Augmentation of NK cell-mediated cytotoxicity to tumor cells by inhibitory NK cell receptor blockers

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Keywords: Ly49, MHC class I, mouse, peptide, phage display library

Abstract

NK cells monitor expression of MHC class I by inhibitory receptors and preferentially kill cells that lose or down-regulate MHC class I expression. One possible mechanism by which tumor cells evade NK cell killing is continued expression of appropriate MHC class I ligands to engage inhibitory receptors on NK cells. We show here that small-mol.-wt blockers against the mouse inhibitory NK cell receptor Ly49A enhance NK cell killing of such tumor cells. We identified Ly49A-binding peptides by selecting phages with the capacity to bind recombinant Ly49A expressed in Escherichia coli from a phage display random peptide library. The Ly49A-binding peptides could also bind Ly49A expressed on mammalian cells. Importantly, the Ly49A-binding peptides blocked Ly49A recognition of its MHC class I ligands H-2Dd and H-2Dk. Moreover, blockade of Ly49A by the peptides enhanced cytotoxicity of Ly49A+ NK cells towards H-2Dd-expressing tumor cells. These results clearly indicate effectiveness of small-mol.-wt blockers of inhibitory NK cell receptors in enhancing NK cell-mediated killing of tumor cells that are otherwise resistant because of MHC class I expression.

Introduction

NK cells kill tumor cells and infected cells spontaneously (1). This killing is regulated by inhibitory NK cell receptors specific for MHC class I molecules (2), providing MHC class I-expressing cells protection from NK cell cytotoxicity (3,4). Cells that are down-regulated of MHC class I due to viral infection or transformation cannot effectively engage inhibitory receptors on NK cells and will be eliminated by NK cells. However, down-regulation of MHC class I does not always accompany transformation or infection of cells. In such cases the MHC class I-expressing transformed or infected cells may escape from NK cell surveillance. Theoretically, blockade of inhibitory receptor recognition of MHC class I ligands will induce NK cell-mediated killing of such cells. Indeed, antibody-mediated blockade of inhibitory NK cell receptors releases NK cells from inhibition and permits killing of MHC class I-expressing targets (3,4).

Inhibitory NK cell receptors for MHC class I include two structurally different groups of receptors. One group of receptors with extracellular Ig-like domains, prominent in humans, includes killer cell Ig-like receptors (KIR) and CD85 (5–9). Another group of receptors with C-type lectin-like domains with homo- or heterodimeric structure includes mouse Ly49 (3,10,11) and murine and human CD94/NKG2A (12–14). Despite the structural differences, Ly49 and KIR are functional homologues in mice and humans respectively. More than 10 Ly49 receptors, including inhibitory as well as activating receptors, have been identified in C57BL/6 mice (15–18). Ly49A, the primary member of inhibitory Ly49 receptors, recognizes H-2Dd and H-2Dk, and expression of these MHC class I on target cells protects the cells from NK cell-mediated lysis (3). Ly49A recognizes a conformational epitope presented on a large area of the MHC class I molecule that includes all the three structural domains of MHC class I: a1/a2 and a3 domains and b2-microglobulin (19–21).

In the present study, we explored specific small-mol.-wt blockers against the inhibitory receptor Ly49A. We used a
phage display peptide library to successfully isolate and identify peptides that have the capacity to bind Ly49A. The peptides have the ability to inhibit the Ly49A recognition of its MHC class I ligands H-2D\textsuperscript{d} and H-2D\textsuperscript{k}. Most importantly, the peptides enhanced Ly49A\textsuperscript{+} NK cell killing of H-2D\textsuperscript{d}-expressing tumor cells. These results suggest that small-mol.-wt substances that block inhibitory NK cell receptors are effective in enhancing NK cell-mediated lysis of tumor cells that are otherwise resistant to NK cell cytotoxicity because of MHC class I expression.

**Methods**

**Mice**
BALB/c and B10.D2 mice were purchased from Nippon SL (Shizuoka, Japan). C57BL/6J mice were from Nippon CLEA (Tokyo, Japan).

**Cells and reagents**
C1498 cells and A20 cells were obtained from ATCC (Manassas, VA) and Cell Resource Center for Biomedical Research, Tohoku University (Sendai, Miyagi, Japan). The cells were maintained in RPMI 1640 medium (Sigma, St Louis, MO) supplemented with 10% heat-inactivated FCS (Intergen, New York, NY), 25 mM HEPES (Dojin, Kumamoto, Japan), 100 μg/ml of penicillin, 100 μg/ml of streptomycin, 2 mM of glutamine and 50 μM 2-mercaptoethanol. C1498 cells transfected with H-2D\textsuperscript{k} were established as described (22). The cells were maintained in the same medium as above supplemented with 0.5 mg/ml of G418 sulfate (Calbiochem-Novabiochem, San Diego, CA). C1498 cells were transfected with the Ly49A expression vector pApuro-Ly49A (a gift from Dr Wayne M. Yokoyama, Washington University School of Medicine, St Louis, MO). The transfectants were selected and cloned in the presence of 2.5 μg/ml of puromycin (Sigma). C1498 cells were stably transfected with the following H-2D\textsuperscript{k} expression vector and were selected and cloned in the presence of 0.5 mg/ml of G418. To construct the H-2D\textsuperscript{k} expression vector, cDNA encoding H-2D\textsuperscript{k} heavy chain was amplified by RT-PCR using poly(A)+ RNA from C3H/He mouse spleen cells as a template with the following primers:

\[
\text{5'}-\text{AAAGGATCCACCATGGGGGCGATGGTA-3'}
\]

and

\[
\text{5'}-\text{CGTGGATCCATTGGAGAGTCAATGAGGA-3'}
\]

PCR with the primers, 5'-GATCCGCGAATTTTCCGACA-TAGTCAA-3' and 5'-CGTGGATCCATTGGAGAGTCAAT-GAGGA-3', using C57BL/6 LAK cell cDNA as a template. The Ly49G2 cDNA was then cloned into pET3C-nbl vector between SmaI and BamHI sites. BL21 (DE3) *Escherichia coli* was then transformed with the above construct for protein expression. The expressed Ly49G2 protein was purified as inclusion bodies, solubilized and refolded. The refolded protein was purified with combination of cation-exchange column chromatography (Uno-S6; Bio-Rad, Hercules, CA) and gel-filtration column chromatography (Superdex-200; Amersham Biosciences, Uppsala, Sweden), and then biotinylated using BirA enzyme (Avidity, Denver, CO).

The anti-Ly49A mAb A1 (24), the anti-Ly49G2 mAb 4D11 (25), the anti-H-2D\textsuperscript{d} mAb 34-2-12 (26) and the anti-HLA-DR mAb L243 (27) were purified from culture supernatants with Protein A- or Protein G-affinity column chromatography using standard methods.

**Selection of Ly49A-binding phages**
The first round of selection was carried out by incubating 10\textsuperscript{11} p.f.u. of a phage library displaying random heptapeptides flanked by cysteine residues (Ph.D.-C7C; NEB, Beverly, MA) with streptavidin–agarose beads (Oncogene, San Diego, CA) at the molar ratio of 4 : 1. Biotinylated soluble Ly49A was prepared as described previously (23). Ly49A tetramer–horseradish peroxidase (HRP) complex was formed by mixing the biotinylated Ly49A and HRP-conjugated streptavidin (Sigma) at the optimum ratio determined by gel-shift assay. When indicated, alkaline phosphatase-conjugated streptavidin (Sigma) was used instead of HRP-conjugated streptavidin. Ly49A tetramer–phycocerythrin (PE) complex was formed by mixing the biotinylated Ly49A and streptavidin–PE (BD Bioscience, San Jose, CA) at the molar ratio of 4 : 1.

Biotinylated soluble Ly49G2 was prepared as described for Ly49A preparation (23) with minor modifications. cDNA encoding an extracellular domain of Ly49G2 was amplified by PCR with the primers, 5'-GATCCGCGAATTTTCCGACA-TAGTCAA-3’ and 5'-CGTGGATCCATTGGAGAGTCAAT-GAGGA-3’, using C57BL/6 LAK cell cDNA as a template. The Ly49G2 cDNA was then cloned into pET3C-nbl vector between SmaI and BamHI sites. BL21 (DE3) *Escherichia coli* was then transformed with the above construct for protein expression. The expressed Ly49G2 protein was purified as inclusion bodies, solubilized and refolded. The refolded protein was purified with combination of cation-exchange column chromatography (Uno-S6; Bio-Rad, Hercules, CA) and gel-filtration column chromatography (Superdex-200; Amersham Biosciences, Uppsala, Sweden), and then biotinylated using BirA enzyme (Avidity, Denver, CO).

**Purification of phages and DNA sequence determination**
After the three rounds of selection, individual phage plagues were transferred to tubes containing diluted culture of ER2738 *E. coli* cells. After 4-h culture at 37°C with constant shaking, the cultures were centrifuged at 1400 g for 10 min. Aliquots of 1.2 ml of the supernatants were mixed with 100 μl of 20% (w/v) polyethylene glycol-8000 containing 2.5 M NaCl and then incubated at 4°C overnight. The phages were precipitated by centrifugation at 15,000 g for 10 min. The phages were used for preparation of single-strand phage DNA and were also used for the following assays. Sequence of the purified phage
DNA was determined using an ABI 310 sequencer (Applied Biosystems, Foster City, CA).

**Ly49A tetramer binding to phages**

Flat-bottomed 96-well plates (Falcon flexible plate; BD Biosciences, San Jose, CA) were coated with 10 μg/ml of anti-M13 phage mAb (Amersham Biosciences) and then blocked with 5% skim milk in TBS-T. After washing the plates with TBS-T, the purified phages were captured on the plates by incubating at 37°C for 1 h. After washing with TBS-T, Ly49A tetramer–HRP complex was added to the wells and incubated at 37°C for 1 h. After washing 10 times with TBS-T, Ly49A tetramer binding to phages was visualized by a chromogenic substrate for HRP (Fast o-phenylenediamine tablet set; Sigma). When indicated, Ly49A tetramer–alkaline phosphatase complex was used instead of Ly49A tetramer–HRP complex. Ly49G2 binding to phages was tested similarly as described for Ly49A.

**Phage binding to Ly49A expressed on mammalian cells**

C1498 cells stably transfected with Ly49A were incubated with 1011 p.f.u. of the purified phages on ice for 1 h in HBSS containing 0.1% NaN_3 and 0.1% BSA (FACS buffer). After washing the cells with FACS buffer, the cells were incubated with 10 μg/ml of anti-M13 mAb on ice for 20 min, then with 10 μg/ml of FITC–goat anti-mouse IgG F(ab')_2. The cells were washed 3 times with FACS buffer and then fixed by 0.5% (w/v) paraformaldehyde in PBS. The cells were analyzed on FACSCalibur with CellQuest software (BD Biosciences).

**Peptide synthesis and cyclization**

The C1 (ACLFNLPWLCGGK), C11 (ACPFQYLPWCGGK), C26 (ACPFSFLPWCGGK) and control (ACHLPFARLCGGK) peptides were synthesized in solid phase by Fmoc chemistry using a peptide synthesizer 9050 Plus PepSynthesizer (Millipore, Belford, MA), and purified by reversed-phase HPLC on a C18 column. The purified peptides were oxidized in the air for cyclization and the cyclized peptides were purified by reversed-phase HPLC. Quality of the peptides was confirmed by MALDI-TOF mass spectrometry.

**Inhibition of Ly49A tetramer binding to H-2D^d, -D^k-expressing cells by peptides.**

H-2D^d, or H-2D^k-transfected C1498 cells were stained with the indicated concentrations of Ly49A tetramer–phycoerytin (PE) complex that was pre-incubated with the indicated concentrations of each peptide. The cells were washed with FACS buffer twice and then fixed with 0.5% paraformaldehyde in PBS. The cells were analyzed on a FACSCalibur with CellQuest software.

**Cytotoxicity assays**

Standard 51Cr-release assays were performed as previously described (22). Briefly, 10^4 51Cr-labeled target cells were incubated with Ly49A+ or Ly49A- LAK cells for 4 h at E:T ratios of 10:1 for H-2D^d-transfected C1498 cells or 20:1 for A20 cells. When indicated, LAK cells were pre-incubated with indicated mAb or indicated peptides for 30 min on ice before incubation with target cells.

**Results**

**Isolation of Ly49A-binding phages**

To obtain potential Ly49A blockers, we selected Ly49A-binding phages using Ly49A-coated agarose beads from a phage library that displays random heptapeptides flanked by cysteine residues. After three cycles of selection, we cloned 36 phages to test their binding to Ly49A tetramer. Out of the 36 phages tested, 25 phages specifically bound Ly49A tetramer, but did not bind HRP-conjugated streptavidin alone, while a control phage with the capacity to bind recombinant human CD94/NKG2A (unpublished result) did not bind Ly49A tetramer (Fig. 1A).

We next examined whether the Ly49A-binding phages have the capacity to bind Ly49A expressed on mammalian cells by flow cytometry (Fig. 1B). All phages with the ability to bind Ly49A tetramer specifically bound to Ly49A-transfected C1498 cells, but not to untransfected C1498 cells. The control phage bound neither Ly49A-transfected nor untransfected C1498 cells. These results establish that the phages selected by recombinant Ly49A expressed in E. coli have the capacity to bind Ly49A expressed on mammalian cells.

**Binding of synthetic peptides displayed by Ly49A-binding phages to Ly49A tetramer**

The Ly49A-binding phages displayed either one of eight varieties of peptides (Fig. 2). All the Ly49A-binding phages share a Phe on the third position and a Leu-Pro-Trp motif of which the location segregated the phages into two groups: one group has the consensus CXFXLPWLC (Type I) and the other has CXFXLPWLC (Type II). To investigate whether the peptides alone can bind Ly49A, we chemically synthesized C1 (Type I) and C11 (Type II) peptides that were further cyclized by disulfide linkage, and tested their capacity to inhibit Ly49A tetramer binding to the phages. Ly49A tetramer binding to the C1 and C11 phages was completely abrogated by addition of the C1 and C11 peptides (Fig. 3) respectively, while a control peptide with the capacity to bind recombinant human CD94/NKG2A did not affect Ly49A tetramer binding to the phages. These results indicate that the C1 and C11 peptides alone can bind Ly49A in the absence of phage coat proteins. Furthermore, the C1 peptide and the C11 peptide inhibited Ly49A tetramer binding to the C11 phage and the C1 phage respectively, indicating the two peptides compete each other for binding to Ly49A. These results suggest that the Type I and Type II peptides bind similar or, at least, overlapping sites on Ly49A.

**Inhibition of Ly49A binding to H-2D^d and -D^k by synthetic Ly49A-binding peptides**

To explore the possibility that the Ly49A-binding peptides may inhibit Ly49A recognition of its MHC class I ligand H-2D^d, we examined effects of three peptides, C1, C11 and C26, on Ly49A tetramer binding to H-2D^d-transfected cells by flow cytometry (Fig. 4A and B). The three peptides were chemically synthesized and cyclized by oxidation. The three peptides inhibited binding of Ly49A tetramer to H-2D^d. The inhibition was dose-dependent, while the control peptide had no significant effect on Ly49A tetramer binding (Fig. 4B). The inhibition was dependent on the cyclic structure formed by the
disulfide bond, because uncyclized linear versions of the C1, C11 and C26 peptides were unable to inhibit Ly49A tetramer binding to H-2Dd (unpublished data). The C11 and C26 peptides with the Type II consensus inhibited Ly49A binding to H-2Dd more potently than the C1 peptide with the Type I consensus, while the two peptides with the Type II consensus showed similar a dose–response. These results clearly indicate that binding of the Ly49A-binding peptides C1, C11 and C26 to Ly49A inhibits Ly49A recognition of H-2Dd, i.e. the peptides work as ‘blockers’.

To further investigate whether the blockade of Ly49A by the peptides is universal among its MHC class I ligands, we...
examined the effect of the peptides on the interaction between Ly49A and its other ligand H-2Dk. Ly49A tetramer bound to H-2Dk expressed on C1498 cells and the binding was inhibited by 100 μM of C1, C11 and C26 peptides by 90.2, 91.3 and 96.3% respectively (Fig. 4C). These results clearly indicate that the Ly49A-binding peptides can block Ly49A binding to its MHC class I ligands such as H-2Dd and H-2Dk.

Enhancement of NK cell cytotoxicity towards H-2Dd-transfected cells by the Ly49A-binding peptides

To investigate the functional consequences of inhibiting Ly49A recognition of its MHC class I ligand by the Ly49A-binding peptides, we examined the effect of the peptides on NK cell-mediated cytotoxicity (Fig. 5). Ly49A+ LAK cells isolated from B10.D2 mice inefficiently killed H-2Dd-transfected C1498 cells. In agreement with the previous results (3), addition of an anti-Ly49A mAb to the system reversed the Ly49A-mediated inhibition and enhanced killing of the H-2Dd-transfected cells. Most importantly, addition of the Ly49A-binding peptides also reversed the inhibition and enhanced NK cell killing of the tumor cells expressing H-2Dd. The enhancement of killing by the peptides was dose dependent, while the control peptide had no significant effect on NK cell killing of the target cells. Similarly, the Ly49A-binding peptide enhanced killing of a BALB/c B cell lymphoma A20 by B10.D2 NK cells in a dose-dependent manner (Fig. 5B). Similar enhancement of NK cell killing of the H-2Dd-transfected cells by the blocker peptides was also observed when Ly49A+ NK cells from BALB/c mice were used (unpublished data), suggesting that the Ly49A-binding peptides are also able to bind and block the BALB/c allelic form of Ly49A. These results indicate that blockade of Ly49A recognition of its MHC class I ligand by the Ly49A-binding peptides augments NK cell cytotoxicity towards the tumor targets that express the MHC class I ligand H-2Dd.

Cross-reactivity of the Ly49A-binding phage C26 to Ly49G2

Despite the similar capacity of C11 and C26 peptides to inhibit physical interaction between Ly49A and H-2Dd (Fig. 4B), the C26 peptide showed greater enhancement of killing of H-2Dd-expressing tumor cells than the C11 peptide (Fig. 5A). The greater enhancement of killing by the C26 peptide over the C11 peptide might due to the inhibition of other inhibitory receptors of Ly49 family. Notably, 40% of Ly49A+ NK cells co-expressed Ly49G2, an inhibitory receptor that recognizes H-2Dd and has the highest homology to Ly49A among Ly49 family molecules (28,29). Indeed, simultaneous addition of the anti-Ly49A mAb A1 and the anti-Ly49G2 mAb 4D11 enhanced killing of the H-2Dd-transfected cells by Ly49A+ NK cells to a similar extent as 100 μM of the C26 peptide did (Fig. 5). This raises the possibility that the C26 peptide may also bind Ly49G2. We tested binding of phages that display the Ly49A-binding peptides (C1, C11 and C26) to soluble Ly49G2.
tetramers that have the capacity to bind H-2Dd-transfected C1498 cells (unpublished data). The C26 phage bound Ly49G2 tetramer, while the C1 and C11 phages failed to bind Ly49G2 tetramer (Fig. 6). These results suggest that the cross-reactivity of the C26 peptide to Ly49G2 likely enables the peptide to enhance NK cell killing of H-2Dd-transfected target cells more effectively than the C11 peptide, despite the similar dose-response of the two peptides in blocking Ly49A.

**Discussion**

In the present study we sought to enhance NK cell killing of MHC class I-expressing tumor cells by interrupting the interaction between the inhibitory NK cell receptor Ly49A and its MHC class I ligand H-2Dd. A similar concept of activating anti-tumor immunity by blocking inhibitory immune cell receptors has also been demonstrated for CTLA-4 on T cells (30). Blocking receptor–ligand interactions may be achieved by substances that bind either one of the receptor or the ligand. We have chosen the receptor Ly49A rather than the ligand MHC class I as a target because substances that bind MHC class I may modify it to induce a cytotoxic T cell response towards the modified MHC class I that may damage normal tissues. The highly polymorphic nature of MHC class I also hinders the idea of choosing MHC class I as a target. Selection of the phages with the capacity to bind the recombinant soluble form of Ly49A from a phage display random peptide library allowed us to discover Ly49A-binding peptides. The Ly49A-binding peptides not only bound Ly49A, but also inhibited Ly49A binding to its ligands H-2Dd and H-2Dk. Most importantly, the peptides released NK cells from MHC class I-dependent inhibition and enhanced killing of H-2Dd-expressing tumor cells by Ly49A + NK cells in an *in vitro* system. Several groups have shown that antibody-mediated blockade of inhibitory NK cell receptors for MHC class I enhances NK cell killing of tumor cells *in vitro* (3,28,31). Koh et al. (31) further demonstrated that the strategy is also effective *in vivo*, using F(ab') 2 fragments from an antibody against the mouse inhibitory NK cell receptors Ly49C and Ly49I. Our data indicate that small-mol.-wt substances such as peptides are also effective in blocking inhibitory receptors to enhance NK cell-mediated tumor cell lysis *in vitro*. Collectively, these studies (including our current study) imply the potential of small-mol.-wt blockers of inhibitory NK cell receptors for application to anti-cancer medicine, even though their effectiveness *in vivo* remains to be examined. Our results also encourage the idea to develop non-peptide small-mol.-wt blockers of inhibitory NK cell receptors, which might be favored for therapeutic purposes in terms of *in vivo* stability.

Another possible consequence of blocking inhibitory receptors on NK cells is induction of NK cell-mediated killing...
of normal cells, which may lead to autoimmune responses and undesired damage to normal tissues, especially myelosuppression, which is also a major side-effect associated with chemotherapy and radiation therapy. It is of note that blockade of Ly49C and Ly49I by F(ab)2 fragments of anti-Ly49C/I mAb does not significantly impair hematopoiesis as reported by Koh et al. (31), even though the possible side-effects of the small-mol.-wt inhibitory receptor blockers need to be tested carefully in in vivo studies. Moreover, the outcome of NK cell recognition of target cells is decided by a balance of opposing signals from activating receptors and inhibitory receptors. Concomitant up-regulation of activating ligands in tumor cells together with blockade of inhibitory receptors on NK cells would render tumor cells more susceptible to NK cell-mediated lysis. Induced expression of ligands for the activating receptor NKG2D in transformed cells and in cells under stress has been reported in human (32) and mouse (33,34).

The Ly49A-binding peptides discovered in this study were classified into two types, Type I and II, distinguished by the locations of the LPW consensus. Cross-inhibition between the two types of Ly49A-binding peptides suggests that they most likely bind similar or at least overlapping sites on Ly49A. Binding of the Ly49A-binding peptides to Ly49A inhibited Ly49A recognition of its MHC class I ligand H-2Dd. However, whether these peptides inhibited the ligand binding simply by competition or in a non-competitive fashion remains to be elucidated. Moreover, there is no apparent similarity between the Ly49A-binding peptides and H-2Dd, at least at the level of primary amino acid sequence, even though it does not exclude the possibility that there is a significant similarity among them at higher-order structure. Structural studies on a Ly49A–Ly49A-binding peptide complex will help to answer these questions and also provide data useful to design non-peptide Ly49A blockers.

One of the Ly49A blockers, peptide C26, showed a larger capacity to enhance NK cell killing of H-2Dd-expressing target cells than the C1 and C11 peptides. This observation apparently correlates with our other finding that solely C26 phages bound Ly49G2 tetramer among the three phages C1, C11 and C26. These results suggest that a blocker with the capacity to bind a wider range of inhibitory receptors would have a larger capacity to reverse MHC class I-mediated inhibition of killing. In this context, the blockers capable of binding larger numbers of inhibitory Ly49 receptors would have better ability to enhance NK cell killing of tumor cells. In contrast, blockade of the activating receptors of the Ly49 family would rather deteriorate NK cell killing of tumor cells. In a preliminary study, we found that the eight varieties of Ly49A-binding phages did not bind cells expressing the activating Ly49 receptors Ly49D or Ly49H (unpublished results).

Expression of inhibitory MHC class I receptors is not limited to NK cells. Subsets of CD8+ T cells and NKT cells, both of which are important effectors against tumor cells and infected cells, also express the inhibitory NK cell receptors: Ly49, CD94/NKG2A and KIR. Melanoma antigen-specific CD8+ T cells express functional inhibitory receptors, such as KIR and CD94/NKG2A, and their potential role in the tumor-specific immune response has been suggested (35–41). Similarly, Ly49 recognition of MHC class I ligands by NKT cells inhibits activation of NKT cells by α-galactosylceramide-primed dendritic cells (42). Therefore, inhibitory NK cell receptor blockers may enhance tumor cell killing by CD8+ T and NKT cells that express inhibitory NK cell receptors. Expression of the inhibitory NK cell receptors on cytotoxic T cells also extends the potential of the inhibitory NK cell receptor blockers to the treatment of viral infection. Recently, Moser et al. (43) demonstrated that CD94/NKG2A expression on polyomavirus-specific CD8+ T cells correlates with genetically defined susceptibilities of C3H/HeN and CBA/J mice to polyomavirus replication and tumor formation. In CBA/J mice, polyomavirus-specific T cells express CD94/NKG2A and are cytotically inactive. The cytotoxicity of those T cells against polyomavirus-infected cells can be restored by interrupting the interaction between CD94/NKG2A and its ligand Qa-1. Similarly, expression of CD94/NKG2A and the inhibitory NK cell receptor KIR2DL3 on HIV-specific CD8+ T cells impairs cytotoxicity against HIV-epitope-coated targets (44). These notions corroborate the possible application of inhibitory NK cell receptor blockers to anti-viral therapy.

In conclusion, our data indicate that the small-mol.-wt substances are effective in blocking recognition of MHC class I ligand by an inhibitory NK cell receptor to enhance in vitro lysis of tumor cells that are otherwise resistant to NK cell lysis because of the MHC class I expression. Our findings together with the previous study showing the effectiveness of blocking inhibitory NK cell receptors in augmenting anti-tumor effects (31) suggest that the strategy of blocking inhibitory NK cell receptors with small-mol.-wt substances may be useful to eradicate tumor or infected cells.

Acknowledgements

We thank Dr Wayne M. Yokoyama for a reagent and helpful comments on the manuscript, and Dr Hajime Karasuyama for a reagent. This study was supported in part by a grant for Research on Health Sciences focusing on Drug Innovation from the Japan Health Science Foundation (11007) (K. Y. and K. S), Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (12672107 and 15590057) (N. M.), a research grant from the Kato Memorial Bioscience Foundation (N. M.), and the Sasakawa Scientific Research Grant from the Japan Science Society (K. T.).

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>KIR</td>
<td>killer cell Ig-like receptors</td>
</tr>
<tr>
<td>LAK</td>
<td>IL-2-activated NK</td>
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<tr>
<td>PE</td>
<td>phycoerythrin</td>
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<td>TBS-T</td>
<td>Tris-buffered saline with Tween 20</td>
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