Brief Definitive Report

DNA Sequence Analysis of NKG2, a Family of Related cDNA Clones Encoding Type II Integral Membrane Proteins on Human Natural Killer Cells

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Summary

We have previously described the isolation of a cDNA clone, designated NKG2, that was expressed in all natural killer (NK) cells tested but not in T or B cells. In the present communication, the original isolate, when used to probe a cDNA library prepared from a $CD3^-$ NK cell clone, was found to crosshybridize with a family of transcripts that fell into four distinct groups designated NKG2-A, -B, -C, and -D. Full-length cDNA sequences were determined for each group, and the DNA and inferred peptide sequences were analyzed. All four transcripts encode type II membrane proteins of 215–233 amino acids. NKG2-A and -B peptides appear to be alternative splicing products of a single gene. NKG2-C is highly homologous with group A, having 94% homology in the external (COOH-terminal) domain and 56% homology throughout the internal and transmembrane regions. NKG2-D is distantly but significantly related (21% amino acid homology) to the first three groups. Therefore, NKG2-A, -C, and -D appear to be encoded by distinct genes within a family of NK cell–specific genes. Peptide sequence homology searches demonstrate that the NKG2 peptides are members of a supergene family that includes several other type II membrane proteins. This family is characterized by the presence of a C-type animal lectin domain, and several of its members have demonstrated transmembrane signaling capability.

NK cells appear to be closely related to T cells based upon the common expression of a number of cell type–specific transcripts and cell surface antigens (1, 2). NK cells and T cells also share expression of perforin and serine proteases, components of the cytolytic machinery that are found almost exclusively in lytic cells. However, the TCR/CD3 complex responsible for triggering lytic activity in T cells is absent from NK cells, and a corresponding structure that recognizes the NK target antigen and triggers NK lytic activity has not been identified. Elucidation of these structures would greatly facilitate an understanding of the in vivo function of NK cells.

We initiated a project to identify, through the use of differential hybridization and cDNA subtraction methodology, genes that are expressed preferentially in NK cells, with particular emphasis on those encoding membrane-bound proteins (1). In an earlier report, we identified 12 groups of crosshybridizing cDNA clones that were expressed in NK cells but not in an EBV-transformed B cell line. One of these groups, NK group 2 (NKG2), was expressed only in NK cells among the panel of cells that we examined. In the current paper, we report that NKG2 is comprised of a group of related transcripts that encode a series of type II membrane proteins.

Materials and Methods

A cDNA library was prepared from B22, a $CD3^-$, $CD16^-$, $CD56^+$ human NK clone that lyses K562 (1). The library was probed with the previously described NKG2 isolate (1) to find additional cDNA clones. DNA sequences of NKG2-A, -B, and -C were determined on templates derived from the original λ-Gem cDNA clones via the asymmetric PCR as described previously (1). Several oligonucleotides were synthesized to serve as sequencing primers for both orientations throughout the length of the cDNA inserts. The DNA sequence of the single NKG2-D isolate was determined by recloning the PCR-amplified insert in both orientations into M13mp19 and using the single-stranded subcloning method (3) to generate overlapping deletions. Data analysis was carried out using the Intelligenetics Suite.

Results and Discussion

DNA Sequence Analysis. The single original isolate of NKG2 described earlier was found to be several hundred bases shorter than the 1.7-kb transcript seen on Northern blots (1). Therefore, that isolate was used to probe the NK cell cDNA library in an effort to identify full-length cDNA clones. The positively reacting plaques in the library showed widely

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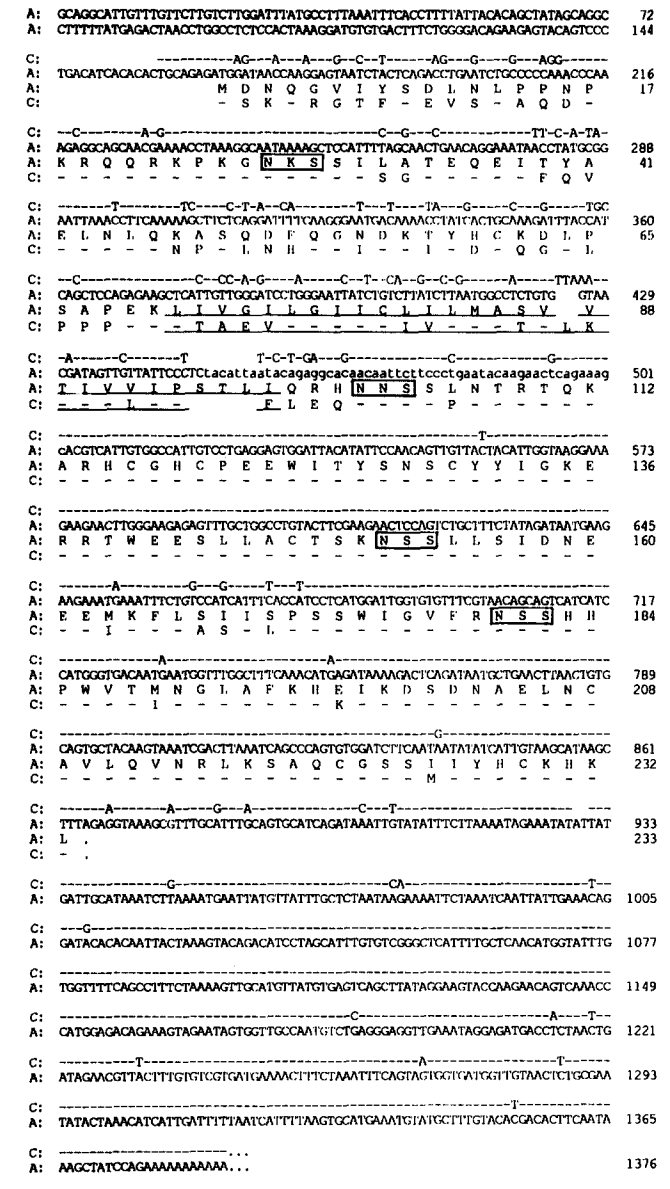

Figure 1. An alignment of the DNA and peptide sequences of NKG2-A and -C. Dashes indicate that residues are identical at these positions. The 54-bp segment in NKG2-A that is absent from NKG2-B is shown in lower case type. The positions of the transmembrane regions are underlined. Sites of potential N-linked glycosylation of the peptides are enclosed within boxes. These sequence data as well as the sequence of NKG2-D are available from EMBL/GenBank/DDBJ under accession numbers X54867 (NKG2-A), X54869 (NKG2-C), and X54870 (NKG2-D).

varying intensities of hybridization, and examples of plaques having differing hybridization intensities were selected for further study. Analysis of the inserts from eight selected clones revealed that several of the clones contained nearly full-length inserts. However, digestion of the inserts with AluI and RsaI revealed four distinct restriction maps; these were designated NKG2-A, -B, -C, and -D.

The clone with the longest insert from each of these groups was sequenced. The DNA sequences of NKG2-A and -C are compared in Fig. 1. A diagram showing the degree and regions

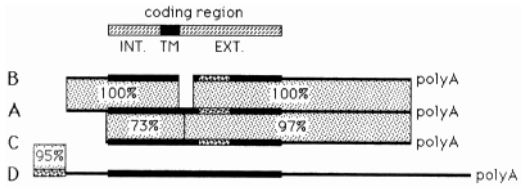

Figure 2. A diagram of the regions of homology in the DNA sequences of the NKG2 transcripts. The shaded areas refer to the percent homology between the adjacent transcripts. The stippled region within the NKG2-A, -B, and -C transcripts is 95% homologous with the 5′ end of NKG2-D. Heavy bars indicate the protein coding regions.

of homology between the different members of the group is shown in Fig. 2. Fig. 2 also shows the position of the longest open reading frame and the positions of the intracellular, transmembrane, and extracellular regions. The membrane orientation with an extracellular COOH terminus will be discussed below. Groups A and B are identical to one another except for the absence of a 54-bp internal segment from group B. These transcripts presumably represent differential splicing products of the same gene. As shown in Fig. 2, the group C transcript consists of two regions that show strong but differential homology to group A. Because of the strong similarities between NKG2-A and -C, we subsequently sequenced several additional independent isolates from these two groups to confirm the observed differences. In contrast, group D, which showed weaker hybridization during screening of the cDNA library, had only a short region of homology with the other groups; a 130-bp segment in the 5′ untranslated region of NKG2-D was 95% homologous with a segment from the coding regions of groups A, B, and C (base pairs 525–653 of NKG2-A; Fig. 1). Although this 5′ segment occurred in the original NKG2-D isolate and was subsequently found in a small percentage of independently isolated clones, Northern blot studies clearly indicate that it is not part of the normal NKG2-D transcript and that its occurrence must result from an unusual RNA processing event (our unpublished data). The remainder of the NKG2-D sequence was only distantly related to the other NKG2 sequences (35% homology). Comparison of the full-length sequences of NKG2-A, -C, and -D with the EMBL and GenBank databases identified no significant homologies at the DNA level.

Reading Frame Analysis. The longest open reading frames of each transcript encoded peptides ranging in size from 215 to 233 amino acids. The hydropathicity profiles of all of the NKG2 peptides were similar, having a single internal hydrophobic region capable of serving as a transmembrane domain and no NH_2-terminal hydrophobic signal peptide. These characteristics are typical of type II membrane proteins, which possess an intracellular NH_2-terminus and an extracellular COOH terminus (4). Each of the NKG2 peptides possessed several potential N-linked glycosylation sites, which were located primarily in the extracellular segment as shown in Fig. 1.

The peptides encoded by each member of NKG2 were compared with one another. The NKG2-B peptide is identical to that of NKG2-A except for the absence of an 18–amino acid segment immediately outside the transmembrane region.

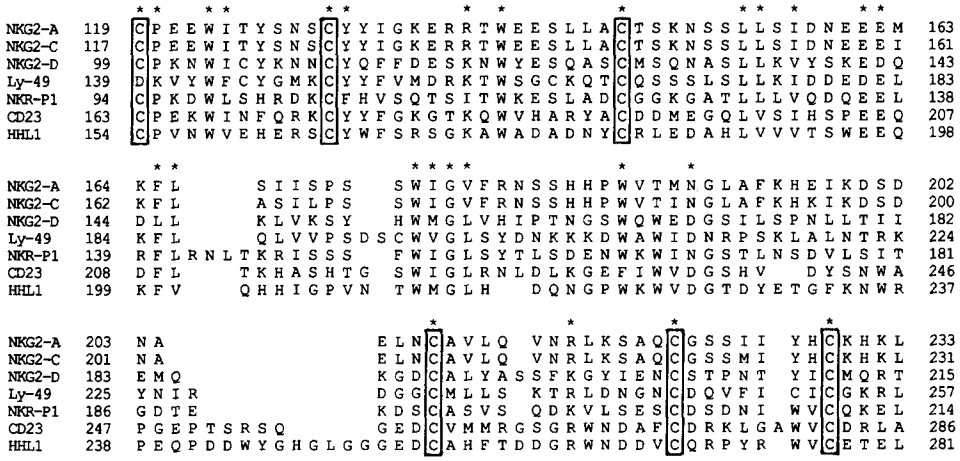

Figure 3. An alignment of the lectin-like domains of the NKG2 proteins and several other members of the supergene family. The amino acid positions within each sequence are shown. Asterisks indicate positions where the residue or the biochemical character of the side chain is highly conserved. The six invariant cysteines are enclosed within boxes. HHL1 refers to human hepatic lectin 1.

NKG2-C showed the strongest homology (94%) with the extracellular segment of NKG2-A and lesser homology (56%) throughout the intracellular and transmembrane segments. NKG2-D displayed distant but significant homology (21%) with groups A, B, and C.

Homology comparisons of the NKG2 peptide sequences with the SwissProt and PIR databases reveal homology with a gene superfamily that is characterized by the presence of a Ca^{2+}-dependent (C-type) animal lectin domain. The peptide sequence homology was confined primarily to the C-type lectin domain with little apparent homology throughout the remainder of the protein. The carbohydrate binding domains share homology throughout a stretch of ~120 amino acids, with the most striking feature being the presence of six invariant cysteine residues at fixed positions (5). Fig. 3 shows a comparison of the NKG2 peptide sequences throughout the lectin-like domain with those of several other members of this gene superfamily that possess the unusual type II membrane orientation (6–9).

Transcript-specific nucleotide probes were prepared by isolating restriction fragments from regions of lowest homology within the cDNA inserts. These probes were used to study tissue-specific expression in T cell clones, cell lines representing NK cells, T cells, B cells, monocytes, and promyelocytes, and several other tissues (data not shown). Each of the transcripts was expressed in the three NK cells that were tested as well as a subset of T cells. NKG2-D was expressed in 9 of 13 T cell clones or lines that were examined, whereas NKG2-A/B was found in three T cells, and NKG2-C was expressed in only one. No expression was observed in any other cell type.

The tissue-specific expression of the NKG2 genes is consistent with the idea that they play an important role in NK cell function. Two recent reports in rat and mouse have each identified a single gene that encodes an NK cell–specific antigen having the type II membrane orientation and a C-type lectin domain (8, 9). The murine Ly-49 alloantigen is confined almost exclusively to a subset of NK cells, and the gene was found to map to the same position on mouse chromosome six as the gene for NK1.1, a commonly used murine NK cell marker (10). However, Ly-49 was clearly shown to be distinct from NK1.1, suggesting that a region on mouse chromosome six may contain a family of NK cell–specific genes. The rat NK cell–specific antigen, NKR-P1, also has a structure similar to the NKG2 proteins (9). NKR-P1 was shown to have transmembrane signaling capability upon crosslinking with its corresponding mAb (11), and it has been proposed as a candidate for the NK cell receptor. Although the Ly-49 and NKR-P1 antigens are members of the same gene superfamily as NKG2, they display very limited amino acid sequence homology with NKG2 and do not appear to be mouse or rat homologues of any of the NKG2 proteins. The possible existence of human homologues to these mouse and rat genes suggests that additional human genes within the NKG2 family might be present.

Several of the type II membrane proteins within the lectin gene superfamily have been shown to act as receptors capable of delivering a transmembrane signal, including NKR-P1 (11), hepatic lectin (5), and CD23 (7). It seems likely that other members of this group, including the NKG2 proteins, will also function as signal-transmitting receptors.

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