Human CD94 Gene Expression: Dual Promoters Differing in Responsiveness to IL-2 or IL-15

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J Immunol 2003; 171:5277-5286; doi: 10.4049/jimmunol.171.10.5277
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Human CD94 Gene Expression: Dual Promoters Differing in Responsiveness to IL-2 or IL-15

Louis D. Lieto, Francisco Borrego, Chi-hyun You, and John E. Coligan

CD94 is a C-type lectin required for the dimerization of the CD94/NKG2 family of receptors, which are expressed on NK cells and T cell subsets. Little is known about CD94 gene expression and the elements that regulate CD94 transcription. In this study, we report that CD94 gene expression is regulated by distal and proximal promoters that transcribe unique initial exons specific to each promoter. This results in two species of transcripts; the previously described CD94 mRNA and a novel CD94C mRNA. All NK cells and CD94+, CD8+ αβ T cells transcribe CD94 mRNA. Stimulation of NK and CD8+ αβ T cells with IL-2 or IL-15 induced the transcription of CD94C mRNA. The distal and proximal promoters both contain elements with IFN-γ-activated and Ets binding sites, known as GAS/EBS. Additionally, an unknown element, termed site A, was identified in the proximal promoter. EMSA analyses showed that constitutive factors could bind to oligonucleotide probes containing each element. After treatment of primary NK cells with IL-2 or IL-15, separate inducible complexes could be detected with oligonucleotide probes containing either the proximal or distal GAS/EBS elements. These elements are highly conserved between mice and humans, which suggests that both species regulate CD94 gene expression via mechanisms that predate their evolutionary divergence. The Journal of Immunology, 2003, 171: 5277–5286.

N
atural killer cells are a class of lymphocytes that express a wide variety of activation receptors that allow them to recognize potential target cells, such as virally infected and tumorigenic cells, without prior sensitization. The consequences of NK cell activation are target cell lysis and/or the production of inflammatory cytokines, such as TNF-α and IFN-γ (1–3). Curiously, many ligands recognized by NK cell activation receptors are proteins expressed by normal cells, which poses an obvious danger to the host (4, 5). To prevent destruction of normal cells and unwanted inflammation, NK cells also express inhibitory receptors that recognize MHC class I molecules on potential target cells (6–8). MHC class I molecules are expressed by almost all normal cells, and inhibitory signals override activation signals by recruiting protein tyrosine phosphatases to the proximity of signal initiation (9, 10). Class I molecules play a sentinel role in protecting cells from the deleterious effects of NK cell recognition. In contrast, once the expression of class I molecules is down-regulated, as is often the case with viral infection or malignant transformation, NK cells are poised to strike by virtue of their recognition of endogenous ligands and virally encoded proteins (5).

In humans, three distinct types of NK cell inhibitory receptors for MHC class I molecules have been defined (6). The first family of receptors are type I transmembrane molecules belonging to the Ig superfamily and are called killer Ig-like receptors (KIR) (2, 10). A second class of receptor, named Ig-like transcripts, also belongs to the Ig superfamily (11). The only member of this family that is expressed on some NK cells is Ig-like transcript 2 (12, 13). The third type of inhibitory receptor is a heterodimer composed of CD94 covalently associated with NKG2A (or its isoformal NKG2B) (14, 15). The ligand for this receptor is the nonclassical class I molecule HLA-E (16–18). The fact that human NK cells express three different types of inhibitory receptors specific for class I molecules suggests that it is critical to tightly control the lytic and inflammatory tendencies of these cells.

In addition to its role in regulating NK cell function, evidence is rapidly accumulating that indicates CD94/NKG2A is also involved in regulating certain T cell functions. In HIV-infected individuals, the percentage of T cells that express CD94/NKG2A is significantly elevated (19, 20). In mice, the percentage of CD8+ T cells that express CD94/NKG2 (A) is dramatically increased after infection with Listeria monocytogenes (LM), polyoma virus, and lymphocytic choriomeningitis virus, such that CD94 can be considered a marker of T cell activation (21–23). In the case of polyoma virus infection, CD94/NKG2 (A) on CD8+ T cells was shown to inhibit effector function; in contrast, no inhibition of effector function was observed for CD8+ T cells in LM- or lymphocytic choriomeningitis virus-infected mice (22–24). This may indicate that inhibition of CD8+ T cell function only occurs in cases of persistent infection in which chronic inflammation needs to be controlled. In addition to regulating effector function, inhibitory receptors may play a role in T cell survival. For example, the acquisition of KIR molecules has been shown to promote the survival of CD8+ T cells (25, 26). In mice, it was recently shown that CD8+ T cells, which express high levels of CD94/NKG2 (A) as a result of LM infection, are protected from apoptosis (21). Thus, CD94/NKG2A and KIR inhibitory receptors not only play a vital role in regulating the lytic and inflammatory responses of NK cells, but almost certainly play an important role in T cell biology as well.

CD94 expression is vital for expression of all members of the CD94/NKG2 family of receptors. Currently, little data exist on what regulates gene expression of CD94/NKG2 receptors in NK or T cells. The CD94 gene is located within a 2.5-Mb region on
chromosome 12p12-p13 known as the NK complex that contains 19 genes coding for C-type lectins, including the NKG2 family (27, 28). It was previously reported that human CD94 had six exons, multiple start sites of transcription, and a TATA-less promoter (29). In this study, we report that dual promoters regulate CD94 gene expression, which leads to the transcription of two types of transcripts that differ in their 5′ untranslated regions (UTR). The promoters show differential sensitivity to IL-2 and IL-15, two cytokines that increase cell surface expression of CD94 on NK cells. In freshly isolated primary NK and CD8/αβ T cells, only the proximal promoter is active. Both promoters contain GAS/EBSS elements, which can form constitutive and inducible DNA-protein complexes. This double element contains a potential binding site for STAT family proteins known as an IFN-γ-activated site (GAS), as well as a potential Ets-binding site (EBS). The proximal promoter also contains an element with no homology to known motifs in close upstream proximity to the GAS/EBS site. Because IL-2 and IL-15 can regulate gene expression through the interactions of transcription factors with GAS and EBS motifs, it is likely that these two cytokines influence CD94 gene expression through the formation of DNA-protein complexes.

Materials and Methods

Cell isolation and culture conditions

Polyclonal NK and CD8/αβ T cells were isolated by negative selection from peripheral blood using the appropriate cell isolation kit (Miltenyi Biotec, Auburn, CA). The purity of the isolated cell populations was confirmed by flow cytometry. NK and CD8/αβ T cells were cultured in IMDM (BioWhittaker, Walkersville, MD) supplemented with 500 U/ml of rIL-2 (Biological Resources Branch, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD) or 5 ng/ml IL-15 (R&D Systems, Minneapolis, MN), 10% human AB serum (Bio-Whittaker), and 1-glutamine (BioSource International, Rockville, MD) at 37°C under an atmosphere of 5% CO2. NK and CD8/αβ T cell clones were generated, as previously described (16). The NK cell line was grown in RPMI 1640 (BioSource International) supplemented with 2 mM l-glutamine, 1 mM sodium pyruvate, 200 U/ml rIL-2, 5 μM plasmocin (Invivogen, San Diego, CA), and 10% FBS at 37°C under an atmosphere of 5% CO2. Whole cell extracts from freshly isolated NK cells or freshly isolated NK cells incubated with IL-2 or IL-15 for 24 h were prepared, as described (16). The proximal promoter deletion mutants were constructed by sequence analysis.

Flow cytometry

Flow cytometric analyses were performed on a FACSort cytofluorometer (BD Immunocytometry Systems, San Jose, CA). Direct immunofluorescent staining was performed using PE-conjugated anti-CD94 (HP-3B1) or PE-conjugated anti-CD56 (PN-IM2073) mAb (Beckman Coulter, Fullerton, CA). A PE-conjugated isotype-matched control mAb was used to monitor background-staining levels.

RNA isolation, 5′ RACE, RT-PCR, and sequence analyses

Total RNA was prepared from fresh primary NK, CD8/αβ T, cultured primary NK, CD8/αβ T, or cultured NKL cells using the RNAqueous Midi kit (Ambion, Austin, TX). RNA concentration was estimated by spectrophotometric analysis. The 5′ RACE was performed with the FirstChoice RLM-RACE kit (Ambion). Two custom CD94-specific antisense primers were used for nested PCR (primer 1, 5′-GAGTAAAGTGCTGGCATATA-3′; primer 2, 5′-CAGAAATTCATTCACTAC-3′). RT-PCR was performed using transcript-specific primers and the Superscript One-Step RT-PCR kit (Invitrogen, Carlsbad, CA). The expression of CD94 transcripts containing exon 1A or full-length exon 1B, as well as β-actin transcripts as an internal control, was determined using 1 μg of total RNA. The following primers were used: CD94, exon 1A, 5′-GCTCATCAAGTCTGAAAGAG-3′; CD94, exon 1B, 5′-GCTTATTCACTGAAAGAG-3′; CD94 exon 6, 5′-AGGTGAAAGATTTCTAC-3′; β-actin sense, 5′-CAGGAGAGATGCCCCAGATC-3′; β-actin antisense, 5′-TTGCTGATCCACCTACTGCTGG-3′. PCR products were electrophoresed on 1× Tris-boric acid EDTA, 1% agarose gels, and visualized with ethidium bromide staining. cDNA was ligated into the pCR2.1 vector using the TA cloning kit from Invitrogen. The ligation products were used to transform Top10F (Invitrogen, Carlsbad, CA). Single colonies were selected. DNA was isolated and PCR amplified using the primer sets described above. Amplification products were isolated and labeled using Big Dye v3.0 terminators (Applied Biosystems, Foster City, CA). Sequence analysis was performed by MWG Biotech (Highpoint, NC). Sequences were aligned with the canonical CD94 transcript using the Blast algorithm from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/).

EMSA

Whole-cell extracts from freshly isolated NK cells or freshly isolated NK cells incubated with IL-2 or IL-15 for 24 h were prepared, as described (30). Briefly, synthetic oligonucleotide probes (Table I) were labeled with [γ-32P]ATP, and binding reactions, gel separation, and detection were performed using 8 μl of whole cell extracts equivalent to 3 × 106 cells. For competition experiments, unlabeled double-stranded oligonucleotides were preincubated with cell extracts for 10 min at room temperature before the addition of the labeled probe.

Analysis of putative regulatory elements

Putative UTR functional elements were searched for using UTRscan (http://big1.hiust.org/UTRscan/). Putative transcription factor binding sites (b) were predicted using 8 μl of whole cell extracts equivalent to 3 × 106 cells. For competition experiments, unlabeled double-stranded oligonucleotides were preincubated with cell extracts for 10 min at room temperature before the addition of the labeled probe.

Table I. Oligonucleotide probes and competitors used in EMSA

<table>
<thead>
<tr>
<th>Sense Sequence (5′→3′)</th>
<th>dGAS/dEBS</th>
<th>pGasp/eEBS</th>
<th>Site A</th>
<th>FcγRI-GAS</th>
<th>Ets-1-PEA</th>
<th>mEts-1-PEA</th>
</tr>
</thead>
<tbody>
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<td>TGAGCACATTGTCCCAGAGGCC</td>
<td>TGATTTGTCTTCGAAAGATCACTCA</td>
<td>TGGCAAGTCATTGGACTTCTCATC</td>
<td>GTGTCCCCAGGAAAGGAC</td>
<td>GATTCGCCAGCAAGAATGCGA</td>
<td>GATTCGCCAGCAAGAATGCGA</td>
<td></td>
</tr>
</tbody>
</table>

* GAS motifs are underlined and EBS motifs are double underlined. Site A is italicized. Mutated nucleotides are in bold type.

* Oligonucleotides were previously described by Rameil et al. (47).

Commercial oligonucleotides were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).
Results

Identification of CD94 transcripts containing an upstream untranslated exon in human NK and CD8\(^{+}\) T cells

Based on primer extension mapping, multiple transcription initiation sites were previously reported for CD94 transcripts (29). Because the actual sequences of the 5' ends of these transcripts were unknown, we performed 5' RACE on total RNA isolated from NKL cells using a system that produces cDNA exclusively from mRNA with capped 5' ends. Sequence analyses verified the existence and location of the previously reported transcription initiation sites. However, several of the transcripts contained an initial sequence of 97 bp, underlined in Fig. 1A, that did not match the sequence of any of the previously reported mRNA isoforms of CD94. Subsequent analysis of the genomic sequence around the CD94 gene revealed that the 97-bp sequence was identical with a sequence of DNA beginning 3628 bp upstream from the translation initiation site and 3419 bp upstream from the consensus start site of the previously identified exon 1 (hereafter referred to as exon 1B). The 97-bp sequence is an untranslated exon that we have termed exon 1A. Transcripts that contain exon 1A make use of an acceptor site within exon 1B located 100 bp upstream from translation initiation site. These transcripts are 63 bp shorter than transcripts initiated from the consensus start site of transcription for exon 1B. A full-length transcript containing exon 1A has been sequenced and reported to GenBank as CD94C, accession number AY227806. All sequenced 5' ends of transcripts containing exon 1A had the same sequence, suggesting that these transcripts were initiated from a single start site of transcription. UTRscan analysis of the 5' UTR of transcripts with (CD94C) and without (CD94) exon 1A did not reveal any functional elements, such as iron response elements or internal ribosome entry sites, that could affect translation rates (31).

Transcript analysis of NK cell clones by RT-PCR shows that both species of CD94 transcripts are present in all clones (Fig. 2A), as well as the NKL leukemia cell line. All of the clones tested and the NKL cell line were positive for CD94 surface expression, as observed by flow cytometry. We also verified that the YT-Indy NK cell line does not express viable CD94 transcripts (data not shown). CD8\(^{+}\) T cell clones show a different pattern of CD94 transcript and protein expression from NK cells (Fig. 2B). Several of the examined clones express both species of CD94 transcript (e.g., T2-03); some express neither (e.g., T2-08); and others only express transcripts with exon 1B (e.g., T2-17). None of the CD8\(^{+}\) T cell clones expressed transcripts that only contained exon 1A. Many of the CD8\(^{+}\) T cell clones that expressed CD94 transcripts do not express CD94 on their surface. The existence of two species of transcripts that contain different 5' initial exons suggested that dual promoters regulate CD94 gene expression.

**FIGURE 1.** The 5' sequences of the CD94C (containing exon 1A) and the CD94 (exon 1B) cDNAs, and the genomic structure of the human CD94 gene showing derivation of these transcripts. A, The underlined portion of the CD94C cDNA identifies the sequence of exon 1A. An asterisk in exon 1B in the CD94 cDNA designates the location of the acceptor splice site for exon 1A in CD94C. Vertical bars indicate the exon-exon boundaries, and the translation initiation site is in bold. Boxes indicate the locations of ATGs upstream of the translation initiation start sites. Only the first ATG in exon 1A fits the Kozak consensus sequence ((A/G)xxATGG). B, A schematic of the CD94 gene structure showing the location of the exons that code for each transcript and the two promoters. The relative gene structure is displayed on the lower bar and the CD94 mRNA structure with a boxed coding sequence is displayed above. The dashed lines indicate how exon 1A and the 5' portion of exon 1B are incorporated into the CD94C transcript.
transcription factors plays critical roles in regulating a variety of NK, NKT, and T cell processes, including growth control, activation, perforin expression, and transformation (33–36). An element without homology to a previously identified regulatory element, as assessed by TFSearch, was identified near the −273 position, hereafter referred to as site A for the convenience of this report.

The distal promoter construct p-3807, which was the smallest promoter construct examined (179 bp), showed a maximal level of luciferase activity in the YT-Indy and NKL cell lines (Fig. 4A). YT-Indy cells that were transiently transfected with p-3807 construct had an average of ~85-fold higher luciferase activity than those transfected with pGL3-basic vector. Although the magnitude of luciferase activity was lower, a similar trend in relative intensity was observed when these constructs were transfected into NKL cells. Transfection of the p-3807 construct into E6-1 Jurkat T cells produced inconsequential changes in luciferase activity in comparison with the cells transfected with the pGL3 basic vector (data not shown). Analysis of the −3807 to −3628 region using TFSearch identified a GAS/EBS motif at −3697 to −3688. This site was mutated (see Materials and Methods) in the p-3807 construct with two single base pair changes within the dGAS/dEBS motif. This mp-3807 construct resulted in a ~50% decrease in luciferase activity in YT and NKL cells compared with the wt p-3807 construct (Fig. 4B).

**FIGURE 2.** NK and CD8^+^ αβ T cells express both CD94 and CD94C transcripts. These mRNAs were detected by RT-PCR and gel electrophoresis. The upper band present in the CD94C and CD94 rows contains a full-length coding sequence for CD94. The lower band contains an alternatively spliced coding sequence that lacks exon 2; see accession number NM007334 for CD94 transcript variant 2 (68). Transcripts were derived from either NK or CD8^+^ αβ T cell clones grown for 3 wk in the presence of IL-2 and feeder cells. Surface expression of CD94 was detected by flow cytometry and is indicated by a positive or negative sign. A, All NK clones tested were CD94+/CD94C+. B, Shown are several representatives of each class of CD8^+^ αβ T cell clone, CD94+/CD94C+, CD94+/CD94C−, and CD94+/CD94C−.

**Differential sensitivity of the distal and proximal promoters to IL-2 or IL-15 stimulation**

A variety of cytokines are known to activate STAT transcription factors via the Janus kinase-STAT pathway (32). Among these cytokines are IL-2 and IL-15, which have been shown to activate and induce proliferation of human NK cells (37, 38). In agreement with previously reported results, we show that treatment with IL-2 or IL-15 up-regulates CD94 surface expression in primary NK cells (Fig. 5A) (39–41). To determine whether transcription of CD94 and/or CD94C was regulated by these cytokines, primary NK cells were cultured with IL-2 or IL-15 for various time points and harvested, and total RNA was isolated. RT-PCR analyses show that CD94C is not transcribed in fresh, primary, resting NK cells or CD8^+^ αβ T cells, which indicates that the distal promoter is not active in these cells (Fig. 5, B and C). After treatment with either IL-2 or IL-15, CD94C transcripts were detectable at 1 h in primary NK cells and showed a steady increase in abundance up to 24 h later. This indicates that either IL-2 or IL-15 can induce transcription from the distal promoter in primary NK cells. CD8^+^ αβ T cells cultured with IL-2 can also express CD94C (Fig. 2). The proximal promoter is active in fresh NK cells and, under all conditions studied, the CD94 transcript appears to be the most abundant, although these results were not quantitated.

**Constitutive and inducible factors bind to CD94 distal and proximal elements in vitro**

The ability of the distal and proximal elements, containing GAS/EBS motifs and the proximal unknown motif (site A), to form protein-DNA complexes was tested by EMSA. Whole-cell extracts from fresh or IL-2-activated primary NK cells were incubated with oligonucleotide probes containing either the dGAS/dEBS, pGAS/pEBS, or site A elements (Table I). One constitutive (dc1) and one IL-2-inducible (dl1) DNA-protein complex were revealed with the dGAS/dEBS probe (Fig. 6A). Similarly, two complexes were observed with the pGAS/pEBS probe, one constitutive (pc1) and one IL-2 inducible (p11) (Fig. 6B). Two constitutive (SAC1, SAC2) DNA-protein complexes were identified with the site A probe (Fig. 6C). EMSA analysis of IL-15-stimulated NK cells indicated that
the DNA-protein complexes were identical with those from the IL-2-stimulated cells for all three probes (data not shown).

Proteins forming complexes with wild-type (wt) probes were further characterized by testing the ability of unlabeled competitor probes containing STAT and Ets binding sites (Table I) to inhibit binding of transcription factors to the wt probes. Competition with an excess of unlabeled wt-dGAS/dEBS eliminated both the dC1 and the dI1 complexes (Fig. 6B). Addition of a competitor containing a known GAS motif, FcyRI-GAS, effectively competed with the wt-dGAS/dEBS probe for formation of the dC1 and dI1 complexes. The mFcyRI-GAS competitor, which has a mutated GAS element, was unable to interfere with formation of these complexes. This suggests that the dC1 and dI1 complexes contain transcription factors that recognize the GAS motif common to both the dGAS/dEBS probe and the FcγRI-GAS competitor. Neither the Ets-1-PEA3 (polyomavirus enhancer activator 3) nor the mEts-1-PEA3 competitors containing a known and mutated EBS motif, respectively, were able to compete with the probe in forming these complexes.

The pC1 and pI1 complexes formed with the pGAS/pEBS probe were eliminated by competition with unlabeled wt competitor (Fig.
Addition of the FcγRI-GAS competitor was able to compete for binding with the wt probe in formation of the pI1 complex, but not the pC1 complex. This suggests that the pI1 complex contains transcription factors that recognize the GAS motif present in the pGAS/pEBS probe and FcγRI-GAS competitor. The Ets-1-PEA3 competitors were ineffective at competing for either complex. This shows that the pC1 complex contains transcription factors specific for the pGAS/pEBS element that do not interact with the FcγRI-GAS or the Ets-1-PEA3 competitor.

The two constitutive complexes bound to the site A probe were removed by competition with the unlabeled wt site A competitor (Fig. 6B). Addition of the FcγRI-GAS competitor was able to compete for binding with the wt probe in formation of the pI1 complex, but not the pC1 complex. This suggests that the pI1 complex contains transcription factors that recognize the GAS motif present in the pGAS/pEBS probe and FcγRI-GAS competitor. The Ets-1-PEA3 competitors were ineffective at competing for either complex. This shows that the pC1 complex contains transcription factors specific for the pGAS/pEBS element that do not interact with the FcγRI-GAS or the Ets-1-PEA3 competitor.

The two constitutive complexes bound to the site A probe were removed by competition with the unlabeled wt site A competitor (Fig. 6C). Located near site A within the probe used for EMSA assays was a putative EBS motif (Table 1). However, competition assays performed with Ets-1-PEA3 competitors failed to inhibit complexes formed with the wt probe.

**Discussion**

We have identified a novel transcript of the CD94 gene, designated CD94C, which contains an untranslated exon 1A located ∼3.5 kb upstream from the initiation of translation (Fig. 1B). The previously described CD94 transcript contains a partially untranslated
FIGURE 6. In vitro complexes formed by oligonucleotide probes containing dGAS/dEBS, pGAS/pEBS, and site A elements, detected by EMSA. Freshly isolated primary NK cells were stimulated with IL-2 and harvested at 24 h. Whole cell extracts were incubated with each probe and resolved by non-denaturing polyacrylamide gel electrophoresis. A. dGAS/dEBS. Time 0, IL-2, Sim-24–h, wt, FcγRI-GAS, mIL-1RI-GAS, ETS-1/1293, mETS-1/1293, lanes 3–5. B. pGAS/pEBS. Time 0, IL-2, Sim-24–h, wt, FcγRI-GAS, mIL-1RI-GAS, ETS-1/1293, mETS-1/1293, lanes 3–5. C. Site A. SAC1, SAC2. Time 0, IL-2, Sim-24–h, wt, ETS-1/1293, mETS-1/1293, lanes 3–5.
FIGURE 7. Analysis of the promoter elements. A, Comparison of the human GAS elements with the consensus sequence. Human GAS elements with the consensus sequence. The differences in the behavior of the pC1 and dC1 complexes in response to competition with the Fc-RII-GAS oligonucleotide competitor (Fig. 6, A and B), mentioned above, may be due to differences in the sequence surrounding each GAS/EBS motif and/or to the differences in the central nucleotide of the GAS motif (Fig. 7A). In the dGAS/dEBS element, the central adenine nucleotide begins a sense-oriented EBS motif. However, in the pGAS/pEBS element, the central thymine motif ends an antisense-oriented EBS motif (Table I). This opposite orientation of the EBS motif between the two elements may lead to differences in the transcription factor complexes that recognize each GAS/EBS element. Such differences may be why the transcription factors in the dC1, dI1, and pI1 complexes bind to the Fc-RII-GAS element, while the factors that constitute the pC1 complex do not. This implies that the pC1 transcription factors do not interact with the GAS motif in the pGAS/pEBS element and instead most likely bind with the EBS motif. These transcription factors probably recognize an element larger than the central EBS motif because they do not bind with the Ets-1-PEA3 competitor.

Evidence suggests that CD94 receptors originated early in the evolution of the chordates, of which vertebrates are a member. A receptor with homology to human CD94, termed BsCD94-1, was recently identified in Botryllus schlosseri, a urochordate (51). BsCD94-1 gene expression changes in response to allorrecognition by B. schlosseri and has been proposed to be a marker of ancestral NK cells. It is suggested that urochordates and vertebrates diverged over 750 million years ago (52). This would indicate that CD94 is an ancient receptor component.

Considering this, it is not surprising that CD94/NKG2 receptors are also found in both primates and rodents (53, 54). Examination of the murine CD94 gene has identified dual promoters that produce two species of transcripts analogous to human CD94 and CD94C (55). Comparison of the human and mouse distal and proximal promoter sequences reveals that the GAS/EBS elements are highly conserved and site A is partially conserved (Fig. 7B). In addition, comparison of the distal promoters revealed that a putative TATA box downstream from the dGAS/dEBS element is also conserved. Because all of the human CD94C cDNAs sequenced had the same 5’ UTR, it is possible that transcription is initiated from this conserved TATA box. Comparison of the MHC I receptors of mouse and human NK cells indicates that CD94 and the NKG2 families existed before the divergence of the rodent and primate lineages ~100 million years ago (56). The presence of functional dual promoters in both species and the conservation of elements within these promoters indicate that they most likely play an important role in the regulation of CD94 gene expression.

Why would regulation of the CD94 gene require two promoters? One possibility is that the two promoters exist to regulate expression in different tissues. For example, distinct promoters have been shown to regulate the expression of the protein kinase DYRK1B gene in different tissues (57). Because CD94 is expressed only by lymphocytes and our data indicate that, at least under the conditions examined, the two promoters do not distinguish expression between NK and T lymphocytes, it is unlikely that the two CD94 promoters exist to regulate cell type expression.

Another possibility is that the two promoters exist to insure expression of the CD94/NKG2A receptor, which may be particularly vital during early stages of development when KIR receptors are not yet expressed. One promoter may require transcription factors that are not present or active early in development, leaving the remaining promoter as the sole regulator of CD94 transcription. A recent study examining human NK cell receptor ontogeny in umbilical cord-derived stem cells showed that contact-dependent ligand stimulation, along with the presence of IL-2 and IL-15, was important for CD94 and KIR expression (39). After stimulation of single progenitor cells, the frequency of CD94+ NK cells was greater than KIR+ NK cells and CD94 expression occurred earlier. In adults, the majority of peripheral blood NK cells express CD94/NKG2A and at least one KIR receptor; however, the majority of the NK cells in synovial fluid in patients with rheumatoid arthritis are CD94+ and KIR2DL1/L2/L3+ (58). Because CD94/NKG2A are likely the only inhibitory receptors expressed by such NK cells, failure to express CD94 in these cells would likely have dire consequences for the host. Thus, dual promoters may exist to insure CD94 expression in cells in which no other inhibitory receptors are expressed. The use of dual promoters to regulate gene expression at different stages during development has been observed for the mouse μ-opioid receptor (59).

The possibility that the dual promoters exist to insure expression of a vital regulatory receptor can be extended to NK cells in general. Analysis of transcription in NK and CD8+ αβ T cells clearly

<table>
<thead>
<tr>
<th>A</th>
<th>Consensus GAS site</th>
<th>dGAS</th>
<th>pGAS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TCCT(A-T)GGAA</td>
<td>TICCGGGAA</td>
<td>TICCGHGGAA</td>
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<table>
<thead>
<tr>
<th>B</th>
<th>Distal Promoter</th>
<th>Proximal Promoter</th>
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<td>Human</td>
<td>Mouse</td>
<td>Human</td>
</tr>
<tr>
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<td>-3722</td>
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<tr>
<td>TATA box</td>
<td>GAS/EBS</td>
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shows that initiation of transcription from the CD94 distal promoter becomes active in cells that are stimulated by exogenous cytokines, such as IL-2 or IL-15. Therefore, the purpose of the distal promoter may be solely to insure CD94 gene expression upon activation of mature cells to guarantee the sensitivity of these cells for detection of MHC class I expression to prevent the targeting of healthy cells.

It is likely that CD94 regulation occurs at several levels. The cell surface expression of CD94 may be regulated at the protein level through sequestrating or receptor recycling (60, 61). It is clear that a sophisticated mechanism of translational regulation exists, especially in T cells in which the presence of CD94 transcripts does not guarantee protein expression (Fig. 2B). CD94 expression is probably influenced by the rate of transcription, mRNA stability, and translation efficiency, all of which may be affected by sequences in the 5′ and 3′ UTRs.

Analysis of the 5′ UTR regions of the CD94 and CD94C transcripts, using predicative algorithms, was unable to identify any functional elements such as iron response elements or internal ribosome entry sites (31). Both CD94 and CD94C have relatively long 5′ UTRs with AUGs upstream of the translation initiation start site (Fig. 1A). The presence of these 5′ UTRs would seem to violate the first AUG rule, stating that translation is initiated at the AUG codon closest to the 5′ end (62). However, in both CD94 and CD94C, the first AUG is followed shortly by an in-frame terminator codon. In this situation, the posttermination ribosomes generally resume scanning and can reinitiate at a downstream AUG site (62–64). Reinitiation is typically inefficient and can reduce translation productivity. It is likely that any issues related to premature initiation of translation affect the processing of the CD94 and CD94C transcripts equally. Moreover, examination of the thermodynamic stability of the 5′ UTRs of both transcripts by analyzing their predicted Gibbs free energy indicates that they both have the same relative propensity to form a stable structure (65, 66). The formation of stable structures can impair the movement of the ribosomal 40S subunits as they scan downstream (67).

In summary, we have characterized two promoters of human CD94, which contain conserved GAS/EBF elements that can form protein-DNA complexes. The two promoters differentially regulate expression of the CD94 gene in response to treatment with IL-2 or IL-15. Clarification of the precise roles of these two promoters and the factors that regulate them is under active investigation.

Acknowledgments

We thank Dr. Kerima Maasho and Robert Valas for technical assistance, and Dr. Brian Wilhelm, Dr. Jean Imbert, and Dr. Konstantin Khalutin for helpful discussion.

References


