A cDNA Clone Expressed in Natural Killer and T Cells that Likely Encodes a Secreted Protein

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Summary

We have isolated a series of cross-hybridizing cDNA clones, as a group designated as NKG5, from a human natural killer (NK) cell clone cDNA library. These clones show a high degree of homology with a previously described gene, 519, which was thought to be T cell specific. A comparison of the full-length cDNA sequence of NKG5 and the published sequence of 519 shows that NKG5 lacks a 242-base segment that is found in 519 and that this deletion leads to the use of a different putative translational start codon. Unlike 519, the predicted NKG5 polypeptide has an NH_2-terminal sequence that is strongly hydrophobic, characteristic of a signal peptide, and lacks any additional hydrophobic regions in the remainder of the peptide, suggesting that NKG5 encodes a secreted protein. Both NKG5 and 519 are expressed in NK and T cells but not in a variety of other hematopoietic cell lines. NKG5 is an abundant transcript and its level of expression is about 40 times that of 519 in NK and T cells. Southern blot and DNA sequence analyses suggest that NKG5 and 519 mRNAs are transcripts from a single gene that has allelic polymorphism.

Natural killer cells and CTL play important roles in protection against tumor and viral infection (1) and they appear to be closely related as evidenced by the sharing of several surface antigens (2) and granule-associated proteins involved in the cytolytic machinery (3–5). In addition, lytic activity against NK-sensitive targets can be induced in CTLs by long-term culture in high levels of IL-2 (6). The close relationship between NK cells and T cells was also apparent in a recent study from this laboratory (7) that sought to identify cDNA clones expressed in NK cells but not in an EBV-transformed B cell line. Of 12 such groups of cDNA clones that were identified, 11 were found to be expressed in at least some types of T cells as well. A partial DNA sequence of one of these groups, designated NKG5, revealed a high degree of homology with, but not identity to, the gene 519, which was previously reported to be expressed in functional T cell lines (8). mRNA of 519 was upregulated 10-fold in PBLs 3–5 d after activation. We reported that NKG5 was expressed at comparable levels in NK cells and T cells and that expression of this gene was also upregulated about 10-fold upon activation of NK cells by T cell growth factor (TCGF).[1]

In this paper we report the full-length cDNA sequence of NKG5 and compare NKG5 and 519 at the levels of DNA sequence, mRNA expression, and predicted protein structure.

[1] *Abbreviation used in this paper:* TCGF, T cell growth factor.

Materials and Methods

Cell Culture. The cloned human NK cell B22 ($CD3^-$, $CD16^-$, $CD56^+$), the NK cell populations EDF ($CD3^-$, $CD16^+$, $CD56^-$) and 221707 ($CD3^-$, $CD16^+$, $CD56^+$) derived from NK cell leukocytosis patients, the $CD4^+$ allospecific CTLs KD15, .3-78, and KD33, and a $CD4^+$ CMV-specific Th clone WRC-16, were cultured as described previously (7). The chronic myelogenous leukemia line K562, the histocytic lymphoma line U937, the leukemic T cell line Jurkat, and the EBV-transformed lymphoblastoid cell line FJO, were cultured in RPMI 1640 containing 10% FCS, glutamine, and antibiotics. The T cell lymphoma line Hut78 was cultured in the same medium as above, with the addition of 10% TCGF. The promyelocytic leukemia line HL60 was cultured in the same medium as above except it contained 20% FCS. One-half of the HL60 was stimulated with 1.25% DMSO at days 1 and 3 and harvested at day 7. DMSO stimulation induces ~50% of the cells to differentiate into more mature myeloid cell forms. The monocyte line THP-1 was cultured in RPMI 1640 containing 10% FCS and 2×10^{-5} M 2-ME.

Isolation and Sequencing of NKG5 cDNA Clones. Nine independent cDNA clones were isolated from the B22 cDNA library as previously described (7). Asymmetric PCR was used to amplify inserts, and the 5'-end sequence (up to 450 nucleotides in length) was determined for seven of the clones. A full-length cDNA sequence was determined by subcloning PstI fragments encompassing the entire NKG5 insert into M13mp19.

Oligonucleotide Probes. Two oligonucleotide probes were used in these studies. Oligomer 1 was complementary to a portion of the 242-base insert (position I-203 to I-237 in Fig. 2 *b*). Oligomer

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/10/1159/05 $2.00
Volume 172 October 1990 1159–1163

2 was complementary to NKG5 (position 239 to 272 in Fig. 2 *b*). Oligonucleotides were end-labeled with γ-[^{32}P]ATP (>5,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) as described (9).

Hybridization. Northern and Southern blot hybridizations were performed as described (7 and 10, respectively). Hybridization with oligonucleotide probes was performed as described (9).

Results and Discussion

A Comparison of the Sequences of NKG5 and 519. We have isolated a group of nine cross-hybridizing cDNA clones, members of which are designated as NKG5, from a cDNA library prepared from a cloned human NK cell (called B22) (7). Partial DNA sequences of seven members of this cross-hybridization group were identical with each other except for the lengths of their 5′ and 3′ ends. A full-length cDNA sequence of NKG5 was determined and is shown, along with the deduced amino acid sequence, in Fig. 1. The longest open reading frame starts with an ATG at position 129 and ends with a TGA at position 564, encoding a 145 amino acid polypeptide. No potential N-linked glycosylation sites were observed in the deduced amino acid sequence. When compared with DNA sequence databases, NKG5 showed strong homology with a previously reported gene, 519. A comparison of the DNA sequence of these transcripts is shown in Fig. 2. The most significant difference between the DNA sequences of NKG5 and 519 is the presence of the 242-base segment in 519 that is absent from NKG5. As shown in Fig. 2 *b*, the presence of this segment interrupts the upstream reading frame (TGA at position I-24); this led Jongstra et al. (8) to propose that the 519 peptide starts with an ATG at position I-236 and ends with the same stop codon as NKG5 (position 564). In addition, several smaller differences were present, including a 3-base insertion in NKG5 within the reading frame, and nine other scattered nucleotide substitutions. Six of these fall within the reading frame and result in four additional amino acid changes. In an attempt to investigate the reason for these sequence differences, we probed the NK cell cDNA library with an oligonucleotide complementary to a portion of the 242-base insert and isolated a clone (B22-519) that contained part of the insert. Except for the presence of the insert, the DNA sequence of this clone was identical to the NKG5 sequence. This result suggests that the scattered base differences between NKG5 and 519 are most likely the result of allelic polymorphism and that the transcript possessing the 242-base insert is derived from the NKG5 gene by an alternative splicing event. The junction sequences flanking the 242-base insert (5′ CAG/C-- --AAG/G 3′) are consistent with conserved sequences at exon splice sites (C or A AG/-- --/G) (11).

The major portions of the NKG5 and 519 peptides are virtually identical; however, the usage of different start codons leads to important differences at the NH_2 termini. The hydropathicity profile of the NKG5 peptide, shown in Fig. 3, reveals that, in contrast to 519, the NKG5 peptide has a strongly hydrophobic NH_2 terminus, characteristic of a signal peptide. Two sites satisfy the signal peptide cleavage criteria reported by von Heijne (12). No other hydrophobic segment capable of serving as a transmembrane domain is present, suggesting that NKG5 encodes a secreted protein.

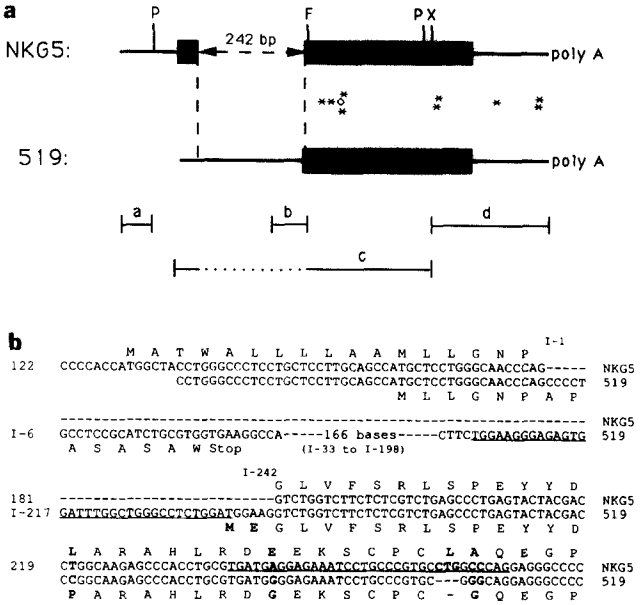

Figure 2. Comparison of NKG5 and 519. (*a*) Diagram of the structure of the NKG5 and 519 mRNA. Black boxes indicate the longest open reading frames. The dashed arrow indicates the position of the 242 base insert. (◇) The position of a three base insert in NKG5; (*) sites of nucleotide substitution. Segments a–d indicate restriction fragments used to probe Southern blots (see Fig. 4). (*b*) Partial sequence comparison of the NKG5 and 519. The numbers to the left indicate nucleotide positions of NKG5. Nucleotide positions of the 242-base insert in 519 are numbered as I-1 to I-242. Dashed lines in NKG5 indicate absence of sequences at these positions. Nucleotide and amino acid differences are shown in bold type. The sequences complementary to oligomer probes are underlined.

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GTATCTGTGGTAAACCCAGTGACACGGGGGAGATGACATACAAAAAGGGCAGGACCTGAGAAAGATTAAG   70

                                                        Met Ala Thr
CTGCAGGCTCCCTGCCCATAAAACAGGGTGTGAAAGGCATCTCAGCGGCTGCCCCACC  ATG GCT ACC  137

Trp Ala Leu Leu Leu Leu Ala Ala Met Leu Leu Gly Asn Pro Gly Leu Val Phe Ser
TGG GCC CTC CTG CTC CTT GCA GCC ATG CTC CTG GGC AAC CCA GGT CTG GTC TTC TCT  194

Arg Leu Ser Pro Glu Tyr Tyr Asp Leu Ala Arg Ala His Leu Arg Asp Glu Glu Lys
CGT CTG AGC CCT GAG TAC TAC GAC CTG GCA AGA GCC CAC CTG CGT GAT GAG GAG AAA  251

Ser Cys Pro Cys Leu Ala Gln Glu Gly Pro Gln Gly Asp Leu Leu Thr Lys Thr Gln
TCC TGC CCG TGC CTG GCC CAG GAG GGC CCC CAG GGT GAC CTG TTG ACC AAA ACA CAG  308

Glu Leu Gly Arg Asp Tyr Arg Thr Cys Leu Thr Ile Val Gln Lys Leu Lys Lys Met
GAG CTG GGC CGT GAC TAC AGG ACC TGT CTG ACG ATA GTC CAA AAA CTG AAG AAG ATG  365

Val Asp Lys Pro Thr Gln Arg Ser Val Ser Asn Ala Ala Thr Arg Val Cys Arg Thr
GTG GAT AAG CCC ACC CAG AGA AGT GTT TCC AAT GCT GCG ACC CGG GTG TGT AGG ACG  422

Gly Arg Ser Arg Trp Arg Asp Val Cys Arg Asn Phe Met Arg Arg Tyr Gln Ser Arg
GGG AGG TCA CGA TGG CGC GAC GTC TGC AGA AAT TTC ATG AGG AGG TAT CAG TCT AGA  479

Vla Thr Gln Gly Leu Val Ala Gly Glu Thr Ala Gln Gln Ile Cys Glu Asp Leu Arg
GTT ACC CAG GGC CTC GTG GCC GGA GAA ACT GCC CAG CAG ATC TGT GAG GAC CTC AGG  536

Leu Cys Ile Pro Ser Thr Glu Pro Leu Stop
TTG TGT ATA CCT TCT ACA GGT CCC CTC TGA GCCCTCTCACCTTGTCCTGTGGAAGAAGCACAG  599

GCTCCTGTCCTCAGATCCCGGGAACCTCAGCAACCTCTGCCGGCTCCTCGCTTCCTCGATCCAGAATCCA  669

CTCTCCAGTCTCCCTCCCCTGACTCCCTCTGCTGTCCTCCCCTCTCACGAGAATAAAGTGTCAAGCAAGA  739

AAAAAA-----
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Figure 1. The nucleotide sequence and predicted amino acid sequence of NKG5. The numbers to the right indicate nucleotide positions. Arrows indicate possible cleavage sites of a signal peptide. The polyadenylation signal is underlined. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54101.

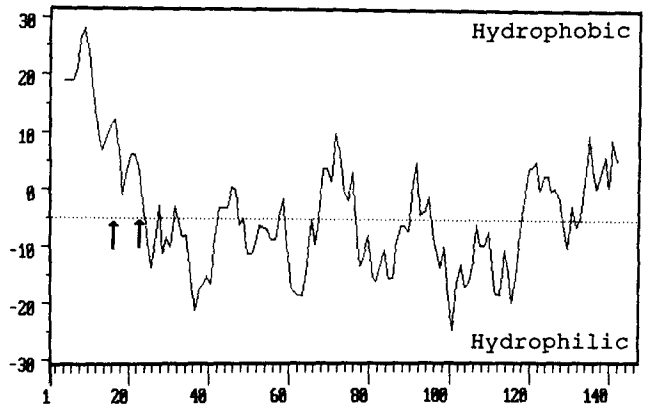

Figure 3. Hydropathicity profile of the predicted NKG5 protein as calculated by the algorithm of Kyte and Doolittle (15), using the Intelligenetics PC Gene program. The numbers on the horizontal axis designate the amino acid residue positions. Arrows indicate two possible cleavage sites of a signal peptide.

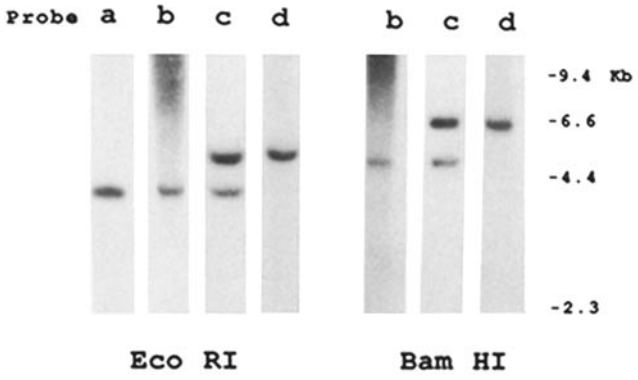

Figure 4. Southern blot hybridization. Genomic DNA samples (10 μg/lane) were digested with EcoRI or BamHI. The blots were hybridized with probes shown in Fig. 2 *a*.

The 519 peptide, in contrast, is hydrophilic, and was proposed to act intracellularly.

The cDNA sequence and the predicted polypeptide sequence were compared with GenBank and EMBL databases and with the SwissPro and PIR databases, respectively. Excluding 519, no significant homology was found.

Genomic Fragments in Southern Blots. EcoRI- or BamHI-digested genomic DNA samples were probed with several fragments derived from different parts of the transcripts (probes a–d in Fig. 2 *a*). The results are shown in Fig. 4. Probe c, which corresponds to the middle of the gene, hybridized to two bands in each digestion. Probe a, a PstI fragment from the 5′ end of NKG5, and probe b, a fragment corresponding to part of the 242-base insert that was obtained by FokI digestion of B22-519, hybridized only to the smaller band in each lane. Both probes correspond to segments of the gene near the 5′ end. In contrast, probe d, an XbaI fragment from the 3′ end of NKG5, hybridized only to the larger band in each lane. These results clearly indicate that the two fragments appearing in each lane on Southern blots are contiguous on the chromosome and constitute a single gene. These also suggest that both NKG5 and 519 mRNAs are transcribed from this gene.

mRNA Expression of NKG5 and 519. Expression of NKG5 and 519 was analyzed with Northern blots in a variety of cell types. The expression patterns of NKG5 and 519 mRNAs were distinguished with transcript-specific oligonucleotide probes. Oligomer 1 is complementary to sequence within the 242-base insert found only in 519 (position I-203 to I-237 in Fig. 2 *b*); oligomer 2 is complementary to a portion of the NKG5 sequence (position 239 to 272 in Fig. 2 *b*). The cDNA sequence of B22-519 demonstrates that oligomer 2 will also hybridize to clones of the B22 library that possess the 242-base insert. Therefore, oligomer 2 hybridizes to both types of transcripts in this cell. As shown in Fig. 5, mRNA

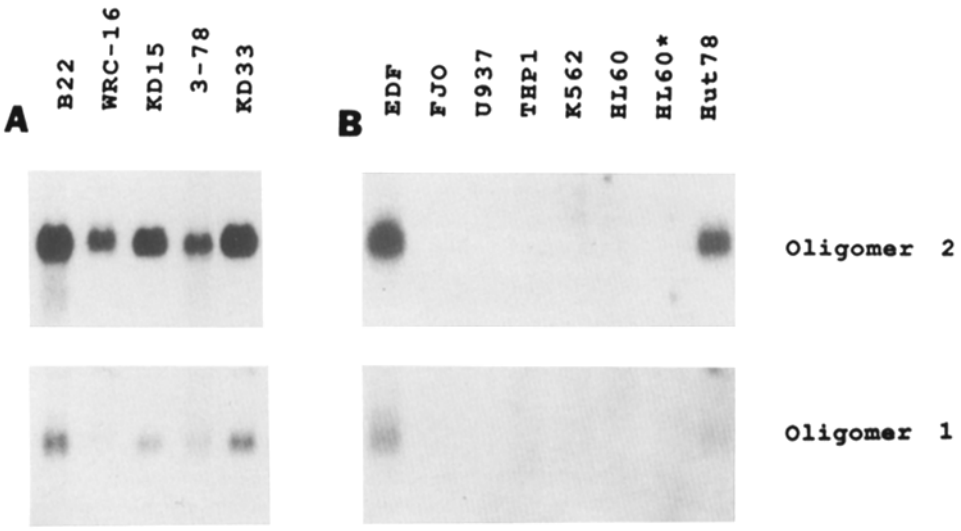

Figure 5. Northern blot hybridization comparing levels of expression in a variety of cell types. Each lane contained 1 μg of poly(A) RNA (*A*) or 20 μg of total cytoplasmic RNA (*B*). HL60*; HL60 was stimulated with DMSO as described in Materials and Methods. Blots were hybridized with oligomer 1 (519-specific) or with oligomer 2 (specific for both NKG5 and 519) probes as indicated. Exposure times: (*A*) overnight (oligomer 2) or 2 d (oligomer 1). (*B*) 7 d (both oligomer 2 and oligomer 1).

representing the 519 transcript in B22 is seen as a faint band with oligomer 1. Hybridization with oligomer 2 revealed a slightly smaller band of much greater intensity representing NKG5 mRNA, plus the much weaker 519 band which, because of its very low intensity, is hidden in the NKG5 transcript. The difference between band intensities seen with oligomer 2 and oligomer 1 is a measure of the amount of NKG5 mRNA.

To obtain frequencies of each cDNA, we screened 100,000 plaques from the NK clone cDNA library with both oligomer probes. NKG5 cDNA and 519 cDNA frequencies are 0.39% and 0.01%, respectively, suggesting that NKG5 mRNA expression is ~40 times that of 519. This ratio is consistent with band intensities on Northern blots (Fig. 5), and could reflect the frequency of the alternative splicing event or differential message stability.

Both NKG5 and 519 mRNA were detected in the NK cell populations EDF (Fig. 5) and 221707 (not shown) at levels comparable to those in B22. Expression of both transcripts was observed in four different $CD4^+$ T cell clones, as well as in the T cell lymphoma line Hut78. The ratio of NKG5/519 mRNA was approximately the same in all cells that expressed these transcripts. No expression of NKG5 or 519 mRNA was observed in FJO, U937, THP-1, K562, HL60 (Fig. 5), or in Jurkat (not shown). These data indicate that NKG5 mRNA is far more abundant than that of 519, and that expression of both transcripts occurs in NK cells and T cells but not in B cells or in nonlymphoid cell lines. Jongstra et al. (8) found a similar distribution in hematopoietic cells, and also reported no expression in several nonhematopoietic tissues including placenta, lung, liver, tonsil, and smooth and skeletal muscles. All of the cells expressing NKG5 were cultured in IL-2 (or TCGF)-containing medium. Also, we have previously demonstrated TCGF-induction of NKG5 expression in an NK cell clone. Jongstra et al. (8) showed that PHA activation of PBLs, a treatment that leads to endogenous production of IL-2, also induced 519. Expression of NKG5/519, therefore, appears to be induced in lymphocytes activated with IL-2.

NKG5 appears to encode a protein that is abundantly expressed in NK and T cells. NKG5 mRNA is expressed at a high level 2–3 d after activation, and its translational start sequence (CCACCATGG) matches exactly the Kozak consensus sequence for eukaryotic translation initiation sites (13). In contrast, 519 was only weakly translated in vitro, due both to a poor match with the Kozak consensus sequence and to the existence of an upstream reading frame (8). The poor translation efficiency of 519, coupled with the low level of 519 message (only 2–3% of the NKG5 level) suggests that little or no protein is encoded by the 519 transcript. It seems likely that the 519 transcript results from a nonproductive splicing event and does not encode a functional product.

Although the function of the NKG5 peptide is not known at this time, several other granular or secreted protein products that are produced by NK and T cells in response to cell activation (14) are known to have important functions. These include proteins associated with cytolytic effector functions, such as serine protease (6, 7) and perforin (5), as well as cytokines like IFN-γ and interleukins 2–6 (14). However, levels of mRNAs for known cytokines generally increase transiently with maximal levels occurring at <10 h after activation. NKG5 appears to fit into a category of several other genes that are induced at later stages, i.e., after 48 h, of lymphocyte activation (14). This group includes secreted and membrane products, the functions of which have not been clearly defined. The high level of expression and the strong upregulation of NKG5 following cell activation suggest that this product has an important role in the NK and T cell response, and efforts to elucidate this function will continue.

We would like to thank Susan Fuad and Bruce Wouda for providing NK cells; Kazumasa Matsuki and Naoyuki Miyokawa for helpful advice; Michael Madden, Celeste Yanisch-Perron, Nancy Reinsmoen, Harriet Noreen, Mary Stewart, Maurine Davidson, Pete Nelson, Craig Litz, Colleen McCarter, and Genia Gordon for technical help; and Connie Greenberg for assistance in manuscript preparation.

This is paper 544 from the Immunobiology Research Center. This work was supported in part by National Institutes of Health grants AI-19007 and DK-13083 and was performed in the Jordan Bazelon Research Laboratories.

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Received for publication 29 May 1990 and in revised form 20 July 1990.

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