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Human and mouse NK cells use different families of receptors to recognize MHC class I (MHC I) on target cells. Although human NK cells express both Ig-like receptors and lectin-like receptors specific for MHC I, all the MHC I-specific receptors identified on mouse NK cells to date are lectin-like receptors, and no Ig-like receptors recognizing MHC I have been identified on mouse NK cells. In this study we report the first MHC I-specific Ig-like receptor on mouse NK cells, namely, murine CD160 (mCD160). The expression of mCD160 is restricted to a subset of NK cells, NK1.1T cells, and activated CD8+ T cells. The mCD160-Ig fusion protein binds to rat cell lines transfected with classical and nonclassical mouse MHC I, including CD1d. Furthermore, the level of mCD160 on NK1.1T cells is modulated by MHC I of the host. Overexpression of mCD160 in the mouse NK cell line KY-2 inhibits IFN-γ production induced by phorbol ester plus ionomycin, whereas it enhances IFN-γ production induced by NK1.1 cross-linking or incubation with dendritic cells. Cross-linking of mCD160 also inhibits anti-NK1.1-mediated stimulation of KY-2 cells. Anti-mCD160 mAb alone has no effect. Thus, mCD160, the first MHC I-specific Ig-like receptor on mouse NK cells, regulates NK cell activation both positively and negatively, depending on the stimulus. The Journal of Immunology, 2005, 175: 4426–4432.
mCD160 is the first MHC I-specific Ig-like receptor found on mouse NK cells or NKT cells.

Materials and Methods

Animals

C57BL/6 (B6), C57BL/10 (B10), B10D2, β2-microglobulin (β2-m)-deficient mice of B6 background, BALB/c, Cd1d-deficient mice of BALB/c background, and C3H/HeJ mice were purchased from The Jackson Laboratory and bred in our animal facility. Fisher 344 rats were purchased from Taconic Farms. Male mice (6–12 wk old) were used in this study. The use of animals for this study was approved by the animal care committee of the University of British Columbia, and animals were maintained in accordance with the guidelines of the Canadian Council on Animal Care.

RT-PCR, protein expression, and purification

RNA was isolated from spleen cells using an RNA extraction kit (Qiagen). RNA was reverse transcribed using an RT kit (Qiagen). For the isolation of full-length mCD160 cDNA, PCR was performed with an initial denaturation at 94°C, followed by 40 cycles of 94°C for 30 s, 65°C for 30 s, and 68°C for 60 s, with a final 7-min extension at 68°C using the primers: 5'-TGAGTATCGTCTGTATCCCTCGAGACCAAGGTTGACGCTT-3' (EcoR I and Bgl II restriction enzyme sites used for cloning are underlined) and HIFI 5'-TGA ATCCTAATTCCGTAAGAGTCGGGTTGTTATGACGCG-3' (EcoR I and Bgl II restriction enzyme sites are underlined). This PCR was performed with an initial denaturation at 94°C, followed by 30 cycles of 94°C for 45 s, 55°C for 30 s, and 68°C for 60 s, with a final 7-min extension at 68°C. The PCR product was digested with EcoR I and BamHI and cloned into the pBluescript KS+ vector. COS cells were transiently transfected with the plasmid DNA using the calcium phosphate transfection method according to the manufacturer's protocol, and mCD160-Fc in the culture supernatants was purified by affinity chromatography using a protein A column (Amersham Biosciences). In some experiments mCD160-Fc was biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (Pierce). A fusion protein consisting of the extracellular domain of H60 and human IgG (H60-Fc) was generated in the same manner and was used as a control.

Generation of anti-mCD160 mAb

Fisher 344 rats were immunized by i.p. injection of 50–100 μg of mCD160-Fc emulsified in IFA (Sigma-Aldrich) three times at 4-wk intervals. After a final i.v. injection of mCD160-Fc, immune spleen cells were fused with the mouse myeloma line SP2/0-Ag14 using the standard protocol. Hybridoma culture supernatants were screened by ELISA using mCD160-Fc-coated plates. Positive hybridomas were subcloned three times. This resulted in one clone producing anti-mCD160 mAb, termed CXN46-3 (rat IgG2b, κ).

Cells, reagents, and flow cytometry

COS-7, K562, RBL-1, Sp2/0-Ag14, M1/42 (anti-pan H-2), 2.4G2 (anti-FCγR), and HO-13-14 (anti-Thy1.2) hybridomas were obtained from American Type Culture Collection. RBL-1 cells transfected with mouse MHC I have been described previously (21). The murine T cell lines RMA, EL4, and RMA-S have been described previously (9). The murine T cell line KY-2 (22) was a gift from Dr. W. Yokoyama (Howard Hughes Medical Institute, Rheumatology Division, Washington University School of Medicine, St. Louis, MO). Anti-murine MHC-class I Abs have been described previously (21). FITC-conjugated anti-CD45.2 mAb AL14A2, anti-Mac-1 mAb M1/70, and anti-Gr-1 mAb RB6-8C5 have also been described (23). All other Abs used in this study were purchased from BD Biosciences.

Cell surface staining, flow cytometric analysis, and cell sorting have been described previously (10). For analysis of mCD160-Fc binding, cells were first incubated with the 2.4G2 mAb or human γ globulin (Sigma-Aldrich) to block FcRs, then incubated with 40 μg/ml biotinylated mCD160-Fc or H60-Fc in HBSS with 2% BSA and 0.09% (w/v) sodium azide at 37°C for 60 min. After washing, the cells were stained with streptavidin-PE on ice for 30 min and analyzed by flow cytometry as described above. In some experiments CD1d-transfected RBL-1 cells were pulsed with α-galactosylceramide for 2 h at 37°C as previously described (24). PE-conjugated K1-OVA 254–267 tetramer was synthesized and used for staining as previously described (25).

Phosphatidylinositol-specific phospholipase C (PIPLC) treatment

Cells were washed three times with PBS, incubated with PIPLC (5 U/ml; Invitrogen Life Technologies) for 1 h at 37°C, and washed again. Control cells were incubated without PIPLC. Cells were then stained with mAbs and were analyzed by flow cytometry.

Immunoprecipitation

The mCD160-transfected K562 cells were incubated with purified CNX46-3 mAb and lysed with lysis buffer (1% Triton X-100, 1% BSA, 150 mM NaCl, and 0.1% NaN3 in 10 mM Tris-HCl, pH 7.5). The cell lysates were centrifuged at 14,000 rpm for 15 min at 4°C, and the cleared cell lysates were incubated overnight at 4°C with protein G beads (Amer- sham Biosciences). The beads were then washed four times with lysis buffer (without BSA and NaN3), and bound proteins were eluted by boiling for 5 min in 100 μl of 2× SDS sample buffer (reducing). The eluted proteins were separated by SDS-PAGE and transferred onto nitrocellulose. After incubation with biotinylated CNX46-3 mAb and peroxidase-conjugated streptavidin, proteins on the blots were detected by the ECL (Amersham Biosciences) method as previously described (26).

N-glycosidase F treatment

After immunoprecipitation as described above, mCD160 cells bound to protein G beads were washed and resuspended in PBS, treated with 500 U of peptide:N-glycosidase F (New England Biolabs) for 6 h at 37°C, eluted with 100 μl of 2× SDS sample buffer, and analyzed as described above.

Transfection

Full-length mCD160 cDNA was subcloned into the pBMC GSNeo (27) and pCEP4 (Invitrogen Life Technologies). The pHFeAronm.C1neo was provided by Dr. M. Kronenberg (La Jolla Institute for Allergy and Immunology, San Diego, CA) (28). Cell lines were electroporated with 10 μg of plasmid DNA by Gene Pulser (Bio-Rad). Transfectants were selected under limited optimal concentrations of G418 or hygromycin (Invitrogen Life Technologies), and cells expressing the transfected genes were isolated by cell sorting. For KY-2, the MSCV IRES GFP (MIG) retrovirus vector (29), provided by Dr. R. K. Humphries (Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada), was used. Full-length mCD160 cDNA was subcloned into the MIG vector, and retrovi- ruses were generated as previously described (29). KY-2 was cocultured with an irradiated mCD160MIG virus-producing cell line in the presence of 200 U/ml IL-2 (PeproTech). After 2 days of infection, nonadherent cells were harvested, and GFP-positive cells were isolated by cell sorting.

Cell culture and cytokine ELISA

The cell culture methods for IL-2–stimulated NK and NK1.1+ T cells and splenocytes stimulated with immobilized CD3 mAb were described previously (9). Bone marrow (BM)-derived dendritic cells (BM-DC) were cultured as previously described (30). Retrovirus-transduced KY-2 lines (1 × 105 cells/well) were cocultured with BM-DC (1 × 105 cells/wells) or stimulated with PMA (50 ng/ml) and ionomycin (1 μM; Sigma-Aldrich) in 96-well, round-bottom plates for 24 h at 37°C. In some experiments, retrovirus-transduced KY-2 lines (2 × 105 cells/well) were incubated with combinations of mAbs (50 μg/ml) immobilized in 96-well, flat-bottom plates for 24 h as previously described (31). The amounts of IFN-γ in the supernatants were measured using Quantikine kits (RD Systems).

Results

Analysis of mCD160 cDNA and protein

The mCD160 cDNA clones were isolated by RT-PCR from splenocytes of B6, BALB/c, and C3H/HeJ mice and sequenced. As reported, the mCD160 cDNA sequence predicts a polypeptide of 4427 aa with the characteristic signal sequence at the N-terminal, a GPI membrane anchor signal sequence at the C-terminal, single Ig domain, and potentially encoding a secreted mCD160, was also isolated. A comparison of our mCD160 cDNA sequences with those in GenBank (an original report in which mouse strain is not indicated: accession no. AF060982; FVB/N: accession no. NM018767; B6: Downloaded from http://www.jimmunol.org/ by guest on April 14, 2017
accession no. AK042093) identified two single nucleotide polymorphisms that result in amino acid changes. In B6 and BALB/c mice, mCD160 has an asparagine in position 76, whereas FVB/N mice, mCD160 has an asparagine in position 76, whereas FVB/N mice and subjected to RT-PCR using specific primers, and PCR products were analyzed by agarose gel electrophoresis. Lane 3, DNA size standard. B, K562 cells transfected with empty pCEP4 vector (control/K562) or with mCD160 cDNA in pCEP4 (mCD160/K562) were stained with either biotinylated CNX46-3 mAb (shaded histograms) or biotinylated isotype-matched control mAb (open histograms), followed by streptavidin-PE, and were analyzed by flow cytometry. C, The mCD160 was immunoprecipitated from mCD160/K562 with CNX46-3 mAb and protein G-coupled beads. The beads were incubated with PBS (lane 1) or N-glycosidase F (lane 2), and the immunoprecipitated proteins were eluted from the beads. They were separated by 10% SDS-PAGE, blotted, probed with biotinylated CNX46-3 mAb plus HRP-conjugated streptavidin, and visualized by chemiluminescence. D, The mCD160-transfected EL4 cells were either treated with PIPLC or left untreated, then stained with anti-CD45 mAb, anti-Thy1, and anti-mCD160 mAb (CNX46-3; shaded histograms) or isotype-matched control mAb (open histograms), followed by streptavidin-PE, and analyzed by flow cytometry.

**Distribution of mCD160 on leukocytes**

The anti-mCD160 mAb CNX46-3 stained NK1.1⁺CD3⁺ cells, NK1.1⁺TCRαβ⁺ cells (40.9 ± 7%; not shown), and ~5% of double-negative cells in thymus, but not other thymocytes (Fig. 2). In spleen, the mAb stained ~20% of NK cells and ~80% of both NK1.1⁺CD3⁺ cells and NK1.1⁺TCRαβ⁺ cells (74.4 ± 3.7%; not shown), whereas other spleen cells were mostly negative (Fig. 2). Murine CD160 was also undetectable on Mac1⁺ cells and Gr1⁺ cells in BM. The proportion of mCD160-positive NK cells increased after culture with high dose IL-2 (1000 U/ml; 20.4 ± 4.7 to 36.2 ± 2.4%; n = 4). In contrast, the proportion of mCD160-positive NK1.1⁺ T cells declined after stimulation with 200 U/ml IL-2 (79.1 ± 5.4 to 40.1 ± 10%; n = 4). Stimulation of T cells with immobilized anti-CD3 mAb induced the expression of mCD160 on ~80% of CD8 T cells, but not on CD4 T cells (Fig. 2).

**Ligand specificity of mCD160**

Binding of mCD160-Fc to the murine T cell lymphoma line RMA and the TAP-deficient variant line RMA-S was compared. The fusion protein bound to RMA cells that express a high level of MHC I, whereas it bound poorly to RMA-S cells, which express a much lower level of MHC I (Fig. 3A). This suggested that mCD160 might bind to mouse MHC I. Therefore, we next tested the binding of mCD160-Fc to a panel of rat basophilic leukemia RBL-1 cell lines transfected with individual murine MHC I, mCD160-Fc bound to both classical and nonclassical MHC I, including D₄, D₅, K₂, Qa-1b, and CD1d to varying degrees (Fig. 3B). The interaction between mCD160 and MHC I was also tested by binding tetrameric MHC I H-2Kb folded with OVA-derived peptide to the human erythroleukemia cell line K562 transfected with mCD160. The tetramer very weakly bound to mCD160-transfected K562 cells, but not to control K562 cells transfected with empty vector (Fig. 3C).

**Modulation of mCD160 expression by MHC I in vivo**

The level of Ly49 expression on NK cells is known to be calibrated by the host MHC I (32). To test whether mCD160 is similarly modulated in vivo, the levels of mCD160 expression on
NK1.1$^+$ T cells in wild-type and $\beta_2 m$-deficient B6 mice were compared. The level of mCD160 expression in wild-type mice was slightly lower than that in $\beta_2 m$-deficient mice (Fig. 4A). The level of mCD160 expression on NK1.1$^+$ T cells also differed between MHC-congenic B10 (H-2b) and B10.D2 (H-2d) mice (Fig. 4A), and the level of mCD160 on NK cells differed between wild-type and $\beta_2 m$-deficient mice as well as between B10 and B10.D2 mice (Fig. 4B). Although these differences were small, they were statistically significant (Fig. 4C). Therefore, mCD160 expression seems to be calibrated by host MHC I in vivo.

Because mCD160 binds CD1d in vitro, the effects of CD1d deficiency on mCD160 expression were examined. We compared NK T cells from wild-type and CD1d-deficient BALB/c mice. Because BALB/c mice do not express NK1.1, NKT cells were identified as DX5$^+$ T cells (33) in this experiment. Very few DX5$^+$ T cells expressed mCD160 in CD1d-deficient BALB/c mice (Fig. 4D), whereas $\sim$25% of NKT cells in wild-type BALB/c mice were mCD160 positive. The expression of mCD160 on DX5$^+$ T cells also significantly differed between wild-type (48.6 $\pm$ 4.5%) and $\beta_2 m$-deficient (22.9 $\pm$ 7.0%) B6 mice (data not shown). It should be noted that NK1.1$^+$ and DX5$^+$ subsets among T cells only partially overlap (33). Because the mCD160 cDNA sequences from BALB/c and B6 mice were identical (see above), the results suggest that the genetic background of the mice, in addition to the MHC, influences the expression of mCD160.

**Functions of mCD160**

By retrovirus-mediated gene transfer into the murine NK cell clone KY-2, we established the KY-2 cell line overexpressing mCD160 (mCD160/KY-2) as well as control virus-transduced KY-2 (MIG/KY-2). NK1.1 expression on these two KY-2 lines was the same (data not shown). PMA plus ionomycin potently stimulated both mCD160/KY-2 and MIG/KY-2 cells. However, the former secreted significantly less IFN-γ than the latter (Fig. 5A). In contrast, upon NK1.1 cross-linking by immobilized mAb, mCD160/KY-2 secreted significantly more IFN-γ than MIG/KY-2 (Fig. 5B). Similarly, upon coculture with BM-DC, mCD160/KY-2 cells secreted more IFN-γ than MIG/KY-2 cells (Fig. 5C). It should be noted that these cell lines produced the same amount of IFN-γ without stimulation. IFN-γ was not detected in the culture of DC alone. Thus, overexpression of mCD160 seems to have both costimulatory and inhibitory effects on KY-2 cells, depending on the stimulus.

To further investigate the role of mCD160 in NK cell activation, the effects of mCD160 cross-linking on NK1.1-mediated stimulation of mCD160/KY-2 cells were tested. Cross-linking of mCD160 by immobilized anti-mCD160 mAb alone had no effect on mCD160/KY-2 cells. However, mCD160/KY-2 cells incubated with immobilized NK1.1 mAb plus mCD160 mAb secreted significantly lower amounts of IFN-γ than those stimulated with immobilized NK1.1 mAb plus isotype control mAb (Fig. 5D). The treatments did not affect the viability of the cells (data not shown). These results indicate that mCD160 cross-linking inhibits NK1.1-mediated activation of KY-2 cells.

We also isolated mCD160$^+$ and mCD160$^-$ primary NK cells and tested cytotoxicity against RMA-S cells. As reported for human CD160 (14, 15), mCD160$^+$ NK cells were more cytotoxic than mCD160$^-$ NK cells (data not shown).

**Discussion**

We have characterized mCD160 using newly generated mAb and mCD160-Fc fusion protein. Similar to the human counterpart, mCD160 is a GPI-anchored protein with a single Ig domain. It interacts with a broad range of MHC I, including CD1d. Among resting lymphocytes, mCD160 is predominantly expressed on NK1.1$^+$ T cells and a subset of NK cells. Thus, mCD160 is the first Ig-like receptor for MHC I identified on mouse NK cells. Although the precise function of mCD160 is still unclear, overexpression of mCD160 in the murine NK cell line KY-2 both enhances and inhibits KY-2 cell activation, depending on the stimulus. Overexpression of mCD160 in KY-2 inhibits stimulation by PMA plus ionomycin, but augments stimulation with immobilized anti-NK1.1 mAb or BM-DC. Cross-linking of mCD160 on KY-2...
TAP-mutated RMA-S cells were incubated with 40 μg/ml biotinylated mCD160-Fc (shaded histograms) or biotinylated H60-Fc (open histograms) plus streptavidin-PE and analyzed by flow cytometry. The expression levels of individual MHC I were determined by anti-pan MHC I mAb M1/42 (shaded histograms) and compared with isotype-matched control Ab staining (open histograms). Representative data from at least four independent experiments are shown. A panel of mouse MHC I-transfected RBL-1 cell lines was stained with biotinylated mCD160-Fc (shaded histograms) or biotinylated H60-Fc (open histograms) plus streptavidin-PE and analyzed by flow cytometry. The expression levels of individual MHC I on the transfected RBL-1 cells were detected by specific anti-MHC I mAbs (shaded histograms) and compared with isotype-matched controls (open histograms). Representative data from at least four independent experiments are shown. C. The mCD160-transfected K562 (shaded histograms) or control vector-transfected K562 (open histograms) cells were stained with 10 μg/ml OVA/Kb tetramer for 1 h at 4°C and analyzed by flow cytometry. Representative data from three independent experiments are shown.

FIGURE 3. Binding of mCD160 to MHC I. A. TAP-sufficient RMA or TAP-mutated RMA-S cells were incubated with 40 μg/ml biotinylated mCD160-Fc (shaded histograms) or biotinylated H60-Fc (open histograms) plus streptavidin-PE and analyzed by flow cytometry. The expression levels of MHC I were determined by anti-pan MHC I mAb M1/42 (shaded histograms) and compared with isotype-matched control Ab staining (open histograms). Representative data from at least four independent experiments are shown. B. A panel of mouse MHC I-transfected RBL-1 cell lines was stained with biotinylated mCD160-Fc (shaded histograms) or biotinylated H60-Fc (open histograms) plus streptavidin-PE as described in A and analyzed by flow cytometry. The expression levels of individual MHC I on the transfected RBL-1 cells were detected by specific anti-MHC I mAbs (shaded histograms) and compared with isotype-matched controls (open histograms). Representative data from at least four independent experiments are shown.
MHC tetramers may not be suitable for the binding of mCD160 or CD8. In addition to the binding of mCD160-Fc to MHC I-transfected cells in vitro, small, but significant, differences in the expression levels of mCD160 on the surface of NKT cells between MHC congenic mouse strains as well as between wild-type and /H9252 2m-deficient mice suggest that MHC I of the host interacts with mCD160 and modulates its level of expression on the cell surface in vivo. Such modulations of NK cell receptors are most evident with Ly49. The Ly49 expression level is highest in MHC I-deficient mice such as /H9252 2m-deficient mice, and it is down-modulated by its interaction with the host MHC I (32). Strong interaction between Ly49 and host MHC I results in low expression of Ly49. The

FIGURE 5. Murine CD160 regulates stimulation of the NK cell line KY-2. A, A bulk population of mCD160-transduced KY-2 (mCD160/KY-2; [ ]) or control virus-transduced KY-2 (MIG/KY-2; [ ]) cells was isolated by cell sorting of GFP-positive cells. The sorted cells were expanded in cultures, 10^5 cells/well were stimulated with PMA (50 ng/ml) and ionomycin (1 μM) for 24 h, and the amount of IFN-γ secreted in the culture supernatants was determined by ELISA. Data are the mean ± SD of triplicate cultures and are representative of three independent experiments. B, The mCD160/KY-2 ( [ ]) and MIG/KY-2 ( [ ]) cells (2 × 10^5 cells/well) were stimulated with immobilized NK1.1 mAb or isotype control mAb for 24 h, and the amount of IFN-γ in the culture supernatants was determined by ELISA. Data are the mean ± SD of triplicate cultures and are representative of three independent experiments. C, The mCD160/KY-2 ( [ ]) and MIG/KY-2 ( [ ]) cells (2 × 10^5 cells/well) were cultured with (DC) or without (control) BM-DC (10^5 cells/well) in microculture wells for 24 h, and the amount of IFN-γ secreted in the culture supernatants was determined by ELISA. Data are the mean ± SD of triplicate cultures and are representative of three independent experiments. D, The mCD160/KY-2 cells (2 × 10^5 cells/well) were stimulated with various combinations of immobilized mAbs in microculture wells. The total concentrations of mAbs to be immobilized were kept at 50 μg/ml in all wells, and two mAbs were mixed in equal proportions. The amount of IFN-γ secreted in the culture supernatants was determined by ELISA. Data are the mean ± SD of triplicate cultures and are representative of three independent experiments.

MHC tetramers may not be suitable for the binding of mCD160 or CD8. In addition to the binding of mCD160-Fc to MHC I-transfected cells in vitro, small, but significant, differences in the expression levels of mCD160 on the surface of NKT cells between MHC congenic mouse strains as well as between wild-type and β2m-deficient mice suggest that MHC I of the host interacts with mCD160 and modulates its level of expression on the cell surface in vivo. Such modulations of NK cell receptors are most evident with Ly49. The Ly49 expression level is highest in MHC I-deficient mice such as β2m-deficient mice, and it is down-modulated by its interaction with the host MHC I (32). Strong interaction between Ly49 and host MHC I results in low expression of Ly49. The

FIGURE 4. Down-modulation of mCD160 in vivo. A, In the left panel, age- and sex-matched, wild-type (WT; thin histogram) and β2m knockout (bold histogram) B6 mouse spleen cells were stained for NK1.1, CD3ε, and mCD160, and mCD160 expression levels on NK1.1^+CD3^+ cells were determined by flow cytometry. Dot-line histograms show staining with isotype-matched control mAb. In the right panel, mCD160 expression levels on NK1.1^+CD3^+ spleen cells from age- and sex-matched B10D2 (thin histogram) and B10 (bold histogram) mice were determined by flow cytometry as in the left panel. Dot-line histograms show isotype control mAb staining. B, The expression of mCD160 on NK cells from the same mice as those in A was analyzed by flow cytometry. Open histograms show isotype control Ab staining, and shaded histograms show staining of mCD160. The bars indicate the gates to calculate the mean fluorescence intensity of mCD160. C, The mean fluorescence intensities (MFI) of mCD160 staining of NK1.1^+CD3^+ cells and NK cells (NK1.1^+CD3^−) from various strains of mice, as described in A and B, were compared. The numbers show the mean fluorescence intensity ± SD of four individual animals. Paired Student’s t test showed statistically significant (p < 0.05) differences between the datasets (*). D, The expression of mCD160 on DX5^+CD3^+ cells from wild-type and CD1d knockout BALB/c mice was analyzed by flow cytometry. Shaded histograms show staining with anti-mCD160 mAb, and open histograms show staining with isotype control mAb. The numbers show average percentage (±SD) of cells stained with anti-m160 mAb obtained from four individual animals.
modulation of mCD160 by host MHC I is less pronounced than that of Ly-49, perhaps reflecting the weak interaction of the former.

The expression of mCD160 among resting lymphocytes is most prominent on NK1.1+ T cells in B6 mice. Because NK1.1+ T cells in β2m-deficient B6 mice also express mCD160, its expression is not limited to CD1d-restricted NKT cells. In contrast, mCD160 is barely detectable on DX5+ T cells in CD1d-deficient BALB/c mice, whereas those in wild-type BALB/c mice express mCD160, suggesting that only CD1d-restricted NKT cells may express mCD160 in this strain. Because mCD160 recognizes CD1d, it may play an important role in the development or functions of CD1d-restricted NKT cells. In addition to NK1.1+ T cells, a subset of NK cells expresses mCD160. Although our results with the mouse NK cells line KY-2 suggest a regulatory function of mCD160 in cytotoxic production, the role of mCD160 in NK cell-mediated cytotoxicity is still unknown. It has been reported that CD1d on target cells inhibits the cytotoxicity of a subset of NK cells (11–13). It remains to be determined whether mCD160 is involved in the inhibition of NK cytotoxicity by target cell CD1d. Future studies, including generation of mCD160-deficient mice, will provide more information on the role of mCD160 in immune responses mediated by NK and NKT cells.

Disclosures

The authors have no financial conflict of interest.

References


MURINE CD160