb and anti-2B4 mAb. All three cell lines; YTS eco, YTS-KIR2DL3, and YTS-KIR2DL3(2YF) showed a similar killing profile against P815 cells coated with anti-2B4 only or against 721.221 cells (Fig. 4B), indicating that the killing machinery in all YTS transfectant is intact and equal among all various transfectants. As expected, when redirected killing experiments were performed with anti-2B4 and anti-KIR2DL3 mAb, inhibition was observed only in the wild-type YTS-KIR2DL3 cells (Fig. 4B). Thus, the mutated KIR2DL3 protein is, indeed, nonfunctional and can be used to test whether the engagement of MHC class I proteins (in this particular case HLA-Cw-08) will affect the 2B4 killing. We next assumed that if indeed the interaction between the mutated KIR2DL3 and the MHC class I proteins on the NK cells inhibit the self-killing of NK cells, blocking of such interactions will result in increased killing of target cells. We used the YTS-KIR2DL3(2YF) cells to avoid inhibition that is mediated via KIR2DL3, and as can be seen in Fig. 4C, indeed blocking of MHC class I with the B1.23.2 mAb, or blocking the receptor with the GLI83 mAb resulted in increased killing, whereas the GB7 mAb had no effect (probably because it is a nonblocking mAb). To further confirm that the MHC class I proteins prevent the 2B4-mediated self-killing of NK cells, we initially demonstrated that YTS eco and YTS/KIR2DL3(2YF) cells express similar levels of the killer receptor 2B4 and its ligands CD48 (Fig. 5A). Next, we...
used two types of assays, CD107a mobilization and BrdU incorporation. The CD107a measures NK cell degranulation and we reasoned that if the interactions between the inhibitory receptor KIR2DL3(2YF) and the MHC class I proteins inhibit self-killing, these YTS cells should be less degranulated. Indeed, as can be seen in Fig. 6A, YTS cells expressing the inhibitory-signal deficient KIR2DL3(2YF) express less CD107a as compared with YTS eco cells. In the second assay we used BrdU, a synthetic analog of thymidine, which is incorporated into the newly synthesized DNA of replicating cells (during the S-phase of the cell cycle) and again predicted that YTS/KIR2DL3(2YF) cells will be more proliferating as compared with YTS cells because self-killing is prevented. As expected, the percentage of the proliferating cells (S-phase) was enhanced in KIR2DL3(2YF) cells (Fig. 5C, upper panel). Furthermore, cell cycle analysis using PI staining detected an apoptotic sub-G1 population that is absent in YTS-KIR2DL3(2YF) cells (Fig. 5C, lower panel).

MHC class I proteins directly bind to 2B4 and the self-killing of YTS cells is mediated via the 2B4 receptor

The above results demonstrate that the interaction between the inhibitory receptor and MHC class I on NK cells inhibits the self-killing of NK cells in a 2B4-dependent manner. To demonstrate that MHC class I proteins interact directly with 2B4, we initially repressed the 2B4 expression using shRNA against the 2B4 receptor (Fig. 6A) and then examine the binding of HLA-A2 tetramer to the various cells. As can be seen in Fig. 6B, efficient binding of the HLA-A2 tetramer was observed to YTS cells expressing 2B4 and when 2B4 expression was reduced, the binding of the HLA-A2 tetramer was also much reduced (Fig. 6B). We next tested whether the reduced 2B4 expression will also lead to a reduction in the self-killing of YTS cells. As can be seen in Fig. 6C, the reduction in 2B4 expression was accompanied with reduced CD107a mobilization to the cell surface, which suggest that 2B4 is the receptor responsible for the self-killing of YTS cells. Finally, to directly demonstrate that 2B4 is involved in the self-killing of YTS cells, we blocked 2B4 interactions with CD48 by using a specific mAb against 2B4 and demonstrated that such blocking resulted in reduced CD107a mobilization (Fig. 6D), whereas, the control CD99 had little or no effect (data not shown). These three assays indicate that 2B4 is directly involved in the self-killing of YTS cells by directly interacting with MHC class I proteins.

The interaction of 2B4 and the MHC molecules is crucial for the inhibition of self-killing

To investigate the mechanism responsible for the MHC class I-mediated inhibition of 2B4 killing, we generated two types of chimeric molecules in which the extracellular portion of the NKp44 receptor was fused either to the transmembrane and cytoplasmic tail of 2B4 (YTS 44-2B4, Fig. 7A) or to the transmembrane and cytoplasmic tail of HLA-Cw7 (YTS 44-Cw7, Fig. 7B), and expressed them in YTS eco cells.

Importantly, cross-linking of YTS 44-2B4 with anti-NKp44 mAb, resulted in efficient killing, which was equivalent to that of 2B4 and the NKp44-2B4-mediated killing was inhibited when MHC class I molecules were engaged (Fig. 7A, lower panel). Thus, the transmembrane and cytoplasmic tail of 2B4 are responsible for the MHC class I proteins-mediated inhibition. As expected, cross-linking of YTS 44-Cw7 with anti-NKp44 mAb did not result in killing, as the tail and transmembrane portions of the MHC class I proteins do not contain any activating signal needed for killing (Fig. 7B, lower panel). Furthermore, cross-linking of YTS 44-Cw7 cells with anti-NKp44 mAb together with the cross-linking of 2B4, did not inhibit the 2B4-mediated killing (Fig. 7B, lower panel).

These results indicate that the transmembrane and the cytoplasmic tail of 2B4 are involved in the MHC class I-mediated inhibition and that such inhibition does not require the tail and the transmembrane portions of the MHC class I. Thus, we hypothesized that the extracellular portion of MHC class I is involved in the 2B4 inhibition and that this inhibition probably occurs through the association of MHC class I proteins with 2B4. To test this

**FIGURE 6.** The self-killing of YTS cells is mediated via the 2B4 receptor. A, ShRNA-mediated downregulation of the 2B4 receptor. FACS analysis of the 2B4 expression postinfection of YTS eco cells with shRNA directed against 2B4 receptor. B, Decrease binding of the HLA tetramer resulting from the reduction of 2B4 expression. C, Reduced CD107a expression after 2B4 downregulation. D, Blocking the 2B4 receptor results in reduced self-killing. Staining YTS eco cells with anti-CD107a mAb after blocking of the 2B4 receptor with a specific mAb. For A–D, “After” indicates after shRNA infection, “Before” indicates before shRNA infection. BG is background with second mAb only.
hypothesis, we immunoprecipitated either the 2B4 protein using specific anti-2B4 mAb or the NKp44-2B4 protein using anti-NKp44 mAb and performed Western blot analysis with anti-MHC class I mAb HC10. As predicted, MHC class I proteins were coimmunoprecipitated with both anti-2B4 and anti-NKp44, suggesting that MHC class I proteins are probably associated with both receptors (Fig. 7C, left blot). Finally, to further strengthen these results we also performed the reciprocal experiments and demonstrated that both the 2B4 or NKp44 receptors were coimmunoprecipitated when the anti–β-2-microglobulin mAb was used (Fig. 7C, right blot).

**Discussion**

In contrast to all other NK receptors, whose ligands are not expressed on NK cells, the CD48 protein and its interacting receptor 2B4 are both expressed on normal hematopoietic cells, including NK cells. This dangerous situation might potentially lead to the self-killing of NK cells. In this study, we demonstrate that the MHC class I proteins expressed on NK cells inhibit the 2B4 self-killing not only by serving as an inhibitory ligand for inhibitory NK receptors but also through the association with 2B4. We demonstrate a direct association between MHC and 2B4 as the binding of MHC class I tetramer was reduced concomitantly with the reduction of 2B4 expression and we could precipitate MHC class I with anti-2B4 and vice versa. The mechanism by which MHC class I affects 2B4 killing is unknown and all signaling molecules we have tested, including SAP, SHP1, SHIP1, FYN kinase, and ERK/pERK were not altered by the MHC class I cross-linking (data not shown).

We also show in this study that in addition to 2B4, MHC class I proteins also interact with NKp44, but not with NKp30 (data not shown). However, the association of MHC class I molecules with NKp44 did not lead to the inhibition of NKp44-mediated killing, but only affects 2B4 killing.

If 2B4 is such a dangerous, potentially self-destructing receptor why do all NK cells express this receptor? One possibility is that 2B4 is important for killing of hazardous pathogens/tumors. Indeed, it was shown that 2B4 is an important modulator of immune cells activity and that overexpression of CD48 enhanced the NK-mediated killing (25, 33). It seems, however, that the most crucial function of 2B4 is during viral infections (34) and the most convincing evidence for this comes from XLP patients. In these patients, SAP is absent, 2B4 can no longer function as an activating receptor, and the patients suffer from fatal immune dysfunction, associated with the inability to control EBV infections (35).

Another difficult question is why should NK cells express CD48, the ligand of 2B4, and be susceptible to self-killing. One possible
explanation is that other proteins also interact with CD48 and that such an interaction would be beneficial for NK cell activity. Indeed, CD48 is also the ligand for CD2, an important costimulatory molecule of lymphocyte activation, including NK cells (36). Triggering of CD2 induces calcium flux and tyrosine phosphorylation that leads to increased proliferation, cytokoty, IFN-γ, and IL-2 release (37). In addition, NK cells are involved in regulating the adaptive T cell responses not only indirectly, by sequestering cytokines, but also through the direct killing of immature dendritic cells (38, 39). NK cells could also serve as APCs, which express MHC class II proteins and other costimulatory molecules after activation and thus could directly prime T cell responses (3). Because it was demonstrated that adhesion of T cells to APCs, or NK cells is increased after CD2 cross-linking (12, 37), the CD48 expression on NK cells might function as another costimulatory molecule when NK cells serve as APCs for priming of T cell responses.

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Disclosures
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