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NKG2D and CD94 bind to multimeric α2,3-linked N-acetylneuraminic acid

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ABSTRACT

Killer lectin-like receptors on natural killer cells mediate cytotoxicity through glycans on target cells including the sialyl Lewis X antigen (sLeX). We investigated whether NK group 2D (NKG2D) and CD94 can bind to sialylated N-linked glycans, using recombinant glutathione *S*-transferase-fused extracellular lectin-like domains of NKG2D (rNKG2Dlec) and CD94 (rCD94lec). Both rNKG2Dlec and rCD94lec bound to plates coated with high-sLeX-expressing transferrin secreted by HepG2 cells (HepTF). The binding of rNKG2Dlec and rCD94lec to HepTF was markedly suppressed by treatment of HepTF with neuraminidase and in the presence of N-acetylneuraminic acid. Moreover, rNKG2Dlec and rCD94lec bound to α2,3-sialylated human α_1-acid glycoprotein (AGP) but not to α2,6-sialylated AGP. Mutagenesis revealed that ^{152}Y of NKG2D and ^{144}F and ^{160}N of CD94 were critical for HepTF binding. This is the first report that NKG2D and CD94 bind to α2,3-sialylated but not to α2,6-sialylated multi-antennary *N*-glycans.

Introduction

Natural killer (NK) cells are large granular lymphocytes that play important roles in innate immunity. The activity of NK cells is regulated by a balance of opposing signals through activating and inhibiting cell-surface receptors of the immunoglobulin and C-type lectin superfamilies [1]. Inhibitory NK cell receptors predominantly recognize major histocompatibility complex (MHC) class I proteins, allowing healthy cells to escape NK cell attack, a phenomenon known as the 'missing self' hypothesis.

CD94 forms disulfide-linked heterodimers with NK group 2 (NKG2) A, B, C, E, or H, and is expressed on most NK cells and on subsets of $CD8^+$ T cells. The ligand for CD94/NKG2A, B and C is leukocyte antigen (HLA)-E, which is constitutively expressed on human cells and Qa-1b in mice. NKG2A and B have two immunoreceptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic domains and are inhibitory receptors, while NKG2C, E and H are activating receptors, having a positively charged residue within their transmembrane region that associates with the immunoreceptor tyrosine-based activating motif (ITAM) containing the adaptor molecule DNAX-activating protein of 12 kDa (DAP12) [2–5]. The activating receptor NKG2D is a homodimer and forms a salt-bridged hexamer with two homodimers of the YINM motif-containing adaptor molecule DAP-10 in humans [6]. NKG2D is expressed on all NK cells, T cell receptor (TCR)-γδ T cells, and TCR-αβ $CD8^+$ T cells [7] and recognizes several major histocompatibility complex (MHC) class I related ligands, including MHC class I related chain family proteins (MIC) A and B [8] and the UL 16-binding proteins (ULBP) 1–4 [9] in humans; as well as H60 [10], the retinoic acid early inducible (RAE-1) [11] and murine ULBP-like transcript 1 (MULT-1) [12] in mice. Although these ligands are usually absent from or expressed at low levels by normal adult tissues, they are induced on heat-shock-stressed, microbial-infected, or tumor-transformed cells. NKG2D is involved in antiviral and anti-tumor immunity.

These C-type lectin-like receptors lack most of the conserved Ca^{2+}-binding residues [13,14], and the glycan ligands for NKG2D and CD94/NKG2s have yet to be resolved. Of the C-type lectin-like receptors on NK cells, several have been reported to recognize glycans: dectin-1 binds to fungal β-glucan [15,16], mouse Ly-49A to fucoidan [17–19], rat natural killer cell receptor-protein 1 (NKR-P1) (CD161) to galactosyl ceramide [20,21] and osteoclast inhibitory lectin (OCIL, lectin-like transcript 1: LLT1) to fucoidan, λ-carrageenan and dextran sulfate [22].

The sialyl Lewis X (sLeX) antigen, NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc-R, expressed on leukocytes, and its interaction with E-selectin on vascular endothelial cells, trigger the extravasation of leukocytes into inflammatory sites [23,24]. Selectin-mediated adhesion of cancer cells to vascular endothelial cells is also involved in cancer metastasis, and the presence of cancer cells expressing sLeX and sLeA is correlated with poor prognosis [25,26]. Conversely, CD94 has also been reported to bind to overexpressed sLeX in shorter *N*-glycans on mouse melanoma B16-FI cells transfected with the fucosyltransferase (*FUT*)-3 gene [27,28]; this can be inhibited by pre-incubation with anti-CD94 and anti-sLeX.

In a previous report [29], we indicated that high-sLeX-expressing K562 cells were more susceptible to lysis by NK-derived KHYG

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cells, and that this susceptibility was suppressed by treatment with anti-sLeX antibody. Here, we prepared recombinant NKG2D and CD94 and their mutants and estimated their binding to plates coated with multivalent sLeX-expressing HepG2-derived transferrin (HepTF) [30], commercially available human transferrin (NorTF), human α_1-acid glycoprotein (AGP) and their glycan-remodeled preparations, revealing that NKG2D and CD94 can bind to α2,3-linked NeuAc on multi-antennary complex-type *N*-glycans.

Materials and methods

Cells and cell culture. Human NK-derived KHYG cells and human hepatoma-derived HepG2 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (Tokyo, Japan). KHYG cells and HepG2 cells were cultured in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) and Dulbecco's modified Eagle's medium (DMEM) (Nissui), respectively, supplemented with 10% heat-inactivated fetal bovine serum (JRH Biosciences, Lenexa, KS) and 0.6 mg/ml L-glutamate (Wako Pure Chemicals Co., Osaka, Japan) in a humidified atmosphere containing 5% CO_2 at 37 °C. In the case of KHYG cells, recombinant interleukin-2 (Shionogi Pharmaceutical Co., Osaka, Japan) was also added to the medium at a final concentration of 100 U/ml.

Preparation of rGST-fused NKG2Dlec and CD94lec. The extracellular domains of NKG2D (NKG2lec, AA 73–216) and CD94 (CD94lec, AA 68–179) were amplified from KHYG cDNA using primers 5′-CACCAT ATGGAGTGCTGTATTCCTAAAC-3′ (forward) and 5′-TTACA CAGTCC TTTGCATGCA-3′ (reverse) for NKG2Dlec and 5′-CACCTACC GGTGCAACTGTTACTT-3′ (forward) and 5′-TTAAATGAGCTGTTG CTTACAG-3′ (reverse) for CD94lec, respectively. PCR conditions were 94 °C for 2 min, 30 cycles of 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 90 s, followed by 68 °C for 5 min. The purified PCR products were ligated into pGEX4T-1 vector (GE Healthcare Bio-Science, Uppsala, Sweden) with Ligation High (Toyobo, Tokyo, Japan).

The recombinant plasmids were transformed into chaperone competent cells pTf16/BL21 (Takara Bio., Otsu, Japan) and positive clones were confirmed by DNA sequencing using a 3730xl DNA analyzer (Applied Biosystems, Foster, CA). The recombinant proteins were induced by 1 mM isopropyl-β-D-thiogalactopyranoside (Promega Co., Madison, WI) at 20 °C overnight. Cells from 1000 ml culture medium were sonicated for 6 s × 6 on ice and the lysates were applied to a GSTrap FF column (GE Healthcare) according to the manufacturer's instructions.

Purification of transferrin from HepG2 culture medium. Transferrin (TF) secreted by human hepatoma-derived HepG2 cells (HepTF) was purified according to a published method [29,30]. Briefly, HepG2 cells were cultured in IS-RPMI medium under a humidified atmosphere containing 5% CO_2 at 37 °C for 1 week. TF was purified from the culture medium by ultrafiltration and affinity chromatography on an anti-human transferrin mouse antibody (SOM4D10, Diatec Co., Oslo, Norway) conjugated column.

Preparation of glycoprotein-coated plates. Glycoproteins, including NorTF (Nacalai Tesque, Kyoto, Japan), HepTF and human AGP (Sigma–Aldrich, Chicago, IL), were coated onto 96-well plates. Each glycoprotein (100 μl; 50 μg/ml) was placed on the plates at 37 °C overnight, and then the plates were blocked with 350 μl of 0.1% bovine serum albumin/phosphate-buffered saline for 2 h at 37 °C. HepTF-coated plates were further treated with 1 U/ml α2,3/6/8 neuraminidase (Seikagaku Co., Tokyo, Japan) in 100 mM MES (pH 6.0) for 2 days at 37 °C. Desialylated-HepTF was blotted with 1 μg/ml CSLEX-1 and anti-Lewis X (73–30) (Seikagaku) followed by 1 μg/ml peroxidase (POD)-conjugated anti-mouse IgG + IgM rabbit antibody (Jackson ImmunoResearch Lab., West Grove, PA).

Binding of rGST-fused NKG2Dlec and CD94lec to glycoprotein-coated plates. Recombinant glutathione *S*-transferase (GST)-fused NKG2Dlec (rNKG2Dlec), rCD94lec, or rGST (100 μl; 0–400 μg/ml) in 20 mM Tris–HCl buffer (pH 7.4) containing 10 mM $CaCl_2$, 0.1% bovine serum albumin, 150 mM NaCl and 0.3% Tween (TBS-T) was incubated on the glycoprotein-coated plates for 1 h at 37 °C. After washing three times with TBS-T, the plates were further incubated with 100 μl of 1 μg/ml POD-conjugated anti-GST antibody (Rockland Immunochemicals, Inc., Gilbertsville, PA) in TBS-T for 1 h at room temperature. After washing with TBS-T, the plates were incubated with 100 μl tetramethylbenzidine (TMB)-1 solution (BioFX Lab., Owings Mills, MD) for 5 min at room temperature. After adding 100 μl of 1 M H_2SO_4, the absorbance was determined at 450 nm using a Model DTX800 plate reader (Beckman Coulter, Fullerton, CA).

For competition of monosaccharides with rNKG2Dlec and rCD94lec binding to HepTF, 0, 10 and 100 mM *N*-acetylneuraminic acid (NeuAc), *N*-acetylglucosamine (GlcNAc), galactose (Gal), fucose (Fuc) and mannose (Man) were added to 10 μg/ml rNKG2Dlec and rCD94lec solutions and their binding to the HepTF-coated plates was determined as described above.

Preparation of plates coated with desialylated-, α2,3-sialylated and α2,6-sialylated glycoproteins. NorTF and human AGP (each 1 mg) were treated with 1 U/ml α2,3/6/8 neuraminidase in 100 mM MES (pH 6.0) for 2 days at 37 °C; 100 μl of 50 μg/ml desialylated NorTF and AGP were immobilized on the plates as described above. The desialylated glycoprotein-coated plates were further treated for 16 h at 37 °C with 100 μl/well of 2.5 μU/μl α2,3-*N*-sialyltransferase (Boehringer, Ingelheim, Germany) or α2,6-sialyltransferase (Calbiochem Co., La Jolla, CA) in 100 mmol MES (pH 7.0) containing 10 mM $MnCl_2$ and 250 μM CMP-NeuAc.

For estimation of de- and re-sialylation, glycoprotein-coated plates were treated with 100 μl of 10 μg/ml biotin-conjugated *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* agglutinin (MAA) (Boehringer), α2,6- and α2,3-linked NeuAc binding lectins, respectively, followed by 100 μl of 1 μg/ml POD-conjugated ExtrAvidine (Sigma–Aldrich). The POD activity was determined as described above.

Mutation of rNKG2Dlec and rCD94lec. One-amino-acid mutated constructs of NKG2D (C203G, Y199A and Y152A) and CD94 (C166G, F114A, and N160A) were prepared using the KOD-plus-Mutagenesis kit (Toyobo) using pGEX4T-1/NKG2Dlec and pGEX4T-1/CD94lec as the templates, respectively, according to the manufacturer's instructions. The primers used for mutant vectors were as follows: NKG2D C203G, 5′-AACGGTTCAACTCCAAATACG-3′ (forward) and 5′-TTCTATATACGGTTTAAAGCTCG-3′ (reverse); NKG2D Y199A, 5′-AGGCGCTATAGAAAACTGTTCAACT-3′ (forward) and 5′-TTAAAG CTCGAGGCATAGAGTG-3′ (reverse); NKG2D Y152A, 5′-TCAGCTCA TTGGATGGGACTAGTA-3′ (forward) and 5′-CTTCACCAGTTTAAGT AAATCCT-3′ (reverse); CD94 C166G, 5′-GGTGAAGATAAAAATCGTT ATATCTGTA-3′ (forward) and 5′-GGATTCATCTAAAGCATTTCCA-3′ (reverse); CD94 F114A, 5′-GCTTACTGGATTGGACTCTCTTACA-3′ (forward) and 5′-TTGTTGACTGGAGCTCATAAAATC-3′ (reverse); CD94 N160A, 5′-GCTGCTTTAGATGAATCCTGTGA-3′ (forward) and 5′-GTTCTTTGTATTAAAAGTTTCAAATG-3′ (reverse).

Results and discussion

Binding of rNKG2Dlec and rCD94lec to transferrin-coated plates

To confirm direct binding of NKG2D and CD94 to glycans, we constructed GST-fused extracellular domains of NKG2D (AA 73–216: rNKG2Dlec) and CD94 (AA 68–179: rCD94lec). HepTF- and NorTF-coated plates were incubated with rNKG2Dlec and rCD94lec to determine their binding. rNKG2Dlec and rCD94lec bound dose-dependently to the HepTF-coated plates, but hardly at all to the NorTF-coated plates (Fig. 1). We have reported previously that HepTF contains bi-, tri- and tetra-antennary N-glycans with α2,3-

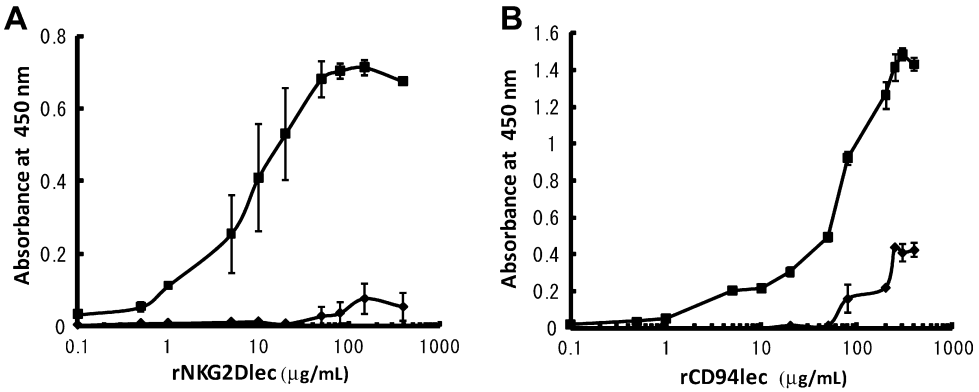

Fig. 1. Binding of rNKG2Dlec and rCD94lec to HepTF. rNKG2Dlec (A) and rCD94lec (B) (0–400 μg/ml) were incubated on NorTF- (closed diamonds) and HepTF-coated plates (closed squares) for 2 h at 37 °C followed by 1 μg/ml POD-conjugated anti-GST for 1 h at room temperature. POD activities were determined using TMB-1 reagent. Nonspecific-GST binding was subtracted from the data. The results are shown as mean ± SD ($n = 3$).

linked NeuAc and α1,3-linked Fuc (sLeX) [30], while *N*-glycans of commercially available human TF (NorTF) are predominantly bi-antennary with α2,6-linked NeuAc and without α1,3-linked Fuc. The results indicate that rNKG2Dlec and rCD94lec can recognize sLeX, α2,3-linked NeuAc and/or α1,3-linked Fuc.

rNKG2Dlec and rCD94lec recognize NeuAc on HepTF

To clarify whether rNKG2Dlec and rCD94lec recognize the sLeX moiety or the α2,3-linked NeuAc residues, we estimated the binding of rNKG2Dlec and rCD94lec to de-sialylated HepTF. After exhaustive treatment with α2,3/6/8 neuraminidase, disappearance and appearance of sLeX and LeX on HepTF was confirmed using CELEX-1 and anti-LeX (data not shown). The binding of both rNKG2Dlec and rCD94lec to the HepTF-coated plates was markedly suppressed by treatment with neuraminidase (Fig. 2A).

We further estimated their binding to HepTF in the presence of excess monosaccharides including NeuAc, Fuc, GlcNAc, Gal, and Man (Fig. 2B,C). Binding of both rNKG2Dlec and rCD94lec to the HepTF-coated plates was markedly suppressed in the presence of 10 and 100 mM NeuAc. The other monosaccharides, Fuc, Gal, GlcNAc, and Man, did not significantly suppress rNKG2Dlec binding to HepTF, while these monosaccharides tended to suppress rCD94lec binding to HepTF at high concentrations. These results indicate that rNKG2Dlec and rCD94lec recognize mainly the NeuAc residues but not α1,3-linked Fuc on HepTF; the tetra-saccharide sLeX moiety is not essential for their recognition.

Multimeric α2,3-NeuAc is critical for rNKG2Dlec and rCD94lec binding

To clarify the glycan structures for NKG2D and CD94 recognition, bi- to tetra-antennary and α2,3- or α2,6-linked NeuAc, we used NorTF and human AGP. Commercially available human TF contains two bi-antennary N-glycans and one *O*-glycan with α2,6-linked NeuAc at the non-reducing ends [31], while human AGP has five N-glycans that are bi-, tri-, and tetra-antennary with predominantly α2,6-linked NeuAc [32,33].

NorTF and AGP, de-sialylated with α2,3/6/8 neuraminidase and coated on the plates, were re-sialylated using α2,3-*N*- or α2,6-sialyltransferase. De- and re-sialylation was confirmed using the α2,6-NeuAc and α2,3-NeuAc binding lectins SNA and MAA, respectively (data not shown), then the binding of rNKG2Dlec and rCD94lec to these glycoprotein-coated plates was determined (Fig. 3). rNKG2Dlec and rCD94lec did not bind to NorTF- and AGP-coated

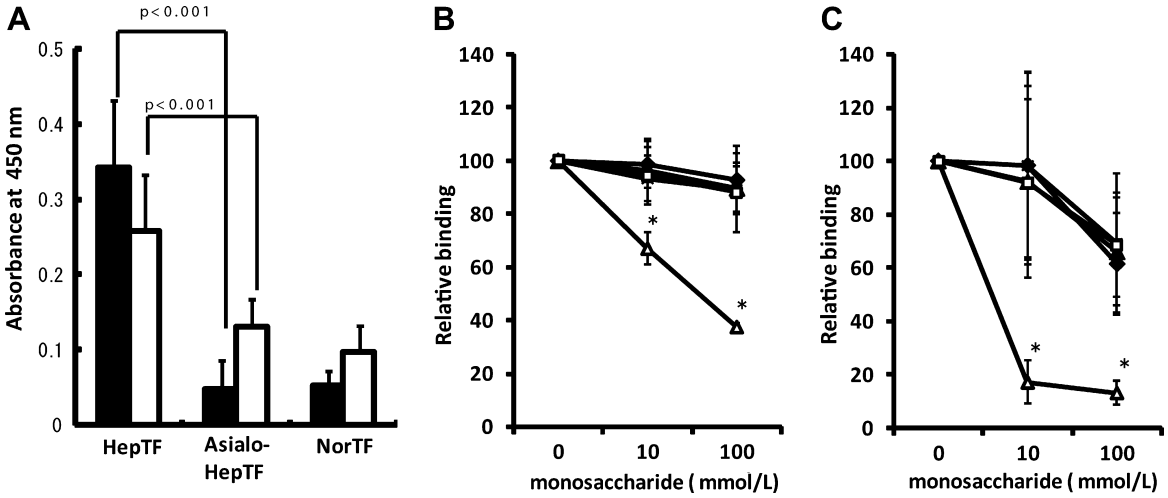

Fig. 2. rNKG2Dlec and rCD94lec binding to neuraminidase-treated HepTF and competition with monosaccharides. (A) The binding of rNKG2Dlec (closed squares) and rCD94lec (open squares) to HepTF-coated plates treated with α2,3/6/8 neuraminidase was determined as described in Fig. 1. (B, C) The binding of 10 μg/ml rNKG2Dlec (B) or rCD94lec (C) was determined as described in Fig. 1, in the presence of 0, 10, and 100 mM monosaccharides: galactose (closed diamonds), *N*-acetylglucosamine (closed squares), fucose (closed triangles), *N*-acetylneuraminic acid (open triangles), mannose (open squares). The binding was normalized to rNKG2Dlec or rCD94lec binding to HepTF as 100. The results are shown as means ± SD ($n = 3$) and asterisks show statistically significant differences ($P < 0.05$).

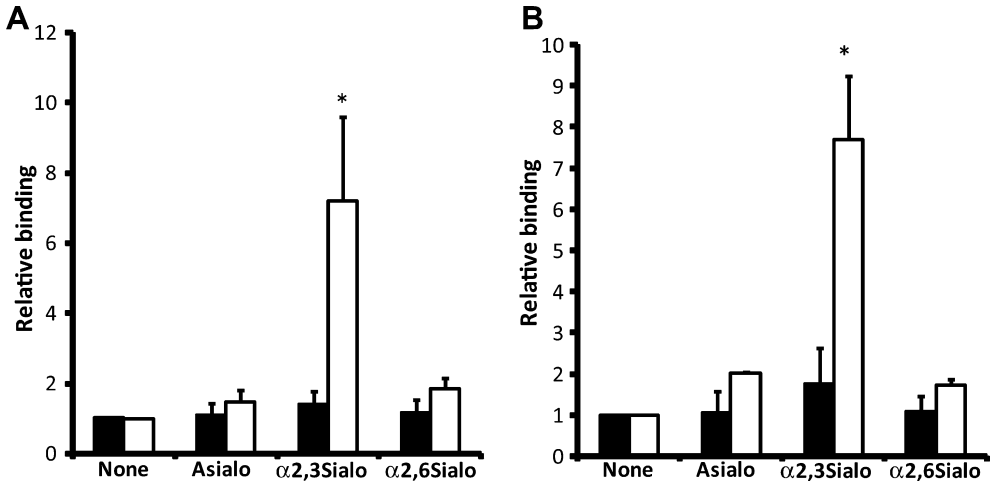

Fig. 3. Binding of rNKG2Dlec and rCD94lec to desialylated, α2,3-sialylated and α2,6-sialylated glycoproteins. NorTF and AGP treated with α2,3/6/8 neuraminidase and coated on the plates were sialylated with α2,3-*N*- or α2,6-sialyltransferase. The binding of rNKG2Dlec (A) or rCD94lec (B) to NorTF (closed squares), AGP (open squares) and their desialylated, α2,3-sialylated and α2,6-sialylated preparations was determined as described in Fig. 1. The binding was normalized to their binding to intact NorTF and AGP, respectively. The results are shown as means ± SD ($n = 3$). Asterisks indicate significant differences compared with binding to non-treated NorTF and AGP ($P < 0.05$). Asialo: glycoproteins treated with neuraminidase, α2,3Sialo and α2,6Sialo: glycoproteins treated with neuraminidase followed by treatment with 2,3-*N*- and 2,6-sialyltransferases, respectively.

plates or asialo NorTF- and AGP-coated plates. However, rNKG2Dlec and rCD94lec could bind significantly to the α2,3-sialylated AGP-coated plates and showed a low level of binding to the α2,3-sialylated TF-coated plates, although they did not bind again to the α2,6-resialylated NorTF- and AGP-coated plates. These results indicate that rNKG2Dlec and rCD94lec recognize α2,3-NeuAc on tri- and tetra-antennary N-glycans of AGP but not on the bi-antennary *N*-glycan of NorTF; they could not recognize α2,6-NeuAc on bi- to tetra-antennary N-glycans.

Mutagenesis study for rNKG2Dlec and rCD94lec binding to HepTF

It has been reported that ^{152}Y, ^{185}Q, ^{197}K, ^{199}Y and ^{201}E on NKG2D are important for its recognition of MICA/B and ULBP1-4 [13,34–36]. Moreover, of these amino acids, ^{152}Y and ^{199}Y on NKG2D are homologous to the NeuAc recognition domain of E-selectin [37] and also to the glycan-recognition domain of dectin-1 [15,17]. ^{203}C in NKG2D is essential for the formation of disulfide links between β-sheets [14]. In CD94, ^{112}Q, ^{114}F, ^{160}N, ^{162}L, and ^{163}D are important for its recognition of HLA-E [38,39]. To uncover the glycan-binding domains in NKG2D and CD94, we prepared NKG2D mutants (Y152A, Y199A, and C203G) and CD94 mutants (C166G, F114A, and N160A), and estimated the binding of these mutants to the HepTF-coated plates (Fig. 4).

The binding of rNKG2Dlec Y152A, but not rNKG2Dlec Y199A, to the HepTF-coated plates was significantly lower than that of wild-type rNKG2Dlec. rCD94lec binding was significantly reduced by mutagenesis of F114A and N160A. Mutations of C203G of NKG2D and C166G of CD94 did not affect binding to the HepTF-coated plates. These results indicate that hydrophobic and/or hydrogen bonding interactions are critical for binding between these molecules and HepTF.

In a previous report [29], we clarified that more high sLeX-expressing K562 cells were lysed than non-expressing cells. Furthermore, KHYG cells were stimulated by plates coated with HepTF, resulting in induced tyrosine phosphorylation of a 17-kDa protein. Therefore, the interaction between NKG2D and their glycan ligands must have a functional role in NK signaling and cytotoxicity.

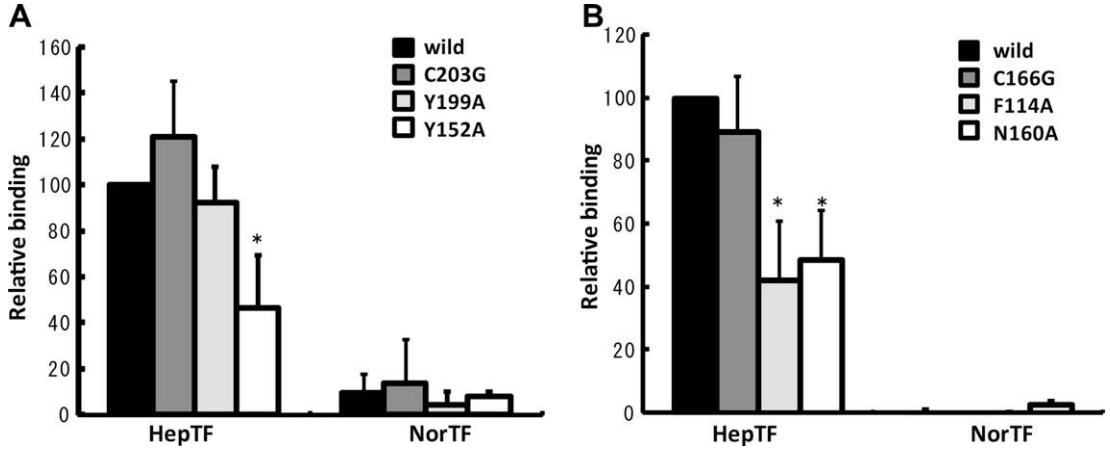

Fig. 4. Mutagenesis of rNKG2Dlec and rCD94lec. The binding of the NKG2D mutants Y152A, Y199A and C203G (A), and the CD94 mutants C166G, F114A, and N160A (B) to the HepTF-coated plates was determined as described in Fig. 1. The results are shown as means ± SD ($n = 3$). Asterisks indicate significant differences compared with the binding of their respective wild-types ($P < 0.05$).

In this paper, we resolved the glycan ligands of NKG2D and CD94 for the first time. Further study is necessary to elucidate the physiological glycan ligands of killer lectin receptors and their roles in innate immunity to cancer progression and metastasis.

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References

[1] L.L. Lanier, NK cell recognition, Annu. Rev. Immunol. 23 (2002) 225–274.
[2] F. Borrego, M. Masilamani, A.I. Marusina, X. Tang, J.E. Coligan, The CD94/NKG2 family of receptors: from molecules and cells to clinical relevance, Immunol. Res. 35 (2006) 263–278.
[3] H.L. Hoare, L.C. Sullivan, C.S. Clements, L.K. Ely, T. Beddoe, K.N. Henderson, J. Lin, H.H. Reid, A.G. Brooks, J. Rossjohn, Subtle changes in peptide conformation profoundly affect recognition of the non-classical MHC class I molecule HLA-E by the CD94-NKG2 natural killer cell receptors, J. Mol. Biol. 337 (2008) 1297–1303.
[4] H. Wada, N. Matsumoto, K. Maenaka, K. Suzuki, K. Yamamoto, The inhibitory NK cell receptor CD94/NKG2A and the activating receptor CD94/NKG2C bind the top of HLA-E through mostly shared but partly distinct sets of HLA-E residues, Eur. J. Immunol. 34 (2004) 81–90.
[5] L. Deng, R.A. Mariuzza, Structural basis for recognition of MHC and MHC-like ligands by natural killer cell receptors, Semin. Immunol. 18 (2006) 159–166.
[6] D. Garrity, M.E. Call, J. Feng, K.W. Wucherpfennig, The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure, Proc. Natl. Acad. Sci. USA 102 (2005) 7641–7646.
[7] K. Ogasawara, L.L. Lanier, NKG2D in NK and T cell-mediated immunity, J. Clin. Immunol. 25 (2005) 534–540.
[8] S. Bauer, V. Groh, J. Wu, A. Steinle, J.H. Phillips, L.L. Lanier, T. Spies, Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA, Science 285 (1999) 727–729.
[9] D. Cosman, J. Müllberg, C.L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin, N.J. Chalupny, ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor, Immunity 14 (2001) 123–133.
[10] A. Diefenbach, A.M. Jamieson, S.D. Liu, N. Shastri, D.H. Raulet, Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages, Nat. Immunol. 1 (2000) 119–126.
[11] A. Cerwenka, A.B. Bakker, T. McClanahan, J. Wagner, J. Wu, J.H. Phillips, L.L. Lanier, Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice, Immunity 12 (2000) 721–727.
[12] L.N. Carayannopoulos, O.V. Naidenko, D.H. Fremont, W.M. Yokoyama, Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D, J. Immunol. 169 (2002) 4079–4083.
[13] R.K. Strong, B.J. McFarland, NKG2D and related immunoreceptors, Adv. Protein Chem. 68 (2004) 281–312.
[14] A.N. Zelensky, J.E. Gready, The C-type lectin-like domain superfamily, FEBS J. 272 (2005) 6179–6217.
[15] J. Brown, C.A. O'Callaghan, A.S. Marshall, R.J. Gilbert, C. Siebold, S. Gordon, G.D. Brown, E.Y. Jones, Structure of the fungal beta-glucan-binding immune receptor dectin-1: implications for function, Protein Sci. 16 (2007) 1042–1052.
[16] K.M. Dennehy, G.D. Brown, The role of the beta-glucan receptor dectin-1 in control of fungal infection, J. Leukoc. Biol. 82 (2007) 253–258.
[17] Y. Adachi, T. Ishii, Y. Ikeda, A. Hoshino, H. Tamura, J. Aketagawa, S. Tanaka, N. Ohno, Characterization of beta-glucan recognition site on C-type lectin, dectin 1, Infect. Immun. 72 (2004) 4159–4171.
[18] B.F. Daniels, M.C. Nakamura, S.D. Rosen, W.M. Yokoyama, W.M. Seaman, Ly-49A, a receptor for H-2Dd, has a functional carbohydrate recognition domain, Immunity 1 (1994) 785–792.
[19] N. Matsumoto, R.K. Ribaudo, J.P. Abastado, D.H. Margulies, W.M. Yokoyama, The lectin-like NK cell receptor Ly-49A recognizes a carbohydrate-independent epitope on its MHC class I ligand, Immunity 8 (1998) 245–254.
[20] M.J. Loza, L.S. Metelitsa, B. Perussia, NKT and T cells: coordinate regulation of NK-like phenotype and cytokine production, Eur. J. Immunol. 32 (2002) 3453–3462.
[21] D. Christiansen, E. Mouhtouris, J. Milland, A. Zingoni, A. Santoni, M.S. Sandrin, Recognition of a carbohydrate xenoepitope by human NKRP1A (CD161), Xenotransplantation 13 (2006) 440–446.
[22] C.T. Gange, J.M. Quinn, H. Zhou, V. Kartsogiannis, M.T. Gillespie, K.W. Ng, Characterization of sugar binding by osteoclast inhibitory lectin, J. Biol. Chem. 279 (2004) 29043–29049.
[23] J.B. Lowe, Glycan-dependent leukocyte adhesion and recruitment in inflammation, Curr. Opin. Cell Biol. 15 (2003) 531–538.
[24] D. Vestweber, J.E. Blanks, Mechanisms that regulate the function of the selectins and their ligands, Physiol. Rev. 79 (1999) 181–213.
[25] J.L. Magnani, The discovery, biology, and drug development of sialyl Lea and sialyl Lex, Arch. Biochem. Biophys. 426 (2004) 122–131.
[26] R. Kannagi, M. Izawa, T. Koike, K. Miyazaki, N. Kimura, Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis, Cancer Sci. 95 (2004) 377–384.
[27] C. Ohyama, S. Tsuboi, M. Fukuda, Dual roles of sialyl Lewis X oligosaccharides in tumor metastasis and rejection by natural killer cells, EMBO J. 18 (1999) 1516–1525.
[28] C. Ohyama, S. Kanto, K. Kato, O. Nakano, Y. Arai, T. Kato, S. Chen, M.N. Fukuda, M. Fukuda, Natural killer cells attack tumor cells expressing high levels of sialyl Lewis x oligosaccharides, Proc. Natl. Acad. Sci. USA 99 (2002) 13789–13794.
[29] K. Higai, A. Ichikawa, K. Matsumoto, Binding of sialyl Lewis X antigen to lectin-like receptors on NK cells induces cytotoxicity and tyrosine phosphorylation of a 17-kDa protein, Biochim. Biophys. Acta 1760 (2006) 1355–1363.
[30] K. Higai, K. Shibukawa, S. Muto, K. Matsumoto, Targeted proteo-glycomics analysis of Sialyl Lewis X antigen expressing glycoproteins secreted by human hepatoma cell line, Anal. Sci. 19 (2003) 85–92.
[31] G. de Jong, H.G. van Eijk, Microheterogeneity of human serum transferrin: a biological phenomenon studied by isoelectric focusing in immobilized pH gradients, Electrophoresis 9 (1988) 589–598.
[32] P. Hermentin, R. Witzel, R. Doenges, R. Bauer, H. Haupt, T. Patel, R.B. Parekh, D. Brazel, The mapping by high-pH anion-exchange chromatography with pulsed amperometric detection and capillary electrophoresis of the carbohydrate moieties of human plasma alpha 1-acid glycoprotein, Anal. Biochem. 206 (1992) 419–429.
[33] S.D. Shiyan, N.V. Bovin, Carbohydrate composition and immunomodulatory activity of different glycoforms of alpha1-acid glycoprotein, Glycoconj. J. 14 (1997) 631–638.
[34] M.A. Holmes, P. Li, E.W. Petersdorf, R.K. Strong, Structural studies of allelic diversity of the MHC class I homolog MIC-B, a stress-inducible ligand for the activating immunoreceptor NKG2D, J. Immunol. 169 (2002) 1395–1400.
[35] S. Radaev, B. Rostro, A.G. Brooks, M. Colonna, P.D. Sun, Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3, Immunity 15 (2001) 1039–1049.
[36] B.J. McFarland, T. Kortemme, S.F. Yu, D. Baker, R.K. Strong, Symmetry recognizing asymmetry: analysis of the interactions between the C-type lectin-like immunoreceptor NKG2D and MHC class I-like ligands, Structure 11 (2003) 411–422.
[37] W.S. Somers, J. Tang, G.D. Shaw, R.T. Camphausen, Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1, Cell 103 (2000) 467–479.
[38] B.K. Kaiser, J.C. Pizarro, J. Kerns, R.K. Strong, Structural basis for NKG2A/CD94 recognition of HLA-E, Proc. Natl. Acad. Sci. USA 105 (2008) 6696–6701.
[39] L.C. Sullivan, C.S. Clements, T. Beddoe, D. Johnson, H.L. Hoare, J. Lin, T. Huyton, E.J. Hopkins, H.H. Reid, M.C. Wilce, J. Kabat, F. Borrego, J.E. Coligan, J. Rossjohn, A.G. Brooks, The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition, Immunity 27 (2007) 900–911.