Murine *Nkg2d* and *Cd94* are clustered within the natural killer complex and are expressed independently in natural killer cells

**EMILY L. HO†, JONATHAN W. HEUSEL†, MICHAEL G. BROWN†,** KEIKO MATSUMOTO†, **ANTHONY A. SCALZO‡,** AND **WAYNE M. YOKOYAMA∗¶**

†Howard Hughes Medical Institute, Division of Rheumatology, Washington University School of Medicine, St. Louis, MO 63110; and ‡Department of Microbiology, University of Western Australia, Nedlands, Western Australia 6007, Australia

Communicated by Emil R. Unanue, Washington University School of Medicine, St. Louis, MO, March 23, 1998 (received for review January 28, 1998)

**ABSTRACT** Natural killer (NK) cells express C-type lectin-like receptors, encoded in the NK gene complex, that interact with major histocompatibility complex class I and either inhibit or activate functional activity. Human NK cells express heterodimers consisting of CD94 and NKG2 family molecules, whereas murine NK cells express homodimers belonging to the Ly-49 family. The corresponding orthologues for other species, however, have not been described. In this report, we used probes derived from the expressed sequence tag database to clone C57BL/6-derived cDNAs homologous to human NKG2-D and CD94. Among normal tissues, murine NKG2-D and CD94 transcripts are highly expressed only in activated NK cells, including both Ly-49A⁺ and Ly-49A⁻ subpopulations. Additionally, mNKG2-D is expressed in murine NK cell clones KY-1 and KY-2, whereas mCD94 expression is observed only in KY-1 cells but not KY-2. Last, we have finely mapped the physical location of the *Cd94* (centromeric) and *Nkg2d* (telomeric) genes between *Cd69* and the *Ly-49* cluster in the NK complex. Thus, these data indicate the expanding complexity of the NK complex and the corresponding repertoire of C-type lectin-like receptors on murine NK cells.

Natural killer (NK) cells are a distinct lymphocyte lineage that functions as a critical component of innate immunity against a wide variety of intracellular and parasitic pathogens and may also mediate tumor surveillance and influence hematopoiesis (1, 2). The activity of NK cells is controlled by inhibitory surface receptors for major histocompatibility complex (MHC) class I molecules (3, 4). Two structural types of NK receptors for MHC class I have been described—type I integral membrane Ig-like killer inhibitory receptors and type II integral-membrane C type lectin-like disulfide-linked dimers, including the human (h) CD94/NKG2 family of heterodimers and the murine (m) Ly-49 family of homodimers (5–8). Both types of NK cell receptors for MHC class I transmit potent inhibitory signals that are dependent upon the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIM) consisting of the consensus sequence YVXYYXX/L in the cytoplasmic domains (9, 10). Receptor cross-linking appears to lead to tyrosine phosphorylation of the ITIM and the subsequent recruitment of the SHP-1 intracellular tyrosine phosphatase that then presumably dephosphorylates tyrosine residues on molecules involved in the activation cascade.

Among the C-type lectin-like receptors, cross-linking of hCD94 with a mAb either inhibited cytolytic activity or induced redirected lysis of various NK clones, leading to confusion about its function in NK cells (11–14). Remarkably, the cDNA sequence of CD94 reveals an extremely short cytoplasmic domain that contains no consensus sequences involved in cell signaling (15). The phenotypic differences observed with CD94 engagement have been recently clarified in studies demonstrating that CD94 forms heterodimers with NKG2 molecules (16, 17). At least five NKG2 family members have been described in humans: NKG2-A/B, -C, -D, -E, and -F (18–20). NKG2-D/A, -C, and -E show 94–95% amino acid homology in the extracellular domain, whereas NKG2-D is less related (21% amino acid homology overall) (20). Functional studies of human NK cell clones revealed that NKG2-A forms a disulfide-linked heterodimer with CD94 that inhibits cytotoxicity toward targets expressing HLA-A, -B, -C, and -G and virus-encoded MHC class I-like homologues (21–25). Notably, NKG2-A contains two ITIMs in its cytoplasmic domain that associate with SHP-1 (26). On the other hand, NKG2-C, which also forms heterodimers with CD94, lacks ITIM sequences and delivers activating signals (26, 27). In mouse NK cells, Ly-49A belongs to a family of highly related molecules that bear significant amino acid identity to each other (65–89%) (28, 29) but are distinct from human CD94 and NKG2 (<30% identity), suggesting that Ly-49 and NKG2/Cd94 are not orthologous. This is also highlighted by previous studies demonstrating that Ly-49 molecules form homodimers rather than heterodimers and that inhibitory Ly-49 molecules bear only one ITIM in the cytoplasmic domain rather than two ITIMs (10, 29). Nevertheless, Ly-49A interacts with H-2Dδ and H-2Dk, resulting in inhibition of NK cytotoxicity and secretion of cytokines (30, 31). Other Ly-49 members, such as Ly-49C and Ly-49G, also transmit inhibitory signals after engagement of specific MHC class I ligands (32, 33). However, the Ly-49D receptor, which lacks cytoplasmic ITIM (29), appears to be a stimulatory NK cell receptor (34). With orthologues for Ly-49 receptors in humans yet to be reported, one hypothesis to reconcile these observations is that the murine Ly-49 receptors are functional orthologues of human CD94/NKG2 molecules and that they substitute for the other in their corresponding species. Recent studies, however, indicate that rat NK cells express Ly-49, CD94, and NKG2 molecules (35–37).

Abbreviations: EST, expressed sequence tag; h, human; IL-2, interleukin 2; ITIM, immunoreceptor tyrosine-based inhibitory motif; NK, natural killer; LAK, IL-2-activated NK cell; m, mouse; MHC, major histocompatibility complex; NKC, natural killer complex; YAC, yeast artificial chromosome; UTR, untranslated region; RFLP, restriction fragment length polymorphism.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF054819 and AF0547714).

© 1998 by The National Academy of Sciences

PNAS is available online at http://www.pnas.org.
The genes encoding the Ly-49 family of receptors reside in the NK complex (NKC) on mouse chromosome 6 (3, 38). Our laboratory has mapped genes encoding other C-type lectin-like receptors on NK cells, including Nkrp1 and Cd69, within a 2-megabase region by constructing a contig of overlapping yeast artificial chromosomes (YACs) containing NKC DNA from C57BL/6 mice (39). In humans, the NKG2 family members and CD94, as well as orthologs to murine Nkrp1 and Cd69, are located on human chromosome 12p12.3–13.1, a region syntenic to mouse chromosome 6, indicating that the NKC has been conserved across species (15, 40–42). Because the NKC contains a very large genomic region in which gene order has been conserved, and recent studies of rat NK cells indicate the presence of NKG2 and CD94 genes (35–37), we hypothesized that orthologs for human NKG2 and CD94 genes were also encoded in the mouse NKC. In this report, we identified cDNA clones for mouse Nkg2d and CD94 genes, determined their expression, and mapped their physical positions within the NKC. Because an individual NK cell can express multiple receptors simultaneously, these studies indicate the complexity of the C-type lectin-like NK cell receptors.

MATERIALS AND METHODS

Animals. C57BL/6J and BALB/cJ mice were purchased from the Jackson Laboratory, whereas C57BL/6T mice were purchased from Taconic Farms. All mice were maintained in a pathogen-free facility at Washington University.

Cell Lines. KY-1 and KY-2 murine NK cell clones were as described (43). Both lines were grown in R10 medium consisting of RPMI 1640 medium (GIBCO/BRL) supplemented with L-glutamine (300 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (50 μM), 10% fetal calf serum (Harlan Breeders, Indianapolis, IN), and recombinant human interleukin 2 (IL-2; Chiron; 800 units/ml), 10% fetal calf serum, sodium pyruvate (1 mM), and streptomycin (100 μg/ml; 100 units/ml). All other cell lines were obtained from the American Type Culture Collection (44). Nonadherent splenocyte suspensions were cultured at 2–4 × 10⁶ cells per ml in 1 × HEBS (137 mM NaCl/5 mM KCl/0.7 mM NaHPO₄/6 mM dextrose/20 mM Hepes base) were directly extracted in 4 M guanidine thiocyanate; mouse tissues and organs were flash frozen and then homogenized in 1 × HEBS prior to RNA extraction. Lyophilized RNA pellets were resuspended in 0.2% diethyl pyrocarbonate in H₂O at concentrations of 100–1,000 μg/ml. For Northern blot analyses, 10–20 μg of RNA samples were electrophoresed in a 1% agarose/2.2 M formaldehyde gel (46). RNA was transferred overnight in 20× SSC onto Hybond-N membranes (Amersham) and fixed by baking at 80°C for 2 h. Membranes were hybridized with [α-³²P]dCTP-labeled probes derived from the full-length murine NKG2-D and CD94 cDNAs or from human β-actin cDNA (CLONTECH) as an internal quality and content control. Stringent washing was performed in 0.2× SSPE/0.1% SDS at 95°C for NKG2-D and human β-actin and at 50°C for CD94.

Southern Blot Analysis. C57BL/6J and BALB/cJ genomic DNA was extracted from mouse liver in proteinase K as described (39). Samples of 2–5 μg were subjected to overnight digestion with various restriction endonucleases and then fractionated in 0.8% agarose. Southern blots were prepared and analyzed (46). DNA was transferred to Hybond-N membranes and membranes were hybridized with [α-³²P]dCTP-labeled full-length mNKG2-D or mCD94 cDNA probes. Stringent washing was performed in 0.2× SSPE/0.1% SDS at 56°C for NKG2-D and at 50°C for CD94.

YAC clones 85D11, 178C4, 95E6, 9C10, and 52A6 are derived from C57BL/6 mice and are known to contain NKC-linked DNA fragments (39). Agarose-plug YAC DNA was prepared and separated by pulsed-field gel electrophoresis as described (39) before transfer and hybridization as described above. Stringent washing was performed in 0.2× SSPE/0.1% SDS at 63°C.

RESULTS
cDNA Cloning of Murine NKG2-D and CD94. By amino acid sequence comparison of hNK2-D and hCD94 with deduced protein sequences from the EST database (47), we identified a clone (621324) highly homologous to hNK2-D and two clones (598616 and 596465) homologous to hCD94. With the EST clone inserts as probes, we screened a cDNA library constructed from C57BL/6J LAK cells and obtained cDNA clones 2.1 and 1.1.3 hybridizing with the mNKG2-D and mCD94 EST probes, respectively. The three EST clone inserts and the cDNA clones were sequenced on both strands, and all three ESTs were determined to be incomplete cDNA sequences when compared with the
full-length human cDNAs (Fig. 1). LAK cDNA clone 2.1 contains a 284-bp 5' untranslated region (UTR), an ORF of 696 bp, and a 139-bp 3' UTR with a poly(A) tail. Upon comparison of this sequence to EST clone 621324 (NK2G2-D-like), a region of complete identity is apparent from nucleotide position 317 and extending 3'. The EST clone, therefore, contains a 5' truncation involving the complete 5' UTR and the 32 nucleotides encoding the initial 11 amino acids (Fig. 1). LAK cDNA clone 1.1.3 contained a short 5' UTR (46 bp), an intact 537-nucleotide ORF, and a 3' UTR (321 bp) that includes a polyadenylation signal and a partial poly(A) tail. On comparison of this sequence to EST clones 598616 and 596465 (CD94-like), it is apparent that these EST clones contain 3' regions of identity to the LAK cDNA and incomplete coding sequences at the 5' end. Moreover, clones 598616 and 596465 have 5' DNA segments that do not correspond to the full-length LAK cDNA. Whether these sequences represent incompletely or alternatively spliced mRNA transcripts or cloning artifacts is not currently known. Nevertheless, we have identified apparent full-length cDNAs corresponding to truncated cDNAs of the homologous to mNK2G2-D and cCD94.

The ORF of cDNA clone 2.1 encodes a type II integral membrane protein with homology to hNK2G2-D and rat NKG2-D (NKR-P2) (60% and 81% amino acid identity, respectively; Fig. 2 and Table 1). The predicted protein product contains 232 amino acids, corresponding to a predicted molecular mass of 27 kDa. Notably, as is observed for the human orthologue, the N-terminal cytoplasmic domain lacks ITIMs that are present in human inhibitory NKG2-A/B receptors (20, 26).

By contrast, murine NKG2-D also contains a 13-amino acid stretch within the cytoplasmic domain that is absent in hNK2G2-D (Fig. 2). Similar to hNK2G2-D and hCD94, the putative 23-amino acid transmembrane domain of mNKG2-D contains charged residues, Arg9, Lys22, and Glu23. The 142-amino acid extracellular domain has two potential glycosylation sites and contains cysteine residues conserved among all of the C type lectins (48, 49), as well as cysteines that are conserved only in the NKG2 family (see below).

LAK cDNA clone 1.1.3 contains a 179-residue ORF encoding a type II transmembrane protein (predicted molecular mass = 20.8 kDa) with high homology to rat and hCD94 (77% and 55% amino acid identity, respectively; Table 1 and Fig. 2). It also contains a partial poly(A) tail. In addition, clones 598616 and 596465 (CD94-like), it is apparent that these EST clones contain 3' regions of identity to the LAK cDNA and incomplete coding sequences at the 5' end. Moreover, clones 598616 and 596465 have 5' DNA segments that do not correspond to the full-length LAK cDNA. Whether these sequences represent incompletely or alternatively spliced mRNA transcripts or cloning artifacts is not currently known. Nevertheless, we have identified apparent full-length cDNAs corresponding to truncated cDNAs of the homologous to mNK2G2-D and cCD94.

Expression of mNKG2-D and mCD94. Northern blot analysis revealed the presence of two mNKG2-D transcripts (2.7 and 1.2 kb) in NK cell clones and IL-2-activated NK cells but not in a panel of other cell lines including those of lymphocyte (C1498, WR19L, YAC-1, and EL-4), monocyte/macrophage (WEHI-
example, a RFLP for mNKG2-D was detected on PvuII C57BLy with multiple individual enzymes revealed RFLPs between nine inbred strains (including C57BLy Southern blot analysis of genomic mouse DNA derived from and BALB (RFLP) between the C57BLy and Ly49a Previous studies on other C-type lectins encoded in the NKC detected in BALBbly reflecting allelic differences. A 3.5-kb transcript was also the very small percentage of NK cells (activation or insensitivity of Northern blots for transcripts in spleen were undetectable, perhaps reflecting a prerequisite for completely spliced transcript. Interestingly, transcripts in the are differences in expression among individual NK cells. Thus, the data indicate that transcripts for both NKG2-D and expression in BALBy derived cells. However, the BALBy c CD94 transcripts were reproducibly slightly smaller than C57BLy transcripts, possibly reflecting allelic differences. A 3.5-kb transcript was also detected in BALB/c LAK, which may represent an incompletely spliced transcript. Interestinately, transcripts in the spleen were undetectable, perhaps reflecting a prerequisite for activation or insensitivity of Northern blots for transcripts in the very small percentage of NK cells (<2.5%) in the spleen. Thus, the data indicate that transcripts for both NKG2-D and CD94 are preferentially expressed in NK cells, although there are differences in expression among individual NK cells.

Physical Mapping of Nkg2d and Cd94 Within the NKC. Previous studies on other C-type lectins encoded in the NKC have demonstrated that many of these genes, including Nkrpl and Ly49u, display restriction fragment length polymorphisms (RFLP) between the C57BL/6 and BALB/c strains (50, 53). Southern blot analysis of genomic mouse DNA derived from nine inbred strains (including C57BL/6 and BALB/c) digested with multiple individual enzymes revealed RFLPs between C57BL/6 and BALB/c for both mNKG2-D and mCD94. For example, a RFLP for mNKG2-D was detected on PvuII digest,

Table 1. Amino acid identity of representative C-type lectins expressed on mouse, human, and rat NK cells

<table>
<thead>
<tr>
<th>Lectin</th>
<th>With mNKG2-D</th>
<th>With mCD94</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat NKG2-D (NKR-P2)</td>
<td>81</td>
<td>28</td>
</tr>
<tr>
<td>hNKG2-D</td>
<td>60</td>
<td>24</td>
</tr>
<tr>
<td>mCD94</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Rat CD94</td>
<td>24</td>
<td>77</td>
</tr>
<tr>
<td>hCD94</td>
<td>29</td>
<td>55</td>
</tr>
<tr>
<td>hNKG2-A</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>hNKG2-C</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>hNKG2-E</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Ly49-A</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>mNKR-P1A</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>mNKR-PIC</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>mCD69</td>
<td>21</td>
<td>23</td>
</tr>
</tbody>
</table>

Compared with murine NKG2-D and CD94. Data are from refs. 15, 19, 20, 35, 36, 40, 50–52. The identities were calculated by using the GAP program available from GCG sequence analysis software (version 8.0).

whereas RFLPs for mCD94 were detected on HindIII and EcoRI digests (data not shown). These RFLPs should be useful for genetic analysis of the NKC.

To more precisely map the location of both genes, the NKG2-D and CD94 cDNA probes were hybridized to Southern blots containing YACs representing our previously assembled >2 megabase contig of the NKC, containing 14 YACs (39). The mCD94 and the mNKG2-D probe hybridized only to YACs 9C10, 95E6, 195A10, and 178C4 (Fig. 4 and data not shown). Note that neither mNKG2-D nor mCD94 probes hybridized to the flanking YACs 85D11 and 52A6, which contain the Nkrpl and the Ly49 gene clusters, respectively. PCR analysis using mNKG2-D and mCD94 sequence-specific primers on YAC DNA templates

Fig. 3. Northern blot analyses of mNKG2-D and mCD94 transcripts. (A) Ten micrograms of total cellular RNA from the indicated cells and cell lines was hybridized with full-length mNKG2-D cDNA. (B) Twenty micrograms of total cellular RNA from the indicated organs and cultured cells were hybridized with full-length mCD94 cDNA. (C) Twenty micrograms of total cellular RNA from the indicated LAK cell populations and cell lines were hybridized with the mCD94 cDNA. All of the blots were rehybridized with a human β-actin probe to control for RNA quality and content, as shown below.
We used murine ESTs homologous to hNKG2-D and CD94 to isolate cDNAs for mouse NKG2-D and CD94 from C57BL/6-derived IL-2-activated NK cells, providing another example of the utility of dbEST as a resource in molecular cloning (47). Although both cDNAs encode molecules with high degree of similarity to other murine NK cell C-type lectin-like receptors, sequence alignment demonstrated that the ORF of LAK cDNA clone 2.1 showed the highest degree of homology to hNKG2-D (60% identity). Notably, murine CD69, a monomorphic C-type lectin also mapped to the NKC (5), has a similar degree of homology to its human orthologue (58% amino acid identity) (40, 51). LAK cDNA clone 1.3 encodes a molecule that is related to hCD94 with similar amino acid identity and is closely related to rat CD94 (55% and 77% amino acid identity, respectively). Therefore, clone 2.1 contains the murine homologue of hNKG2-D, and clone 1.3 is the murine homologue of hCD94.

The deduced murine NKG2-D and CD94 polypeptides share several features with the well-characterized Ly-49 family of NK cell receptors (38). These features include structural aspects, such as type II integral membrane protein orientation and C-type lectin homology. Although human homologues of Ly-49 have yet to be described, and CD94/NKG2 molecules share functions with Ly-49, including NK cell selective expression, MHC class I specificity, and inhibitory activity, our data clearly indicate that murine NK cells can coexpress Ly-49, CD94, and NKG2-D molecules. Although definitive protein expression analysis awaits the development of specific serological reagents, the RNA expression patterns of these molecules indicate that the repertoire of murine C type lectin NK cell receptors is larger than initially appreciated.

The function of mCD94, because it also contains a short cytoplasmic domain, is likely to be similar to hCD94, which requires a partner chain (16, 17). Consistent with this hypothesis, murine CD94 contains charged residues in its transmembrane domain, as does hCD94, suggesting that it will also pair with mouse NKG2 molecules. Although we have not yet identified the mouse homologues for the other human NKG2 family members, recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25). Recent studies indicate that such homologues are expressed on rodent NK cells (37). The specificity of human CD94/NKG2-A molecules has been controversial with apparent specificity for a wide range of HLA-A, -B, -C, and -G molecules (21–25).
strates it is expressed in NK cells that lack CD94 expression, suggesting it may pair with another partner chain. Like the human and rat orthologues, the cytoplasmic domain of mNK2-D lacks conserved ITIM sequence motifs, indicating that mNK2-D may not function as an inhibitory receptor but rather may be stimulatory, as has been demonstrated for hNK2-C (26, 36). Thus, these data suggest that murine NK2-D molecules may function as activation receptors that are independent of CD94.

Nkg2d and Cd94 are positioned within ~50 kb of each other in the mouse NKC, indicating the presence of another C-type lectin cluster. This is supported by data that demonstrate the human NKG2A/B/-C/-D/-E, and -F genes are tightly clustered with human CD94 within the NKC on chromosome 12p (54). This predicts that additional members of the mouse NK2 family will reside in this cluster, which we are currently investigating. Presently, we have shown that the murine Nkg2d and Cd94 loci are located in the NKC between Cd69 and the Ly49 gene cluster. Disser et al. (35, 55) have reported an essentially identical synteny position for rat Cd94 in the NKC on rat chromosome 4. Additionally, the human NKG2A/-C, and -E and Cd94 loci have been mapped to the NKC by using gene-specific primers and PCR (56). Therefore, the identification of the CD94/NKG2 gene cluster further illustrates the conservation and complexity of the NKC.

Importantly, several phenotypic traits have been genetically linked to the NKC. In rats, the lymphocyte alloreactivity is mediated by NK cells that can apparently recognize MHC allolarigens (55). This gene, termed Nka, is linked to the rat NKC and segregates with rat Ly494 separately from rat Nkp1, Cd94, and Nkg2d, raising the possibility that the Ly49 activation or inhibitory receptors may be responsible for recognition in this system. In mice, three additional phenotypic markers have been linked to the NKC. Chok encodes the capacity of C57BL/6-derived NK cells to kill Chinese hamster ovary target cells that is lacking in BALB/c-derived cells (A. Idris and W.M.Y., unpublished results). Cmv1 controls NK-mediated host resistance to otherwise lethal murine cytomegalovirus infection (57). Similarly, Rmp1 controls host resistance to mousepox virus (58). CD94 and/or NK2D may be related to any of these traits not only because they map physically and genetically to the same region but also because they are known to contribute toward NK cell target recognition and effector function. Therefore, the identification of murine CD94 and NK2-D contributes toward understanding the complex nature of NK cell regulation and function.

Note Added in Proof. The EST cloning of murine CD94 recently was described by Vance et al. (59).

We thank Beatrice Plougastel for helpful discussions and critical review of this manuscript. We gratefully acknowledge the use of the Frederick Biomedical Supercomputing Center, National Cancer Institute, Frederick, MD. This work was supported by grants from the National Institutes of Health and Barnes–Jewish Research Foundation. E.L.H. is a recipient of the American Heart Association Medical Student Fellowship 9501021A. J.W.M. is a fellow of the Howard Hughes Medical Institute. M.G.B. is a recipient of a National Research Service Award from the National Institute of Allergy and Infectious Diseases. W.M.Y. is an investigator of the Howard Hughes Medical Institute.