



## BRIEF COMMUNICATION

# The NKG2 natural killer cell receptor family: comparative analysis of promoter sequences

C Brostjan<sup>1</sup>, Y Sobanov<sup>2</sup>, J Glienke<sup>3</sup>, S Hayer<sup>2</sup>, H Lehrach<sup>3</sup>, F Francis<sup>3,4</sup> and E Hofer<sup>2</sup>

<sup>1</sup>Department of Surgery, Vienna General Hospital 8G9.05, University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria;

<sup>2</sup>Department of Vascular Biology and Thrombosis Research, Vienna International Research Cooperation Center, University of Vienna, Brunnerstraße 59, A-1235 Vienna, Austria; <sup>3</sup>Max-Planck-Institute for Molecular Genetics, Ihnesstraße 73, D-14195 Berlin, Germany

*The NKG2 receptor family is crucially involved in target cell recognition by natural killer cells and comprises several activating as well as inhibitory family members. We have established approximately 3 kilobases of upstream promoter sequences of the human NKG2-C, -E and -F genes and have carried out a comparative analysis with available NKG2-A sequences. We found extended regions of homology which contain numerous putative transcription factor binding sites conserved in the NKG2 genes. However, variation in Alu insertion among family members has led to promoter structures unique to the respective family members, which could contribute to differences in transcriptional initiation as well as gene-specific regulation.* Genes and Immunity (2000) 1, 504–508.

**Keywords:** natural killer cell receptors; NKG2 genes; promoter sequences; transcriptional regulation

Natural killer (NK) cells recognize target cells that have lost or severely reduced their surface expression of MHC class I.<sup>1</sup> NK cell activation and cytotoxicity is regulated via a multitude of NK cell receptors recognizing class I molecules.<sup>2</sup> Co-expression of activating as well as inhibitory receptors seems to allow adaptation to self class I levels as well as fine-tuning of NK cell responses.<sup>3</sup> NK cell receptors can be grouped in two subtypes: killer immunoglobulin-like receptors (KIRs) have been characterized in the human system,<sup>4</sup> while lectin-like NK cell receptors have been described in the mouse as well as humans and are generally type II transmembrane proteins containing an extracellular C-type carbohydrate recognition domain.<sup>5</sup> Two multigene families of lectin-like

receptors have been identified, which are termed Ly49<sup>6</sup> and NKG2.<sup>7</sup>

The human NKG2 family comprises seven members referred to as NKG2-A, -B, -C, -D, -E, -F and -H, with A/B and E/H being splice variants of the same genes.<sup>7–9</sup> Even though occasionally generated via trans-gene splicing of a common NKG2-F/2-D transcript,<sup>9,10</sup> NKG2-D is only remotely related to the other NKG2 family members and constitutes a separate class of lectin-like receptors. All other NKG2 members share substantial sequence homology and NKG2-A, -B, -C, -E, as well as -H have been shown to form disulfide-linked heterodimers with the invariant CD94 protein.<sup>8,11</sup> Activating family members (NKG2-C and potentially -E, -F, -H) are characterized by the presence of a charged amino acid residue in the transmembrane domain mediating interaction with DAP-12, an adapter molecule containing an immunoreceptor tyrosine-based activation motif (ITAM).<sup>12,13</sup> In contrast, inhibitory NKG2 proteins (NKG2-A and -B) carry immunoreceptor tyrosine-based inhibition motifs (ITIMs).<sup>12,14</sup> Despite their distinct signaling capabilities, NKG2-A, -B and -C receptors have been found to recognize the same ligand, the non-classical HLA class I molecule HLA-E.<sup>15,16</sup>

We have previously characterized gene structure and genomic organization of human NKG2 members.<sup>10,17,18</sup> We found that NKG2-A, -C, -E and -F are closely linked (as listed) and of the same transcriptional orientation.<sup>10</sup> NKG2-A contains an additional 5' untranslated exon not detected in transcripts of the other NKG2 members.<sup>19</sup> Expression of NKG2 genes is limited to NK cells and a subset of cytotoxic T-cells (Tc).<sup>20</sup> Individual NKG2 members can be expressed separately or in combinations on NK as well as Tc subsets,<sup>12</sup> a process which seems to be regulated transcriptionally and is thus reflected in mRNA

---

Correspondence: Erhard Hofer Department of Vascular Biology and Thrombosis Research, Vienna International Research Cooperation Center, University of Vienna, Brunnerstraße 59, A-1235 Vienna, Austria  
E-mail: Erhard.Hofer@univie.ac.at

According to the Human Gene Nomenclature Database (www.gene.ucl.ac.uk/nomenclature) the various gene loci described in this manuscript have obtained the following symbols: KLRC1 (NKG2-A/-B), KLRC2 (NKG2-C), KLRC3 (NKG2-E/-H), KLRC4 (NKG2-F) and KLRD1 (CD94).

The nucleotide sequence data reported in this paper have been submitted to the GenBank / EMBL nucleotide sequence databases and have been assigned the accession numbers AF238468 (NKG2-C), AF238469 (NKG2-E) and AF238470 (NKG2-F).

This work was supported in part by grants from the Austrian National Bank (Jubiläumsfonds No 7567) and the European Commission (BIO-CT95-0062).

<sup>4</sup>Present address: Institut Cochin de Génétique Moléculaire, Unité 129 de l'Inserm, CHU Cochin-Port-Royal, 24 Rue du Faubourg Saint Jacques, F-75014 Paris, France.

Received 20 June 2000; revised 27 August 2000; accepted 29 August 2000

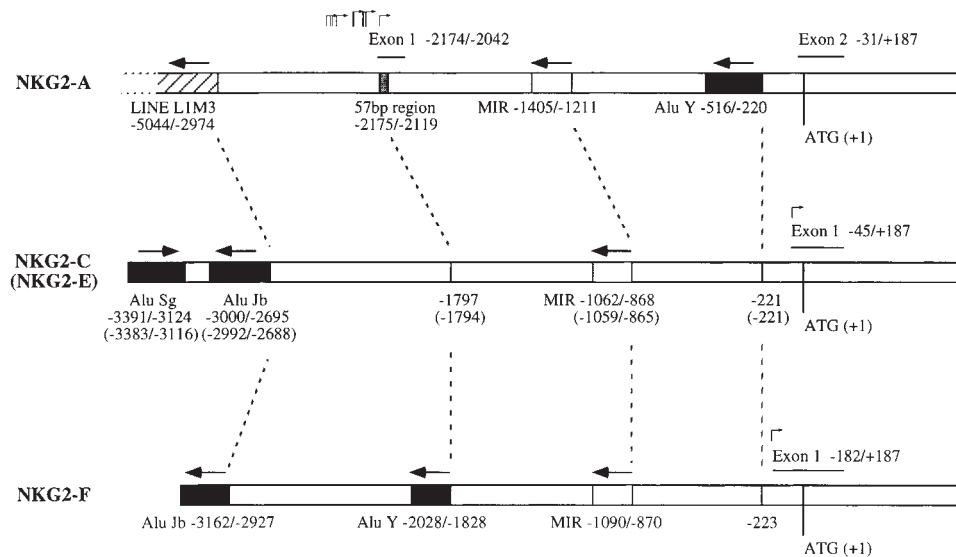
expression.<sup>20</sup> Development of an NK-like phenotype, ie, *de novo* expression of *CD94/NKG2-A* can be induced by cytokines such as IL-2, IL-15 and TGF- $\beta$  on immature thymocytes or Tc subsets.<sup>21,22</sup> *NKG2* mRNA levels can be further increased by prolonged treatment of NK cells with IL-2 or IFN- $\alpha$ , thus pointing to further regulation at the transcriptional level.<sup>20,23</sup> However, to date little is known about the promoter regions of *NKG2* genes, which should harbor various regulatory elements mediating cell type-specific expression as well as cytokine responsiveness.

Thus, we have now characterized the 5' promoter sequences of the *NKG2-C*, *-E* and *-F* genes and have carried out a comparative analysis with available *NKG2-A* sequences.<sup>19</sup> Upstream sequences of the human *NKG2-C*, *-E* and *-F* genes were established by double-strand sequencing of the respective subclones B46 (*NKG2-C*), E-15 (*NKG2-E*) as well as shot gun clones (*NKG2-F*) generated from a P1 artificial chromosome clone, PAC D21184, previously described by us to contain the entire *NKG2-C*, *-E*, *-F* and *-D* genes.<sup>10</sup> We found the *NKG2-F* regulatory region to be in close proximity with the 3' end of the *NKG2-E* gene.<sup>10,19</sup> The genomic sequence of *NKG2-A* (GenBank file AF023840) including putative regulatory regions has previously been published.<sup>19</sup> Alignment of sequences was carried out using the MacVector 6.5.1 analysis program (Oxford Molecular Ltd., Oxford, UK) and revealed substantial homology among all family members over a range of approximately 3 kilobases (Figure 1). The *NKG2-C* and *-E* regions were found to be highly similar (98% identity). Upstream sequences of *NKG2-F* were 75% identical with *NKG2-C* and *-E*, while

*NKG2-A* differed to a greater extent showing slightly closer homology to *NKG2-F* (60%) than to *NKG2-C* and *-E* (57%).

A search for repetitive DNA elements within the established sequences (using the RepeatMasker program at <http://ftp.genome.washington.edu/RM/RepeatMasker.html>) revealed a high frequency of *Alu* repeats in *NKG2* upstream regions (Figure 1). *Alu* sequences, which constitute roughly 5% of the human genome, occur at a frequency of approximately 1 element per 4 kb.<sup>24</sup> Promoter regions display a higher frequency of *Alu* insertions,<sup>25</sup> which correlates with our observation of 2 *Alu* elements present in 3 kb of the established *NKG2-C*, *-E* and *-F* sequences. *NKG2-A* contains a single *Alu* insertion, which is approximately 200 bp upstream of the start codon. The site of insertion as well as the *Alu* subtype and orientation were clearly distinct for the *NKG2* members examined, suggesting insertion events following duplication of the genes. Further repetitive sequences identified include a *MIR* element conserved in all family members as well as a long interspersed repeat (*LIM3*) interrupting the *NKG2-A* upstream region at approximately -3000 (start codon defined as +1).

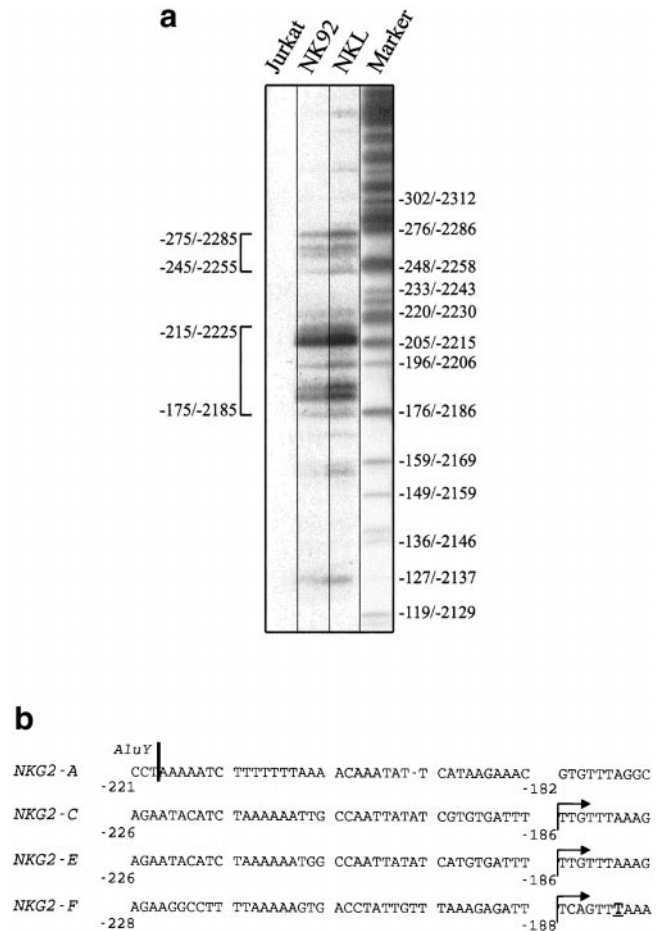
Data previously obtained by us applying 5' RACE reactions and sequencing of *NKG2* cDNA clones revealed cDNA start sites for *NKG2-C* and *-E* (-45) as well as *NKG2-F* (-182) in the region immediately preceding the translation initiation codon and did not give any indication of an upstream untranslated exon.<sup>10</sup> In contrast, most *NKG2-A* transcripts initiate at a distance of about -2.2 kb from multiple start sites and include a 5' untranslated exon, which is subsequently spliced at -2042/-31.<sup>19</sup>



**Figure 1** Schematic drawing of *NKG2* upstream nucleotide sequences. Nucleotide sequences were established using the ABI Prism 373A DNA Sequencer (PE Biosystems, Foster City, CA, USA) and were based on plasmid clones B46 (covering *NKG2-C* upstream sequences and exons 1, 2) and E15 (containing *NKG2-E* upstream sequences and exons 1 to 4). Both clones were derived from the P1 artificial chromosome (PAC) D21184 previously identified by us to cover the entire human genomic region of *NKG2-C*, *-E*, *-F* and *-D*.<sup>10</sup> *NKG2-F* upstream sequences were established by analysis of a PAC D21184 derived shotgun library.<sup>10</sup> Putative regulatory regions of *NKG2-C* (3642 bp), *NKG2-E* (3634 bp) and *NKG2-F* (3169 bp) were subsequently aligned and analyzed for the presence of repetitive elements using the MacVector 6.5.1 software (Oxford Molecular Ltd, Oxford, UK) and RepeatMasker at <http://ftp.genome.washington.edu/RM/RepeatMasker.html>. Regions of extended homology among the family members *NKG2-A*, *-C*, *-E* and *-F* are indicated by white bars and dashed lines connecting the corresponding areas. Repetitive DNA elements are drawn in black (*Alu* elements), dotted (*MIR* elements) or striped (*LIM3* element) boxes; their orientation is given by large arrows. Transcript start sites (according to 5' RACE cDNA analysis) are indicated by small bent arrows. All numbering refers to the corresponding start codons defined as A<sup>+</sup>TG. Numbers given for *NKG2-C* differ minimally from *NKG2-E* nucleotide counts (put in parentheses).

A minority of *NKG2-A* transcripts seems to be generated from a downstream TATA box at -44, which is not conserved in *NKG2-C*, *-E* or *-F* sequences. In the case of *CD94*, no 5' untranslated exon has been identified, and in the absence of a functional TATA box multiple start sites from a promoter preceding the first translated exon have been described.<sup>26</sup> To further characterize *NKG2* transcriptional initiation, we performed primer extension analysis with RNA obtained from NK cell lines NKL and NK92. We applied oligonucleotides which specifically bound to *NKG2-A*, *-C*, *-E* or *-F* cDNA (as tested in sequencing reactions). Primer extension experiments for *NKG2-A*, which have not been reported previously, were repeatedly carried out with an oligonucleotide priming at +4 (PE-A) and gave a characteristic band pattern specific for NK cell mRNA (Figure 2a). The pattern could be reproduced with a second oligonucleotide (PE-A<sub>up</sub>) priming within *NKG2-A* exon 1 (data not shown). Observed transcript start sites correlate with multiple cDNA 5' ends identified by Plougastel *et al*<sup>19</sup> via 5' RACE and sequencing of *NKG2-A* cDNA clones. Most cDNAs contain approximately 200 bp of 5' untranslated sequence, which initiates about 2.2 kb upstream of the start codon and is subsequently spliced at positions -2042 and -31. In accordance, the primer extension experiment as shown in Figure 2a displayed products with varying length of 5' untranslated region (mostly between 175 to 215 and with lower frequency between 245 to 275 nucleotides) reflecting multiple initiation points from the *NKG2-A* upstream promoter.

In the case of *NKG2-C*, *-E* and *-F*, quantitative RT-PCR (using ABI Prism 7700 Sequence Detection System, PE Biosystems, Foster City, CA, USA) demonstrated detectable but yet 10-fold (NKL) to 100-fold (NK92) lower mRNA levels when compared to *NKG2-A* (data not shown). For this reason, no NK cell-specific primer extension products could be obtained for *NKG2-C*, *-E* or *-F*, although repeatedly attempted with various oligonucleotides. In the absence of available primer extension data, we performed a computational promoter prediction by neural network (NNPP program at <http://dot.imgen.bcm.tmc.edu:9331>), which led to the identification of a putative transcript start site conserved among *NKG2-C*, *-E* and *-F* and located only 6 bp upstream of the *NKG2-F* cDNA start observed in our previous work<sup>10</sup> (Figure 2b). Probability scoring was high, ranging from 0.93 (*NKG2-C*) to 0.99 (*NKG2-E*) and 0.97 (*NKG2-F*); no other start sites were predicted within 1 kb upstream of the start ATG. High sequence similarity in this region argues indeed for a common transcript start site of *NKG2-C*, *-E* and *-F*. The putative transcript start site was not preserved in *NKG2-A* sequences, which display a unique *Alu* insertion shortly before the corresponding region, thus suggesting that this promoter area is not involved in the transcriptional initiation of *NKG2-A*. A second line of evidence indicating that *NKG2-A* and *NKG2-C*, *-E*, *-F* do preferentially employ different promoter regions, became apparent upon alignment of upstream *NKG2-A* exon 1 with the matching sequences of *NKG2-C*, *-E* and *-F*: We observed substantial differences in the area of *NKG2-A* transcript initiation, even though adjacent regions were highly homologous (Figure 1). While the *NKG2-F* gene harbors an *Alu* insertion, *NKG2-C* and *-E* sequences lack a 57-bp motif within the region corresponding to the *NKG2-A* upstream promoter and exon 1. This divergence



**Figure 2** *NKG2* transcript start sites. (a) Primer extension analysis of *NKG2-A* transcripts. NK cell lines NKL (generously supplied by MJ Robertson<sup>30</sup> and NK92<sup>31</sup> (Immune Medicine Inc, Vancouver, Canada) were grown in RPMI1640 medium containing 10% heat-inactivated human serum, 1 mM sodium pyruvate, 50 μM β-mercaptoethanol and 1000 U/ml human recombinant IL-2 (kind gift of Novartis Research Institute, Vienna, Austria). Total cellular RNA was extracted using TRIzol reagent (Life Technologies, Paisley, UK) according to manufacturer's instructions. Primer extension experiments were performed with 20 μg of total RNA hybridized to [γ-<sup>32</sup>P]ATP labeled oligonucleotides (100000 cpm) essentially as described.<sup>32</sup> Extension products were analyzed on a 6% polyacrylamide gel in comparison with DNA sequencing reactions (USB T7 Sequenase 2.0 Sequencing Kit, Amersham Pharmacia Biotech, Little Chalfont, UK). The oligonucleotides applied read as follows: PE-A 5'GTA GAT TAC TCC TTG GTT ATC<sup>3'</sup> (*NKG2-A*, +24 to +4), PE-A<sub>up</sub> 5'ATA GCT GTG TAA TAA AAG GTG<sup>3'</sup> (*NKG2-A*, -100 to -120), PE-C 5'GAA GGT TCC TCT TTG TTT AC<sup>3'</sup> (*NKG2-C*, +24 to +5), PE-E/F 5'GAA GGT TCC TCT TTG TTT AT<sup>3'</sup> (*NKG2-E* and *-F*, +24 to +5), PE-F 5'GAG GCT GAG TAG TAA TGT TCA T<sup>3'</sup> (*NKG2-F*, -112 to -133). Primer extension analysis was successfully carried out for *NKG2-A* transcripts of NKL and NK92 cells (negative control: Jurkat cells). Data are shown for PE-A in comparison with a size marker based on the ddGTP-lane of a PE-C sequencing reaction. Numbers indicate the corresponding positions in *NKG2-A* cDNA/genomic sequences with respect to the start codon (+1). (b) Putative transcript start sites of *NKG2-C*, *-E* and *-F* genes identified by NNPP analysis via <http://dot.imgen.bcm.tmc.edu:9331>. Homologous regions of *NKG2-A*, *-C*, *-E* and *-F* sequences are aligned. Predicted transcript start sites for *NKG2-C*, *-E* and *-F* are indicated by bent arrows. The published cDNA start site of *NKG2-F* is underlined, the position of *Alu* insertion in *NKG2-A* is indicated by a bold line.

**Table 1** Putative transcription factor binding sites conserved in *NKG2-C*, *-E* and *-F* upstream regions. Sites were identified using the GCG Wisconsin sequence analysis package version 8.1 (Genetics Computer Groups, Madison, WI, USA) and TESS analysis software available via <http://dot.imgen.bcm.tmc.edu:9331>. Occurrence of sites within 1, 2 or 3 kb of the start codon is listed

Transcription Factor	Number of sites conserved in <i>NKG2-C</i> , <i>-E</i> and <i>-F</i> sequences within		
	-3000	-2000	-1000
AFP-1	0	0	1
AP-1	1	2	2
C/EBP	1	3	1
c-ets1	0	1	0
c-mos	1	0	0
CP-1	0	0	1
GATA-1	1	0	2
HFH-1	0	1	0
HiNF-A	0	1	0
HNF-5	2	0	0
MBF-1	1	0	0
MEF-2	1	1	0
NF-AT1	0	0	1
NF-GMa	1	0	0
Oct	2	5	1
PEA-1	0	0	1
PEA-3	0	1	3
Pit-1a	1	4	2
S8	0	0	1
Sp1	0	2	1
SRF	0	2	0
TCF-1	2	7	6

Numbering refers to the *NKG2-C* start codon as +1. A complete list of sites and positions in *NKG2-C*, *-E* and *-F* sequences is available from the authors.

might in turn diminish transcriptional initiation at the related regions of the *NKG2-C*, *-E* and *-F* genes. Based on the NNPP analysis, the high sequence homology among *NKG2-C*, *-E*, *-F* and the findings of several cDNA clones initiating within the region corresponding to *NKG2-A* intron 1, we propose that these three family members employ conserved transcript start sites at -186/-188.

On the assumption that transcription of activating *NKG2* family members might be regulated in a similar fashion, we analyzed *NKG2-C*, *-E* and *-F* upstream regions for putative transcription factor binding sites that

were conserved in sequence and location among the family members. Among the predicted cis-regulatory elements, binding motifs for TCF-1, Oct, Pit-1a, C/EBP, AP-1, PEA-3, GATA-1 and Sp1 were the most frequent (Table 1). Several of these predicted transcription factor binding sites were also identified in *CD94* and *NKG2-A* regulatory regions and include TCF-1, GATA-1, C/EBP and PEA-3.<sup>19,26</sup>

We further analyzed putative cis-regulatory elements within the *Alu* elements unique to the respective family members (Table 2) and found the *Alu* sequences to be an abundant source of potential transcription factor binding sites. While insertion of *Alu* elements in promoter regions could possibly lead to abrogation of transcriptional activity, it has also been demonstrated that *Alu* elements can provide regulatory sequences functioning within the context of a gene promoter.<sup>24</sup> Putative transcription factor binding sites<sup>27</sup> as well as the ability to influence nucleosome positioning<sup>28</sup> contribute to *Alu*-derived regulation. The evolutionarily more recent subtypes of *Alu* repeats are known to harbor retinoic acid receptor response elements,<sup>29</sup> which we detected in all *NKG2*-contained *Alu* sequences. Other recognition motifs mapping to the *NKG2* *Alu* elements include putative binding sites for AP-1, AP-3, CREB/ATF, C/EBP, Sp1, NF-1/CTF or LyF-1 and may contribute to differences in gene regulation among family members.

We tested for possible transactivators of *NKG2* gene expression by stimulation of NK cell lines with various cytokines and subsequent mRNA analysis via Northern blotting or quantitative RT-PCR. We performed stimulation experiments of NKL and NK92 cells, which were kept in culture without IL-2 for 36 h and subsequently exposed to different concentrations of IL-2, IL-12, IL-15, IFN- $\alpha$ , TGF- $\beta$ , LPS, TNF- $\alpha$ , TPA, ionomycin or combinations thereof. Incubation times were kept short (0.25 to 5 h) to possibly detect direct transcriptional activators. Even though the NK cell lines NKL and NK92 were responsive to cytokine treatment as shown by the reproducible and strong induction of *c-myc* or *IFN- $\gamma$*  mRNA by stimuli like IL-2, IL-15 or TPA, we could not detect any regulation of *NKG2* transcript levels under the various conditions applied (data not shown). We thus conclude that with respect to the NK cell lines NKL and NK92, *NKG2* transcript levels are unaffected by short-time cytokine stimulation, possibly due to constitutive

**Table 2** Putative transcription factor binding sites localized within the various *Alu* elements that differ among *NKG2* family members (based on GCG and TESS analysis of upstream regulatory regions). Recognition elements identified in *NKG2-E* but not *NKG2-G* *Alu* repeats are marked by asterisks

<i>NKG2-A</i> <i>Alu Y</i>	<i>NKG2-C/E</i> <i>Alu Sg</i>	<i>NKG2-C/E</i> <i>Alu Jb</i>	<i>NKG2-F</i> <i>Alu Jb</i>	<i>NKG2-F</i> <i>Alu Y</i>
AP-1	E12/TCF3	AP-1	AP-1	E12/TCF3
C/EBP	NF-E1/YY1	ARP-1/COUP $\beta$ *	HiNF-A	HFH-2
CREB/ATF	NF-E2	GATA-1	NF-1/CTF	NF-1B
E12/TCF3	NF-GMa	HFH-1/HNF-3	Nfe	NG-E2
GATA-1	Pur factor	HFH-2	NF-GMa	NF-GMa
LyF-1	RARE	INSAF/IEF-1	RARE	RARE
NF-E2	ROR $\alpha$ 1	NF-GMa	Sp1	SRY/TDF
RARE	Sp1	Oct*		TCF-1
Sp-1	TCF-1	RARE		TEF-2/AP-3
TCF-1		TCF-1		

upregulation of the genes in these lymphoblastoid cell lines. The elucidation of transcriptional control mechanisms acting on the *NKG2* genes would thus require the use of NK cell clones or NK precursor cells which could be differentiated in culture.

## Acknowledgements

We would like to thank Jacqueline McBride, Markus Teige, Astrid Pesendorfer and Christof Bull for helpful discussions and technical support. Furthermore, we are grateful to Rudolf Oehler for critically reviewing the manuscript.

## References

- Karre K. How to recognize a foreign submarine. *Immunol Rev* 1997; **155**: 5–9.
- Lanier LL. NK cell receptors. *Annu Rev Immunol* 1998; **16**: 359–393.
- Lopez Botet M, Bellon T. Natural killer cell activation and inhibition by receptors for MHC class I. *Curr Opin Immunol* 1999; **11**: 301–307.
- Moretta A, Moretta L. HLA class I specific inhibitory receptors. *Curr Opin Immunol* 1997; **9**: 694–701.
- Ryan JC, Seaman WE. Divergent functions of lectin-like receptors on NK cells. *Immunol Rev* 1997; **155**: 79–89.
- Takei F, Brennan J, Mager DL. The Ly-49 family: genes, proteins and recognition of class I MHC. *Immunol Rev* 1997; **155**: 67–77.
- Houchins JP, Yabe T, McSherry C, Bach FH. DNA sequence analysis of *NKG2*, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J Exp Med* 1991; **173**: 1017–1020.
- Bellon T, Heredia AB, Llano M *et al*. Triggering of effector functions on a CD8<sup>+</sup> T cell clone upon the aggregation of an activating CD94/kp39 heterodimer. *J Immunol* 1999; **162**: 3996–4002.
- Plougastel B, Trowsdale J. Cloning of *NKG2-F*, a new member of the *NKG2* family of human natural killer cell receptor genes. *Eur J Immunol* 1997; **27**: 2835–2839.
- Glienke J, Sobanov Y, Brostjan C *et al*. The genomic organization of *NKG2C*, *E*, *F*, and *D* receptor genes in the human natural killer gene complex. *Immunogenetics* 1998; **48**: 163–173.
- Lazetic S, Chang C, Houchins JP, Lanier LL, Phillips JH. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and *NKG2* subunits. *J Immunol* 1996; **157**: 4741–4745.
- Houchins JP, Lanier LL, Niemi EC, Phillips JH, Ryan JC. Natural killer cell cytolytic activity is inhibited by *NKG2-A* and activated by *NKG2-C*. *J Immunol* 1997; **158**: 3603–3609.
- Lanier LL, Corliss B, Wu J, Phillips JH. Association of DAP12 with activating CD94/*NKG2C* NK cell receptors. *Immunity* 1998; **8**: 693–701.
- Carretero M, Palmieri G, Llano M *et al*. Specific engagement of the CD94/*NKG2-A* killer inhibitory receptor by the HLA-E class Ib molecule induces SHP-1 phosphatase recruitment to tyrosine-phosphorylated *NKG2-A*: evidence for receptor function in heterologous transfectants. *Eur J Immunol* 1998; **28**: 1280–1291.
- Braud VM, Allan DS, O'Callaghan CA *et al*. HLA-E binds to natural killer cell receptors CD94/*NKG2A*, B and C. *Nature* 1998; **391**: 795–799.
- Lee N, Llano M, Carretero M *et al*. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/*NKG2A*. *Proc Natl Acad Sci USA* 1998; **95**: 5199–5204.
- Sobanov Y, Glienke J, Brostjan C, Lehrach H, Francis F, Hofer E. Linkage of the *NKG2* and *CD94* receptor genes to *D12S77* in the human natural killer gene complex. *Immunogenetics* 1999; **49**: 99–105.
- Bull C, Sobanov Y, Röhrdanz B, O'Brien J, Lehrach H, Hofer E. The centromeric part of the human NK gene complex: linkage of *LOX-1* and *Ly49L* with the *CD94/NKG2* region. *Genes Immun* 2000; **1**: 280–287.
- Plougastel B, Trowsdale J. Sequence analysis of a 62-kb region overlapping the human *KLRC* cluster of genes. *Genomics* 1998; **49**: 193–199.
- Yabe T, McSherry C, Bach FH *et al*. A multigene family on human chromosome 12 encodes natural killer-cell lectins. *Immunogenetics* 1993; **37**: 455–460.
- Bertone S, Schiavetti F, Bellomo R *et al*. Transforming growth factor- $\beta$ -induced expression of CD94/*NKG2A* inhibitory receptors in human T lymphocytes. *Eur J Immunol* 1999; **29**: 23–29.
- Mingari MC, Vitale C, Cantoni C *et al*. Interleukin-15-induced maturation of human natural killer cells from early thymic precursors: selective expression of CD94/*NKG2-A* as the only HLA class I-specific inhibitory receptor. *Eur J Immunol* 1997; **27**: 1374–1380.
- Mori S, Jewett A, Cavalcanti M, Murakami Mori K, Nakamura S, Bonavida B. Differential regulation of human NK cell-associated gene expression following activation by IL-2, IFN- $\alpha$  and PMA/ionomycin. *Int J Oncol* 1998; **12**: 1165–1170.
- Szmulewicz MN, Novick GE, Herrera RJ. Effects of *Alu* insertions on gene function. *Electrophoresis* 1998; **19**: 1260–1264.
- Jendraschak E, Kaminski WE. Isolation of human promoter regions by *Alu* repeat consensus-based polymerase chain reaction. *Genomics* 1998; **50**: 53–60.
- Rodriguez A, Carretero M, Glienke J *et al*. Structure of the human *CD94* C-type lectin gene. *Immunogenetics* 1998; **47**: 305–309.
- Kazakov VI, Tomilin NV. Increased concentration of some transcription factor binding sites in human retrotransposons of the *Alu* family. *Genetica* 1996; **97**: 15–22.
- Englander EW, Howard BH. Nucleosome positioning by human *Alu* elements in chromatin. *J Biol Chem* 1995; **270**: 10091–10096.
- Vansant G, Reynolds WF. The consensus sequence of a major *Alu* subfamily contains a functional retinoic acid response element. *Proc Natl Acad Sci USA* 1995; **92**: 8229–8233.
- Robertson MJ, Cochran KJ, Cameron C, Le JM, Tantravahi R, Ritz J. Characterization of a cell line, NK1, derived from an aggressive human natural killer cell leukemia. *Exp Hematol* 1996; **24**: 406–415.
- Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia* 1994; **8**: 652–658.
- Ausubel FM, Brent R, Kingston RE *et al*. *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc: New York, 1995.