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Transcriptional Control of Murine CD94 Gene: Differential Usage of Dual Promoters by Lymphoid Cell Types

Brian T. Wilhelm,‡‡ Josette-Renée Landry,‡‡ Fumio Takei,‡‡ and Dixie L. Mager‡‡

The CD94 gene product is involved in controlling NK cell activation, and is one of a family of immune receptors that is found in the NK gene complex in both humans and mice, adjacent to members of the NKG2 family. CD94 forms a heterodimeric complex with several members of the NKG2 family on the surface of NK, T, and NKT cells. These complexes recognize the nonclassical MHC class I molecules HLA-E and Qa-1 in humans and mice, respectively. The mechanism for cell type-specific expression of CD94 and other genes from the NK gene complex has not yet been elucidated. In the current study, we show that the murine CD94 gene has two promoters, one of which is upstream of a previously unidentified exon. We illustrate by quantitative real-time PCR that lymphoid cell types use these two promoters differentially and that the promoter usage seen in adult cells is already established during fetal development. We determined that the differential promoter usage by NK cells appears to be susceptible to perturbation, as both the murine NK cell line LNK, as well as cultured C57BL/6 NK cells showed altered promoter usage relative to fresh NK cells. Furthermore, the promoter activity observed in transfection assays did not correlate with expression of the endogenous CD94 gene, suggesting the involvement of chromatin structure/methylation in transcriptional regulation. Our detection of DNase I hypersensitive sites at the CD94 locus that are present only in a cell line expressing endogenous CD94 supports this hypothesis. The Journal of Immunology, 2003, 171: 4219–4226.

Natural killer cells are cytolytic cells that function as part of the innate immune system. Target cells become susceptible to killing by NK cells in part by their down-regulation of cell surface MHC class I expression (1–4). The receptors that regulate the activity of NK cells have been identified in both humans and mice and can be grouped into two main classes based on protein structure. In mice, the largest family of mouse receptors belong to the lectin-like Ly-49 multigene family (5), whereas in humans, members of the killer Ig-like receptors (KIRs) that are part of the Ig superfamily perform the same role (5, 6). Although C-type lectin receptors are present in humans and Ig-like receptors are present in mice, these two species appear to favor the predominant use of only one of the two groups of receptors for interaction with MHC class I molecules, based on the number and variation of genes in each family. In apparent agreement with this theory, it was believed that the only receptors that functioned as homologues between the two species were the CD94/NKG2 family of C-type lectin genes, which in both species interact with nonclassical MHC class I molecules (7, 8). Two recent publications, however, have shown that the KIR receptors are also present in mice (9, 10), and therefore may also function in a similar fashion in both species.

Because of their initial characterization, experimental evidence has demonstrated a role for the heterodimeric CD94/NKG2 receptors in the regulation of immune responses in NK cells (11–14) as well as subsets of T cells (15, 16). Interestingly, while some of the NKG2 gene products, such as NKG2A, form inhibitory heterodimers with CD94, others such as NKG2C and E form activating complexes in association with CD94 and a signaling adaptor molecule DAP12 (14, 17). Despite the data collected on their function, relatively little is known about how the expression of these genes is regulated. Previous studies have shown that nearly all fetal mouse NK cells express CD94/NKG2 on their surface (18) before there is any detectable expression of the Ly-49 genes, with the exception of Ly-49e (19, 20). This high frequency of expression decreases after birth, when Ly-49 expression begins to approach adult levels (21, 22), and so it appears that there is a developmental switch between the predominant receptors expressed by mouse NK cells. It has been subsequently demonstrated that CD94/NKG2 expression in fetal NK cells is responsible, at least partially, for their ability to distinguish between MHC I\textsuperscript{hoch} and MHC I\textsuperscript{high} target cells (23, 24). The requirement for CD94/NKG2 expression for self-tolerance by NK cells is not absolute; however, as a recent report noted, the in-bred mouse strain DBA/2J, which develops normally, does not express CD94/NKG2 on the cell surface (25).

Aside from changes in expression during development, it has been shown that altered expression of CD94/NKG2 may lead to, or exacerbate, pathological conditions. In experiments using the oncoengenic polyomavirus, it was found that mice susceptible to tumor development had mature antiviral CD8\textsuperscript{T} T cells whose CD94/NKG2A expression was up-regulated as a result of the infection (26). This up-regulation was sufficient to impair the cytotoxic action of T cells allowing tumor development. A similar loss of NK-mediated cytotoxicity and up-regulation of CD94/NKG2 expression was seen with NK cells cocultured with renal carcinoma cells (27).

At the level of transcriptional regulation, the promoter of the human CD94 gene has been previously characterized by primer extension to the region just upstream of the translation start codon in the first exon (28), and our group previously reported that the
sequence in this region shows a high level of conservation with the mouse CD94 gene (29). In the current study, we extend these findings by demonstrating that a short region upstream of the translation start codon of either the mouse or human CD94 gene can act as a strong lymphoid-specific promoter. We have also identified a novel upstream promoter of mouse CD94 that is active in various lymphoid cells. Our results indicate that the two CD94 promoters are used differentially by lymphoid cell types beginning during fetal development. A role for altered chromatin/methylation patterns in the transcriptional regulation of CD94 is also suggested by our DNase I hypersensitive site (HSS) experiments. Finally, in the course of our investigation, we have precisely determined the nature of the CD94 defect in DBA2/J mice. The present work therefore provides insight into how CD94 is transcriptionally regulated.

Materials and Methods

Mouse strains

Fetal C57BL/6J pups and 1- to 2-mo-old C57BL/6J mice and DBA2/J mice (The Jackson Laboratory, Bar Harbor, MI) were used for all experiments. The EL-4 cell line was cultured in DMEM supplemented with 5% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin. The LNK cell line was obtained from K. Nakanishi through the laboratory of S. Anderson (National Cancer Institute, Frederick, MD) and was cultured in RPMI 1640 containing 50 µM 2-ME, nonessential amino acids, 5% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, t-glutamine, HEPES, and IL-2 (8000 IU/ml; Chiron Canada, Ville St-Laurent, Quebec, Canada). An IL-2-independent version of the CTL-L-2 cell line was obtained from T. Gonda (Hanson Centre for Cancer Research, Adelaide, Australia) and was grown in RPMI 1640 containing 50 µM 2-ME, nonessential amino acids, 5% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, and t-glutamine. NIH 3T3 cells were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin. LNK and CTL-L2 cell lines were transfected using the DEAE-dextran transfection kit and protocol supplied (Aus肠胃 Biosciences, Baie d’Urfe, Quebec, Canada), and EL-4 and NIH 3T3 cells were transfected using Lipofectamine (Invitrogen Life Technologies, Burlington, Ontario, Canada), as previously described (31) or according to the manufacturer’s protocol, respectively. All transfections were performed twice in duplicate for each cell line tested. To control for variations in transfection efficiency, dual transfections with the PRL-TK vector backbone. HSS enhancer constructs were generated by digesting the constructs created above with BamHI and ligating the PCR product of the entire first intron of the CD94 gene (using primers in Table I) into the BamHI site. All constructs were sequenced to confirm sequence integrity.

Transfections and cell culture

The EL-4 cell line was cultured in DMEM supplemented with 5% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin. The LNK cell line was obtained from K. Nakanishi through the laboratory of S. Anderson (National Cancer Institute, Frederick, MD) and was cultured in RPMI 1640 containing 50 µM 2-ME, nonessential amino acids, 5% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, t-glutamine, HEPES, and IL-2 (8000 IU/ml; Chiron Canada, Ville St-Laurent, Quebec, Canada). An IL-2-independent version of the CTL-L-2 cell line was obtained from T. Gonda (Hanson Centre for Cancer Research, Adelaide, Australia) and was grown in RPMI 1640 containing 50 µM 2-ME, nonessential amino acids, 5% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, and t-glutamine. NIH 3T3 cells were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin. LNK and CTL-L-2 cell lines were transfected using the DEAE-dextran transfection kit and protocol supplied (Aus肠胃 Biosciences, Baie d’Urfe, Quebec, Canada), and EL-4 and NIH 3T3 cells were transfected using Lipofectamine (Invitrogen Life Technologies, Burlington, Ontario, Canada), as previously described (31) or according to the manufacturer’s protocol, respectively. All transfections were performed twice in duplicate for each cell line tested. To control for variations in transfection efficiency, dual transfections with the PRL-TK Renilla luciferase vector were performed, and all firefly luciferase values were normalized by the Renilla activity. The activity of the constructs was made relative to an empty PGL3B vector (modified with additional upstream poly(A) signals) with the SV40-promoted PGL3B as a positive control.

Cultured splenocytes

Spleens were homogenized, and RBC were removed from single suspensions by a 20-s lysis with ice-cold distilled water. Remaining cells were washed twice with PBS and incubated for 1 h in a medium-filled packed

Table I. Primers used

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD94 5’ race</td>
<td>CTGGATTTGGGGCACAAGAAAGGCTTG</td>
</tr>
<tr>
<td>CD94 5’ race nested</td>
<td>GCAGAAACAAGAATTACCTTTGAC</td>
</tr>
<tr>
<td>CD94 RT exon 1b sense</td>
<td>CTCCTGAAGATACCTTCTAGG</td>
</tr>
<tr>
<td>CD94 RT exon 1b antisense</td>
<td>TGGATTTATCAGCAAAACTCCCAAAG</td>
</tr>
<tr>
<td>CD94 RT exon 1a antisense</td>
<td>CAACGGTCGCGCATCTGCAAAGGAC</td>
</tr>
<tr>
<td>GADPH RT sense</td>
<td>TGTCAGAGATTGTGTTGTTGTT</td>
</tr>
<tr>
<td>GADPH RT antisense</td>
<td>AACGAGCCCTCTTCAAGAC</td>
</tr>
<tr>
<td>CD94 construct exon 1a 5’ long</td>
<td>CTCCAGACATACATCAGCAG</td>
</tr>
<tr>
<td>CD94 construct exon 1a 5’ short</td>
<td>GGCTGGTTGGTGATTCCTCTTCC</td>
</tr>
<tr>
<td>CD94 construct exon 1a 3’ base</td>
<td>GGCTGTCATTACATCCTGGATCATAG</td>
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<td>CD94 construct exon 1b 5’ long</td>
<td>CAAGACTCTCTAGCAACCCCTATGTTG</td>
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<tr>
<td>CD94 construct exon 1b 5’ short</td>
<td>CGGTGATCTTGAGAAGGCTGAGAG</td>
</tr>
<tr>
<td>CD94 construct exon 1b 3’ base</td>
<td>CGGCTGATCTTTAGGCAAAATATG</td>
</tr>
<tr>
<td>Human CD94 construct 5’</td>
<td>GAAGACTCTGGACACATACCTCCTGCAAGGA</td>
</tr>
<tr>
<td>Human CD94 construct 3’</td>
<td>GGAGTACGGGAGTACGAGGAGGAC</td>
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<td>CD94 exon 3</td>
<td>AAATCAATCTGGCAGTTCCTCAGA</td>
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<td>CD94 exon 1a</td>
<td>AATTCTACAGTGGTGGTTGGAGAAG</td>
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<td>CD94 exon 4</td>
<td>AAACACATACATCCTACATTGAC</td>
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<td>CD94 exon 6</td>
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<td>CD94 RT exon 5 sense</td>
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<tr>
<td>CD94 RT exon 6 antisense</td>
<td>TTTTACAGGATGCAGGAAAGG</td>
</tr>
<tr>
<td>CD94 HSS enhancer 5’</td>
<td>CGGGGACCTCTCTGAGTATCCCTAGG</td>
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<tr>
<td>CD94 HSS enhancer 3’</td>
<td>CGGAGTCACCTCCCTAGTGATCCTAG</td>
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<tr>
<td>DBA2/J defect 5’</td>
<td>AAAAGAGAGTTTGCTTATG</td>
</tr>
<tr>
<td>DBA2/J defect 3’</td>
<td>AAATCAGTCTGGCAGTTCCTCAG</td>
</tr>
</tbody>
</table>

a RT, Reverse transcription.
nylon wool column. Cells were slowly eluted from the column, washed, and cultured in RPMI 1640 containing 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, L-glutamine, and IL-2 (8000 IU/ml). After 3 days, the medium was changed and all nonadherent cells were removed by washing twice with PBS. Cells were cultured for another 3–6 days before RNA extraction.

Flow cytometry

Adult splenocytes and fetal (18–20 days postcoitus) liver samples were prepared as single-cell suspensions, stained with labeled Abs, and analyzed by flow cytometry. Cell suspensions were pretreated with a culture supernatant containing anti-FcRγII/H9253, 2.4G2 to prevent FcR binding by labeled Abs. Primary mAbs directed against the following cell surface markers were used: NK1.1 (PK136) PE, CD94 (18d3) biotin, NKG2A/C/E (20d5) FITC, CD49b (DX5) PE, CD3 (H9280) (145-2c11) FITC, and a secondary reagent, streptavidin-PE, used with the CD94 Ab, were all purchased from BD Biosciences (Palo Alto, CA). All of the flow cytometry and sorting was performed on a FACS Vantage SE (BD Biosciences) using CellQuest software.

Northern blot analysis

RNA was isolated from EL-4 and LNK cells using TRIzol, as described by the supplier (Invitrogen Life Technologies). Following the elimination of remaining genomic DNA with DNase I (Invitrogen Life Technologies), 10 μg of RNA from EL-4 and LNK cells was electrophoresed on a 1.2% agarose, 5% (v/v) formaldehyde, 1× MOPS buffer gel and transferred to a Zetaprobe membrane (Bio-Rad, Mississauga, Ontario, Canada). The Northern blot was hybridized in ExpressHyb (BD Biosciences) at 68°C with a CD94 cDNA fragment corresponding to the nearly full-length transcript. The membrane was washed twice for 20 min in 2× SSC, 0.05% SDS at room temperature, followed by two washes of 20 min in 0.1× SSC, 0.1% SDS at 50°C. To confirm the amounts of mRNA loaded in each lane, the blots were rehybridized with a human 1.9-kb actin cDNA fragment.

Quantitative real-time PCR

RNA to be used for real-time PCR was isolated, as described above, and treated with DNase I (Invitrogen Life Technologies) to remove any possible DNA contamination and reverse transcribed, as previously described (30), using random hexamers. The RNA from FACS-sorted NK, T, and NKT cells was diluted based on the absolute cell numbers collected (5 × 10^7–5 × 10^8) so that the RNA concentrations were identical before reverse transcription. Quantitative real-time PCR was performed using 1 μl of the prepared CDNA along with 22 μl of water, 1 μl of each of the two primers, and 25 μl of the 2× Sybr green PCR master mix (PE Applied Biosystems, Foster City, CA) and the following amplification conditions: 30 s at 95°C, 30 s at 65°C, and 30 s at 72°C for 40 cycles on a Bio-Rad iCycler. Primers were designed according to PE Applied Biosystems recommendations, and differences in primer amplification efficiency were calculated, as recommended. For each experiment, dissociation curve analysis was performed to verify the presence of only a single PCR product. The relative quantification of the transcripts was derived using the comparative threshold cycle method (PE Applied Biosystems User Bulletin 2, ABI PRISM 7700 Sequence Detection system) supplied by the manufacturer. All quantitative real-time PCR experiments were performed twice in duplicate.

FIGURE 1. A, Sequence of the novel upstream exon and surrounding region. The sequence of the 5' region of the mouse CD94 gene is shown where the translational start codon in exon 1b is underlined and the bold sequence corresponds to the upstream exon 1a region. Bent arrows above and below the sequence lines represent the end points of one or more sequenced 5' RACE products that originated in exon 1a or 1b, respectively. Splice donor and acceptor sites are shown by vertical lines marked by SD and SA, respectively, and predicted transcription factor binding sites are shown as boxed regions of sequence with the factor names below. B, A schematic diagram of the mouse CD94 gene showing the exons of the gene as large numbered boxes with the novel 5' exon shown as 1a. The distance between the two alternative first exons is shown, and the regions of DNA cloned for promoter constructs are shown to scale below.
DNA fragment from the proximal promoter region of the proteinase K digestion overnight. DNA was then cleaved with the appropriate restriction enzyme, electrophoresed, Southern blotted, and probed. A DNA fragment from the proximal promoter region of the Lck gene previously shown to indicate HSS in the EL-4 cell line (33) was used as a positive control for the technique. The murine CD94 gene has a novel upstream exon that is expressed by the cell line LNK, but not EL-4. A, FACS profiles of the EL-4 and LNK cell lines stained with anti-CD94 and anti-NKG2A/C/E Abs. B, Northern blot of RNA collected from the EL-4 and LNK cell lines. In the top panel, a fragment of the actin cDNA was used as a probe. In the bottom panel, a portion of the CD94 cDNA was used as a probe.

DNase I HSS scan

The DNase I HSS assay was performed, as previously described (32). Briefly, DNA was collected from LNK and EL-4 cell lines, in which cell pellets were lysed using a Dounce homogenizer in RSB Nonidet P-40 (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet P-40). Nuclei were collected and further homogenized. The nuclei were then treated with varying concentrations of DNase I before inactivation and proteinase K digestion overnight. DNA was then cleaved with the appropriate restriction enzyme, electrophoresed, Southern blotted, and probed. A DNA fragment from the proximal promoter region of the Lck gene previously shown to indicate HSS in the EL-4 cell line (33) was used as a positive control for the technique. The CD94 probes used were the 1.2-kb proximal promoter construct insert and the 600-bp distal promoter construct insert. Hybridization conditions were performed as per the ExpressHyb (BD Biosciences) protocol.

Results

The mouse CD94 gene has a novel upstream exon

To identify the transcriptional start point of the CD94 gene, we performed 5’ RACE on splenocyte cDNA. The clones that were isolated contained sequence that extended beyond the previously presumed 5’ end of the gene. We determined that the sequence originated from a previously unidentified exon in the 5’ untranslated region (UTR) of CD94. The novel 278-bp exon, called exon 1a, is ~3.3 kb upstream of the previously identified first exon, hereafter named exon 1b (Fig. 1). No canonical promoter sequences such as TATA or CAAT boxes could be identified upstream of the novel exon. Although rat expressed sequence tags sequences in this region (29), but it is not clear which, if any, of the regions exhibited high activity in all lymphoid cell lines tested. We have previously described (34), was positive for CD94/NKG2 expression at the cell surface by FACS (Fig. 2A).

To test for promoter activity of the region upstream of the novel exons 1a and 1b, we first attempted to identify a cell line that expresses CD94 endogenously, so as to have a permissive environment for promoter characterization. Only one NK cell line, LNK, which had previously been described (34), was positive for CD94/NKG2 expression at the cell surface by FACS (Fig. 2A).

We generated reporter constructs (as described in Materials and Methods) containing the sequence 5’ of exon 1a or 1b, which were then transfected into the CD94+ LNK cell line, as well as other lymphoid and nonlymphoid cell lines. The results of the luciferase transfection assays are shown in Fig. 3. Although none of the CD94 constructs tested had activity in the NIH 3T3 cell line, the 5’ promoter (upstream of exon 1a) exhibited limited activity, and the constructs from the 3’ promoter (upstream of exon 1b) had high activity in all lymphoid cell lines tested. The promoter activity of the region 5’ of exon 1b appears to be conserved between the human and mouse genes. The human CD94 construct that contains the 800 bp upstream of the human CD94 translational start codon exhibited high activity in all lymphoid cell lines tested. We have previously characterized the similarity between the human and mouse genes in this region (29), but it is not clear which, if any, of the regions conserved between species are involved in transcriptional regulation.

Interestingly, the promoter activity of the CD94 fragments did not correlate with cell surface expression of the endogenous gene. Both the CTLL-2 and EL-4 cell lines that were negative for CD94
expression by FACS (Fig. 2A and data not shown) had high luciferase activity. Through Northern blots (Fig. 2B) and quantitative real-time PCR (data not shown), we determined that this lack of CD94 expression in EL-4 cells is the result of a transcriptional defect rather than a problem with cell surface presentation. The high promoter activity observed, combined with the lack of endogenous gene expression, indicates that the trans-acting factors that bind to the promoter region are present, but that alterations in chromatin structure or methylation patterns might repress expression of the endogenous CD94 gene. This possibility is supported by the DNase I HSS scans discussed below.

The two CD94 promoters have lymphoid cell type-specific usage that is established before birth

In an effort to characterize the usage patterns of the two CD94 promoters, freshly isolated adult splenocytes and fetal liver cells from C57BL/6 mice were FACS sorted into NK, NKT, and T cell fractions from which RNA was collected. Real-time quantitative PCR was performed using primers that would distinguish between CD94 transcripts that originated from the upstream promoter (containing exons 1a and 1b) and those originating from both promoters (containing exons 1b and 2). The latter was used to represent total CD94 transcripts encoding functional proteins, as the translational start codon is in exon 1b.

As illustrated in Fig. 4A, the usage of the upstream CD94 promoter varied by lymphoid cell type, with adult NK cells using it almost exclusively. Conversely, T cells that expressed CD94 did so primarily from the exon 1b promoter. NKT cells, which express markers characteristic of both NK and T cells, had expression from both promoters. Furthermore, the trends in promoter usage seen in the adult cell types were present in the same late-stage fetal cell types, albeit to a lesser extent. Based on this observation, it appears the cell type-specific promoter usage begins at an early stage of development, with adult patterns being reached shortly after birth.

Discrepancies between the promoter usage observed by real-time PCR and 5′ RACE clones obtained from cultured NK cells led us to investigate the promoter usage by the LNK cell line as well as cultured NK cells. Adult mouse NK cells were isolated from bulk splenocytes using a nylon wool column and cultured in medium containing IL-2 for 7 days before collection of RNA. Real-time quantitative PCR was performed on these samples as well as on RNA collected from the LNK cell line, to compare the promoter usage of the cultured cells. Fig. 4B shows that, relative to freshly isolated NK cells, both cultured cells and the LNK cell line have significantly decreased exon 1a promoter usage. The decrease in exon 1a promoter usage in cultured NK cells was coordinate with a general down-regulation of CD94 expression after culturing (data not shown). Promoter usage of other cultured lymphoid cell types was not determined.

DNase I HSS correlate with endogenous CD94 expression

The fact that transient transfections of constructs containing the downstream CD94 promoter (exon 1b) had high activity in lymphoid cell lines regardless of endogenous expression led us to look for DNase I HSS in expressing and nonexpressing cells. We first verified that our procedure could identify DNase I HSS by detecting the previously described HSS at the distal promoter of the Lck gene in EL-4 cells (31) (data not shown). The region around CD94 was then examined in LNK vs EL-4 cells, and evidence for HSS in the expressing LNK cell line was obtained (Fig. 5). Initial CD94-specific hybridizations performed with the 1.2-kb fragment

![](http://www.jimmunol.org/)

**FIGURE 4.** A. Quantitative real-time PCR results of differential promoter usage by cell type. The cell types tested are indicated along the horizontal axis, while the vertical axis shows the percentage of the total transcripts that contain exon 1a. Fetal and adult cell types are indicated by shading patterns in the legend on the bottom of the figure. B. Quantitative real-time PCR results of differences in promoter usage in fresh cells compared with cultured cells. The figure indicates the percentage of CD94 transcripts that contain exon 1a in freshly isolated NK cells, NK cells cultured for 7 days (as described in Materials and Methods), or the LNK cell line. The error bars in A and B represent the pooled SD for the four samples (two reactions performed in duplicate) for each cell type.

![](http://www.jimmunol.org/)

**FIGURE 5.** DNase I HSS analyses of the EL-4 and LNK cell lines. A. Shows Southern blots prepared from DNase I-treated genomic DNA from EL-4 and LNK cell lines, digested with BglII. The expected size of the BglII fragment detected by the probe used is 9.3 kb, and the size ladder for the Southern blot is shown between the two blots. Numbered arrows on the right-hand side of the blot indicate the hypersensitive fragments generated in LNK DNA. B. Shows a scale diagram of the mouse 

Klr-1 and CD94 locus with the 9.3-kb BglII fragment indicated by the line under the exons of the genes. The positions of the major (1) and minor (2) HSS are indicated by numbered vertical arrows.
from the exon 1b promoter region (Fig. 5A) indicated that there were two HSS. The exon 1b promoter fragment initially used as a probe did not precisely locate the HSS, although the two sites could be qualitatively described as major and minor. To clarify the position of these sites, further hybridizations were conducted, using XhoI genomic digests and the short exon 1a promoter insert as a probe (data not shown). The positions of the sites derived from these hybridizations are indicated in Fig. 5B. The presence of these sites appears to correlate with CD94 expression, as they are detectable in the CD94-expressing cell line LNK, but not EL-4, which does not express CD94.

To further characterize the nature of the HSS, the region containing the stronger HSS (Fig. 5, site 1) corresponding to the second intron of the CD94 gene was placed into the enhancer position of the PGL3B vector of the previously tested shorter exon 1a (–600 bp) and exon 1b (–500 bp) constructs. In transfections of the four cell lines previously tested (Fig. 3), none of the new enhancer constructs showed activity higher than the original construct. Additionally, a multiple sequence alignment of this region in the human, rat, and mouse genes did not reveal any significant region of sequence conservation. Given the lack of effect of this region on reporter gene expression, and as it has been reported that transient transfections of DNase I HSS can show enhancer activity (35), our results suggest that if the CD94 DNase I HSS has a functional role, it may be necessary for it to be in the proper genomic context to have activity.

**The CD94 gene in the DBA2/J mouse strain is partially deleted**

Because of our interest in the transcriptional regulation of CD94 expression, we sought to characterize the nature of the defect in a recently reported mouse strain (DBA2/J) that lacked detectable protein and RNA expression of CD94 by FACS and Northern blotting, respectively (25). Surprisingly, quantitative real-time PCR products from either of the sets of exon 1a/b and 2 primers were generated in the same ratio in the DBA2/J substrain as in a CD94-expressing strain (C57BL/6) relative to an internal control (GADPH) (data not shown). In response to this finding, new primers were designed to test for the presence of the last two exons (5 and 6) of the CD94 transcript. Real-time PCR indicated that the product from these primers could not be amplified from the DBA2/J strain, whereas a product was easily detected from B6 mice. The results of a series of RT-PCR amplifications from FACS-sorted (DX5/NK1.1+ CD3−) adult NK cells from DBA2/J and B6 mice are shown in Fig. 6A. Although products from exons 1a to 3 were generated from both strains, products amplified from exons 4–6 or exons 2–6 could only be generated using B6 cDNA. These results suggest that there is some defect in the 3′ end of the CD94 gene in DBA2/J that does not prevent transcription, but does prevent translation and cell surface presentation of the protein. To clarify the nature of the defect, we PCR amplified, cloned, and sequenced a section of the 3′ end of the DBA2/J CD94 gene. This sequence, a portion of which is shown in Fig. 6B, shows that there is a 2.4-kb deletion of sequence (in comparison with B6) that includes exon 6 of the CD94 gene (Fig. 6C). The sequenced region spanning the deletion is available under GenBank accession AY331150. Why the CD94 transcripts were not detected in the previous report is not clear; however, at least one of the probes used in the published Northern blot was from the 3′ end of the CD94 transcript that would not be able to detect the deleted transcripts. As the defect in this mouse strain was not related to transcriptional regulation, the expression of CD94 in these mice was not investigated further.

**Discussion**

Previous work on NK receptor genes has suggested that their transcriptional control is complex. Even in cases in which trans-acting factors involved in NK gene cluster (NKC) gene regulation have been identified (36, 37), the effects seen have not been the same for all members of a given gene family. Our current work with the mouse CD94 promoter provides further evidence that genes within the NKC have a complicated pattern of transcriptional control.

The identification of a novel upstream CD94 promoter and the observation that various lymphoid cells use the two promoters differentially also offer a theoretical mechanism for the expression of NK receptors on non-NK cells.

It has been observed that small subsets of T cells express receptors usually found on NK cells in both humans (38) and mice.
of the endogenous structs had high activity in lymphoid cell lines that lack expression infection results in which the downstream (exon 1b) promoter con-
one promoter and T cells use another, does not explain our trans-
sites or response elements in the promoter regions of these genes.
many cytokines have not been defined, it is not possible to confirm the
naling in the microenvironment. As the full signaling pathways for
found to have similar activity as IL-15 (47). It is therefore possible
expression of CD94 has been demonstrated to be necessary to induce ex-
sion 1b promoter on developing human (46) and murine (47) NK cells; however, high dose IL-2 in the mouse system has also been
expression of the active CD94 gene was independent of the activity of IL-15 (42), similar to a recent report in which a majority of pathogen-specific CD8\(^+\) T cells up-regulated expres-
sion of CD94/NKG2A independent of the activity of IL-15 (40). In the past, IL-15 has been demonstrated to be necessary to induce expres-
upstream promoter (exon 1a) regions of the two promoters has been described for several of the
promoters has been described for several of the immune system genes with
complex promoter structure that appears to regulate cell type-specific
characteristics, including alternative first exons whose use varies dur-
the CD8\(^+\) T cell activity in viral infections (26), although this effect appears to be virus specific (40, 41). It has also been
shown that several cytokines, including IL-12 (42), TGF-\(\beta\) (43), IL-15 (44), and IL-10 (45), are capable of inducing expres-
CD94 gene clearly has a complex promoter structure that appears to regulate cell type-specific activity. Previously published reports of immune system genes with multiple promoters illustrate that the complex structure of the mouse CD94 promoter is not unique (33, 53, 54). Indeed, the usage of dual promoters has been described for several of the Ly-49 genes in a situation that might be analogous to CD94. The distal promoter of some Ly-49 genes was shown to have promoter activity in fetal cells and in bone marrow cells, possibly linking its usage to the initiation of Ly-49 expression in NK cells (55). In addition, the mouse NKR-P1C gene has also recently been shown to have a novel upstream noncoding exon that is differentially used during NK cell development, as well as a DNaSE I HSS upstream of the gene (35). It is very interesting that many NK receptor genes, at least in mice, share several common characteristics, including alternative first exons whose use varies during development as well as the presence of DNaSE I HSS, although this has not yet been demonstrated to occur in the case of Ly-49 genes. It will be interesting to characterize the transcriptional mechanisms of the murine NKG2 genes to see whether their promoter structures and usage are similarly complex or whether transcriptional regulation of CD94 is predominant in assuring proper cell surface expression of CD94/NKG2 dimers.

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References