NK Lytic-Associated Molecule: A Novel Gene Selectively Expressed in Cells with Cytolytic Function

Miroslaw Kozlowski, Jeff Schorey, Toni Portis, Vitalii Grigoriev and Jacki Kornbluth

*J Immunol* 1999; 163:1775-1785; ;
http://www.jimmunol.org/content/163/4/1775

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
NK Lytic-Associated Molecule: A Novel Gene Selectively Expressed in Cells with Cytolytic Function

Miroslaw Kozlowski, Jeff Schorey, Toni Portis, Vitalii Grigoriev, and Jacki Kornbluth

NK cells are lymphocytes that kill tumor or virus-infected cells without prior sensitization (1, 2). However, it is well established that the cytolytic activity of NK cells can be enhanced by a number of cytokines, including IL-2, IFN-α (α, β, and γ), IL-12, and IL-15 (3–6). These cytokines activate NK cells to kill with greater efficiency and broader specificity. IL-2-activated NK cells are responsible for the majority of the lymphokine-activated killing activity observed in vivo (7). High doses of IL-2, with or without administration of lymphokine-activated cells, can induce tumor regression in experimental animals and in some patients (8, 9). However, a number of side effects and toxicities associated with high dose IL-2 therapy have been observed in treated cancer patients that have limited its success and utility (10–12).

A better understanding of the molecular and cellular events associated with cytokine enhancement of NK function may provide a means for generating and maintaining cells with maximal cytolytic activity and therefore facilitate the use of activated NK cells in cancer, and perhaps also viral, immunotherapy. It has been demonstrated that the augmentation of NK lytic activity by IL-2 or IFN requires de novo RNA and protein synthesis (13, 14). However, it is not clear which gene products are responsible for the increased killing activity and expanded target repertoire. Activated NK cells have increased expression of perforin and granzymes A and B (15, 16). The up-regulation of adhesion molecules CD2, CD11a, and CD54 upon stimulation may also be partially responsible for increased NK lytic activity (17). To further define the events responsible for cytokine augmentation of NK function, we have examined changes in NK gene expression upon IFN-β and IL-2 stimulation.

In the studies presented in this paper the NK clone 3.3 was used as a model to study changes in gene expression in cytokine-activated NK cells. This cloned line is phenotypically and functionally representative of the majority of NK cells in the peripheral blood (i.e., CD16+β, CD56+, CD3−, CD4−, CD8−, TCR γδ−) (18, 19). Like peripheral blood NK cells, the cytolytic activity of NK3.3 is enhanced after treatment with IL-2 or IFN-β. We have previously found that the protooncogene c-myc is up-regulated by both IL-2 and IFN-β in NK3.3 cells and appears to play an important role in the regulation of NK cytolysis (20). To identify new genes associated with cytokine augmentation of NK function, mRNA isolated from IFN-β-stimulated NK3.3 cells was used to construct a cDNA library. Differential screening was performed to identify a set of novel genes with elevated expression in IFN-β-stimulated NK cells compared with unstimulated cells. Sequence analysis revealed 46 novel genes. This paper describes the characteristics of one of these novel clones, which has been designated NKLAM, for natural killer lytic-associated molecule.3

Materials and Methods

Cell lines

The derivation and characterization of the human NK clone, NK3.3, have been described in detail previously (18, 20–22). This IL-2-dependent NK clone is maintained in continuous culture at 2.5–3 × 10⁶ cells/ml in NK medium, composed of RPMI 1640 supplemented with 25 mM HEPES buffer, 2 mM (1%) glutamine, 15% heat-inactivated FBS, and 15% Lymphocult-T as a source of IL-2 (Lymphocult-T, Biotest Diagnostics, Den- ville, NJ). The NK-sensitive, erythroleukemia cell line K562, the T cell tumor line MOLT-4, the B lymphoblastoid cell line JY, the myeloma cell line ARH77, the breast cancer cell line ZR-75–1, the fibroblast cell line A23, and the neuroglioma line H4 were maintained in complete medium composed of RPMI 1640 supplemented with 25 mM HEPES, 2 mM glutamine, and 10% heat-inactivated FBS. All cell culture reagents, unless specified, were purchased from Life Technologies (Gaithersburg, MD). The fibroblast cell line A23 was provided by Dr. Sam Goldstein (University of Arkansas for Medical Sciences, Little Rock, AR), and COS-7 cells were purchased from American Type Culture Collection (Manassas, VA). These cell lines were maintained in DMEM supplemented with 10% FBS. Human liver mRNA was isolated from tissue obtained by postmortem autopsy. rIL-2 was a gift from Hoffmann-La Roche (Nutley, NJ), and rIFN-β was a gift from Triton Biosciences (Alameda, CA).

Abbreviations used in this paper: NKLAM, NK lytic-associated molecule; RPS3, ribosomal protein S3; AbN, Ab to the N-terminus of NKLAM; ABC, Ab to the C-terminus of NKLAM; S, sense; AS, antisense; ODN, oligodeoxynucleotides.

1 Abbreviations used in this paper: NKLAM, NK lytic-associated molecule; RPS3, ribosomal protein S3; AbN, Ab to the N-terminus of NKLAM; ABC, Ab to the C-terminus of NKLAM; S, sense; AS, antisense; ODN, oligodeoxynucleotides.
Isolation and purification of peripheral blood monocytes, T cells, and NK cells

PBL were isolated from healthy donors by centrifugation on Ficoll-Hypaque density gradients. Monocytes were obtained by two rounds of adherence followed by scraping. The purity of this population was between 80–96%. Monocytes were incubated with or without IFN-β (10,000 U/ml) for 24 h. T cells were separated by E-rosetting as previously described (23). SRBC were subsequently lysed from rosetted cells using ACK lysis buffer (150 mM NH₄Cl, 1 mM KCl, and 0.1 mM Na₂EDTA) to obtain enriched T cells. For generation of CTL, CD8⁺ T cell subpopulations were separated from E rosette-purified T cells using anti-CD8 Ab and antimonos IgG magnetic beads (Advanced Magnetics, Cambridge, MA). CD8⁺ T cells were then incubated with irradiated (5000 rad) allogeneic JY B lymphoblastoid cells in mixed lymphocyte cultures. Equal numbers of CD8+ and CD8- T cells were used in a 1:1 ratio. T cells-enriched populations were routinely >96% CD3⁺ by flow cytometric analysis. NK-enriched populations were at least 85% CD16⁺ and/or CD56⁺ and <1% CD3⁺.

Construction of cDNA libraries

NK3.3 cells that had been incubated in medium without IL-2 for 24 h were stimulated for 4 h with 5000 U/ml of IFN-β. Total RNA was then isolated by guanidinium isothiocyanate extraction as previously described (24), and poly(A)⁺ mRNA was selected on an oligo(dT)-cellulose column (Boehringer Mannheim, Indianapolis, IN). In the generation of the first library, RNA molecules greater than 500 bases were used as templates for the synthesis of DNA:RNA hybrids using random primers. To generate a second, NKLAM-specific library, synthesis of the first strand of cDNA was performed using a specific primer derived from the 5′ sequence of the original NKLAM cDNA clone (5′-GACGGGCCATATCGCATGTC-3′). Double-stranded cDNA was synthesized, and EcoRI linkers were added to the cDNAs and ligated to ASAP II vectorarms (Strategene, La Jolla, CA). The DNA was packaged within phage and used to infect XL1-Blue (Stratagene) coli cells. In the original cDNA library, differential screening of 10⁵ plaques containing cDNA inserts was performed by transferring plaque DNA onto duplicate nitrocellulose filters. 3²P-labeled cDNA synthesized from poly(A)⁺ mRNA from IFN-β-treated and IL-2-starved NK3.3 cells were used as probes. The second library was screened using the original NKLAM cDNA as a probe.

Sequencing

Bluescript plasmids containing the cDNA inserts were excised from positive phages by coinfection with helper phage VCSM13 (Stratagene). In some cases, fragments of NKLAM were cloned into M13 to generate ssDNA for sequencing. Dideoxy sequencing using Sequenase or Taq polymerase (U.S. Biochemical Corp., Cleveland, OH) was performed on NKLAM single-stranded and double-stranded cDNA as described by the supplier. Sequencing reactions were initiated using vector-specific or NKLAM-specific oligonucleotide primers. Sequence analysis was performed using the InfiniGeneics PcGene program and GCG Wisconsin package. GenBank, EMBL, PIR, and SwissProt databases were searched using FASTA and BLAST algorithms for homologies (25–27). Additional programs (Psignal, RaoArgos, Tmpred, and PROSITE) were used to predict features of the NKLAM protein.

Cytolytic assays

NK and CTL assays were performed as previously described (18, 20). All conditions were set up in triplicate wells at E:T cell ratios ranging from 30:1 to 2:1. ³⁵Cr release was measured after 4 h of incubation. The percent specific lysis was defined as:

\[
\text{Percent specific lysis} = \frac{(\text{experimental release} - \text{spontaneous release})/\text{maximum release} - \text{spontaneous release})}{\times 100.
\]

Spontaneous release was measured from target cells incubated alone. Maximum release was determined by adding detergent (0.1 M hexadecytrimethylammonium bromide) to target cells. The SD of triplicate values in all experiments did not exceed 10%.

Northern blots

Total RNA was isolated using TRI Reagent (Molecular Research Center, Cincinnati, OH), and poly(A)⁺ RNA was isolated using the RiboSep mRNA Isolation Kit (Collaborative Biomedical Products, Bedford, MA) according to the manufacturer’s instructions. RNA was electrophoresed through 1.5% agarose/6% formaldehyde gels. After gel transfer of RNA to Magna Charge nylon membranes (Micron Separations, Westboro, MA) and UV cross-linking, blots were prehybridized and then hybridized as previously described (20) using 3²P-labeled NKLAM cDNA. All radiolabeled cDNA probes were prepared by random priming (DECA prime DNA labeling kit, Bethesda, MD) and had sp. act. of at least 1 × 10⁶ cpm/μg DNA. 3²P-labeled dCTP was purchased from Amersham (Arlington Heights, IL). The blots were washed and then exposed to Kodak XAR5 (Eastman Kodak, Rochester, NY) or Fuji (Fuji, Tokyo, Japan) film at −70°C under intensifying screens. Blots for quantitative analysis were exposed to a PhosphorImager screen and then analyzed using a PhosphorImager SF machine (Molecular Dynamics, Sunnyvale, CA). Control probes used in these studies include cDNAs for IFN-γ and ribosomal protein S3 (RPS3). Both were obtained from the American Type Culture Collection. Quantitative analysis of RNA was performed by normalizing hybridization to the levels of RPS3 RNA.

Recombinant NKLAM protein expression

Recombinant NKLAM proteins were generated as fusion proteins containing thioredoxin and a polyhistidine moiety using the pET TRX Fusion System 32 (Novagen, Madison, WI). NKLAM gene sequences encoding aa 31–319 and 272–587 were cut out from cloned cDNA, BambHt linkers (New England Biolabs, Beverly, MA) appropriate for in-frame expression of NKLAM sequences were ligated to the fragments. These products were ligated into the BamHI site of the pET32 vector. Vectors containing fragments in the correct orientation were used to transform expressing E. coli strain BL21 (DE3) pLysS (Novagen). Recombinant proteins were expressed and purified using metal affinity chromatography according to the pET TRX Fusion System 32 manufacturer’s recommendation.

Full-length NKLAM was cloned into the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). This vector was used to generate in vitro translated NKLAM using the TNT-coupled reticulocyte lysate system, according to the manufacturer’s protocol (Promega, Madison, WI). To study NKLAM expression in vivo, COS-7 cells were transfected with pcDNA3 containing NKLAM. Briefly, COS-7 cells were transfected with pcDNA3 containing NKLAM using a Life Technologies Cell Porator, followed by selection in G418-containing medium for 48 h. Whole cell extracts from these transfected cells were prepared by cell lysis with 1% SDS and sonication. The extracts were denatured and reduced by boiling in SDS and DTT-containing Laemmli buffer (28) for use in immunoblotting experiments.

NKLAM Ab production and purification

Rabbits were immunized with the purified recombinant NKLAM proteins. Sera were prepared, and Abs were affinity purified exactly as described previously (29), except an additional column with immobilized bacterial thioredoxin was used for serum absorption. These Abs to thioredoxin were eluted and used as control sera. Abs to residues 31–319 (N-terminal part of NKLAM protein) were designated AbN; Abs to residues 273–587 (C-terminal part of NKLAM protein) were designated AbC.

Immunoprecipitation and Western blotting

Immunoprecipitation was performed as described previously (29). NK3.3 cells were incubated overnight in RPMI 1640 medium containing 10% FBS. Cytoplasmic extracts prepared from these cells are referred to as unstimulated cell extracts. After changing to IL-2-containing NK medium, an aliquot of NK3.3 cells was incubated for an additional 6 h. Cytoplasmic extracts prepared from these cells were eluted and used as control sera. Abs to residues 31–319 (N-terminal part of NKLAM protein) were designated AbN; Abs to residues 273–587 (C-terminal part of NKLAM protein) were designated AbC.
precipitated with protein A-agarose (Life Technologies), boiled and reduced in SDS buffer containing DTT, and resolved on polyacrylamide gels using the Laemmli buffer system (28). Prestained m.w. markers ( Benchmark precast protein ladder,older, lot J26151) were obtained from Life Technologies. Protein was transferred to polyvinylidene difluoride membranes (30), probed with primary Abs (0.5 μg/ml), and developed with peroxidase-conjugated secondary Abs and SuperSignal Substrate (Pierce, Rockford, IL).

NKLM antisense experiments

Phosphorothioate-modified NKLM sense (S) and antisense (AS) oligodeoxynucleotides (ODN) were generated (Cruachem, Dulles, VA) and used to inhibit NKLM expression in NK3.3 cells and CTL. In the experiments presented in Figs. 8–10, DNase I-treated NKLM AS, corresponding to nt 694–713 of NKLM, were used. Additional S and AS constructs, corresponding to nt 684–701, were also employed. These were among several S and AS ODN made and tested; the two AS ODN used in the experiments presented were found to be the most effective in selectively reducing the level of NKLM mRNA in NK3.3 cells (data not shown). Myc antisense phosphorothioate-ODN (designated Myc AS; 5′-AACCTGAGGCGGCAT-3′) were used as a control. This ODN corresponds to the region of c-myc mRNA at the AUG start codon and has been used by others to suppress c-myc expression. ODN were ethanol precipitated twice and resuspended in RPMI medium before use. Before addition of oligonucleotides, NK3.3 cells or CTL were cultured for 24 h in fresh, complete NK medium. The cells were then transferred to medium without IL-2. IL-2 NKLM S, AS, and Myc AS ODN were added to the cells at a concentration of 15 μM and introduced into the cells by electroporation. Electroporation was performed in a 0.4-cm gap electroporation chamber in 1 ml of RPMI 1640 medium with 10% FBS at a cell concentration of 4 × 10^9/ml. For electroporation of NK3.3 cells, the Cell-Porator was set at a capacitance of 800 μF and a voltage of 250. Electroporation settings for CTL were 1180 μF and 280V. After electroporation, cells were allowed to rest in the electroporation chamber for 1 h. Cells were then placed in IL-2-supplemented medium at a concentration of 2 × 10^9/ml. Two sets of cells were electroporated without ODN; one set was incubated with IL-2-containing medium (E), and the other set was incubated without IL-2 to act as an unstimulated control (−). After incubation for various periods of time, the cells were counted and examined for viability by trypan blue dye exclusion, and tested for cytolytic activity against the NK-sensitive erythroleukemia cell line K562 or the CTL-specific target JY using 4-h ⁵¹Cr release assays, then an aliquot was prepared for RNA isolation and characterization.

Purification of cytolytic granules

Cytoplasmic granules were purified from NK3.3 cells according to the procedure described previously (31) with modifications. In brief, cells were collected, washed with ice-cold PBS followed by washing with ice-cold PIPEG buffer (10 mM PIPES (pH 6.8), 4 mM EGTA, 2 mM benzamide, and 1% sucrose), and resuspended in PIPEG buffer supplemented with 1 mM PMSF, 50 μg/ml aprotinin, and 5 μg/ml leupeptin. Cells or NK3.3 cells in serum-free RPMI medium before use. Before addition of oligonucleotides, NK3.3 cells or CTL were cultured for 24 h in fresh, complete NK medium. The cells were then transferred to medium without IL-2. IL-2 NKLM S, AS, and Myc AS ODN were added to the cells at a concentration of 15 μM and introduced into the cells by electroporation. Electroporation was performed in a 0.4-cm gap electroporation chamber in 1 ml of RPMI 1640 medium with 10% FBS at a cell concentration of 4 × 10^9/ml. For electroporation of NK3.3 cells, the Cell-Porator was set at a capacitance of 800 μF and a voltage of 250. Electroporation settings for CTL were 1180 μF and 280V. After electroporation, cells were allowed to rest in the electroporation chamber for 1 h. Cells were then placed in IL-2-supplemented medium at a concentration of 2 × 10^9/ml. Two sets of cells were electroporated without ODN; one set was incubated with IL-2-containing medium (E), and the other set was incubated without IL-2 to act as an unstimulated control (−). After incubation for various periods of time, the cells were counted and examined for viability by trypan blue dye exclusion, and tested for cytolytic activity against the NK-sensitive erythroleukemia cell line K562 or the CTL-specific target JY using 4-h ⁵¹Cr release assays, then an aliquot was prepared for RNA isolation and characterization.

Characterization of NKLM

Clones with unique sequences were used as probes to screen RNA from IL-2, IFN-β, and starved NK3.3 cells by Northern blot analysis. One of the unique cDNA clones, designated NKLM, hybridized to a 2.9-kb RNA transcript that was expressed at a 5- to 8-fold higher level in both IL-2- and IFN-β-stimulated NK3.3 cells compared with unstimulated cells. Since this cDNA clone was not full length, the library was rescreened using this clone as a probe. This resulted in 20 additional overlapping clones, but none contained the complete 5′ end of NKLM. Therefore, a second NK 3.3 cDNA library was created using NKLM-specific primers. From this library, three additional overlapping clones containing the missing 5′ end of NKLM were identified, and the entire gene was sequenced (Fig. 1).

The full-length NKLM cDNA is 2874 nt. It contains a single open reading frame of 1771 nt, encoding 587 aa, a 240-bp 5′-untranslated region, and 836-bp 3′-untranslated region, followed by a 37-bp poly(A) tail (Fig. 2A). The presumed start codon (ATG), located at position 241, has a Kozak consensus sequence critical for initiation of translation (32, 33). Sequence analysis of NKLM
using BLAST and FASTA programs revealed significant homology to three sequences in GenBank. The alignments with NKLAM are shown in Fig. 3; only the regions of significant homology are depicted. The gene with the highest homology to NKLAM encodes a blood-meal-induced protein of 663 aa associated with mosquito reproduction. Another gene with high homology to NKLAM is the C17H11.6 gene product of the nematode Caenorhabditis elegans. Its function is unknown. The BLAST program also identified a murine clone (MGEG-154) with high homology to two stretches of sequence at the 3′ end of NKLAM. This clone, isolated from mouse ovary and overexpressed in mouse testis, has been reported to hybridize strongly to two RNA transcripts of 4.2 and 3 kb (34). It is likely that the portion of MGEG-154 homologous to NKLAM is responsible for hybridization to the 3-kb transcript. The function of this gene is also unknown.

NKLAM contains a potential signal sequence, with a predicted cleavage site between aa 45 and 46 identified on the basis of the (−3, −1) rule (35). Computer analysis of the NKLAM amino acid sequence performed with the RaoArgos and TMpred programs detects three well-defined transmembrane segments located between aa 358–380, 409–430, and 513–531, therefore identifying a strongly preferred model of NKLAM as a transmembrane protein with a predicted size of 62 kDa (Fig. 2B) (36, 37). Sequences corresponding to the first two putative transmembrane regions are highly homologous among NKLAM, the mosquito protein, the C. elegans sequence, and GEG-154. Using the PROSITE patterns database, several potential sites for protein modification were identified in NKLAM (38). These include sites for N-linked glycosylation at aa 248 and 562, and several potential amidation and N-myristylation sites throughout the molecule. There is also a potential cAMP- and cGMP-dependent protein kinase phosphorylation site, three potential protein kinase C phosphorylation sites, and two potential casein kinase II phosphorylation sites in the putative nontransmembrane domains of the NKLAM protein.

The predicted N-terminal region of NKLAM contains 26 cysteines within a 213-aa region from 119 to 332, which fall into three cysteine-rich clusters. Sequence analysis of these clusters revealed two potential zinc binding domains located between aa 115–161 and 282–333. These two domains match the consensus sequence of the CXC4 ring finger family (C-X2-C-X(9–27)-C-X(1–3)-H-X(2–3)-C-X2-C-X(4–48)-C-X2-C, where C and H denote cysteine and histidine, and X represents other amino acids) (39–41). An additional cysteine-rich protein domain located between aa 202 and 252 does not perfectly match other well-defined cysteine-rich clusters and may form a novel zinc finger domain. The three cysteine-rich domains appear to be highly conserved, in that they are also found in the homologous mosquito, C. elegans, and mouse GEG-154 sequences (Fig. 3). BLAST sequence analysis of this cysteine-rich region of NKLAM revealed additional homology to other proteins with ring finger domains. The best similarity was seen to KIAA.

**FIGURE 2.** A, Schematic diagram of NKLAM cDNA. It consists of a 240-bp 5′-noncoding region, 1761-bp coding region, and 3′-untranslated region with poly(A) tail. The predicted ATG start codon is shown, compared with the Kozak consensus sequence. B, Schematic diagram of the predicted NKLAM protein. It indicates the position of a potential signal sequence, three predicted transmembrane regions, and two potential sites of N-linked glycosylation.

**FIGURE 3.** Alignment of the predicted NKLAM protein with similar areas in homologous proteins in mosquito, C. elegans, and the mouse. Accession numbers are I64695, 1813871, 1707258, and 1730145, respectively. Numbers in columns indicate the position of the amino acids in each of the proteins. Sequences in boldface denote the regions of greatest homology.

**TABLE 1.** 

<table>
<thead>
<tr>
<th>Protein</th>
<th>Accession Numbers</th>
<th>3′-Untranslated Region</th>
<th>Coding Region</th>
<th>5′-Untranslated Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKLAM</td>
<td>I64695</td>
<td>119 – 332</td>
<td>240</td>
<td>1 – 118</td>
</tr>
<tr>
<td>C. elegans</td>
<td>1813871</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
<tr>
<td>Mouse</td>
<td>1707258</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
<tr>
<td>Mouse</td>
<td>1730145</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
</tbody>
</table>

**TABLE 2.** 

<table>
<thead>
<tr>
<th>Protein</th>
<th>Accession Numbers</th>
<th>3′-Untranslated Region</th>
<th>Coding Region</th>
<th>5′-Untranslated Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKLAM</td>
<td>I64695</td>
<td>119 – 332</td>
<td>240</td>
<td>1 – 118</td>
</tr>
<tr>
<td>C. elegans</td>
<td>1813871</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
<tr>
<td>Mouse</td>
<td>1707258</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
<tr>
<td>Mouse</td>
<td>1730145</td>
<td>120 – 332</td>
<td>1761</td>
<td>1 – 1750</td>
</tr>
</tbody>
</table>
a predicted protein from a cDNA clone isolated from human KG-1 myeloblast cells (accession no. D79983) and the ARI ring finger protein found in the central nervous system of Drosophila melanogaster (accession no. X98309). The location, distribution, and orientation of these three cysteine-rich domains in the three proteins are also similar, suggesting a functional relationship.

**Regulation of NKLAM expression and cytolyis in NK3.3**

The kinetics of induction of NKLAM mRNA expression and corresponding cytolytic function of NK3.3 cells after stimulation with IL-2 and IFN-β were assessed. In these experiments, NK3.3 cells were first cultured in IL-2-free medium for 18–24 h (starved) and then stimulated with IL-2 (200 U/ml) or IFN-β (10,000 U/ml) for the indicated times. Cells were harvested for RNA isolation and Northern blot analysis; the cytolytic activity of NK3.3 cells using the NK-sensitive target K562 was also monitored concurrently in 51Cr release assays. As shown in Fig. 4, A and B, NKLAM RNA expression increases within 2 h of IFN stimulation, peaks at 4–6 h (6-fold increase), and then declines. This closely parallels the kinetics of IFN-mediated augmentation of killing (Fig. 4C). When cells are stimulated with IL-2, NKLAM RNA levels also increase within the first hour and rise 5- to 6-fold over a 6- to 8-h period. However, unlike IFN stimulation, where levels then drop, with IL-2 stimulation, NKLAM levels continue to rise, although much more slowly, throughout the 6- to 12-h period (Fig. 5, A and B). This pattern of kinetics exactly parallels the kinetics of IL-2 augmentation of NK3.3 cytolytic activity (Fig. 5C). Therefore, although the patterns of IL-2- and IFN-mediated augmentation of killing and NKLAM expression are different for each cytokine, the kinetics of cytokine induction of NKLAM and cytolyis are identical.

The levels of two other species of RNA were measured in parallel. RPS3 encodes the ribosomal structural protein S3 (42). This RNA has a very long half-life, is extremely stable and constant throughout the cell cycle, and therefore acts as a good control of total RNA levels. Its expression is not significantly altered by cytokines or cell activation. Levels of RNA were normalized using the RPS3 probe in the quantitation of NKLAM. The other RNA that was measured encodes IFN-γ; it was used to monitor NK cell activation. Ye et al. (43) reported that NK3.3 cells produce IFN-γ after IL-2 treatment. Here, we show that both IL-2 and IFN-β induce the expression of IFN-γ mRNA in NK3.3 cells. (Figs. 4 and 5) The kinetics of induction of IFN-γ mRNA in NK3.3 cells upon IL-2 and IFN-β stimulation closely parallel the kinetics of induction of NKLAM. This suggests that there may be a common regulatory pathway for IFN-γ and NKLAM RNA induction in NK cells.

To determine whether the increase in NKLAM mRNA in cytokine-stimulated NK3.3 cells was accompanied by a commensurate increase in NKLAM protein production, we employed a combination of immunoprecipitation and Western blot analysis using polyclonal Abs to NKLAM raised in rabbits. Extracts from unstimulated and IL-2-stimulated NK3.3 cells were immunoprecipitated with Abs raised against the N-terminal part of NKLAM (AbN, see Fig. 6, lanes 2 and 3) and Abs to thioredoxin (Fig. 6, lane 1). The precipitated material was analyzed by Western blot with Abs to the C-terminal part of NKLAM (AbC). As a positive control, the in vitro translation product of NKLAM was run on SDS-PAGE and immunoblotted with AbC (Fig. 6, lane 4). Additional controls include whole cell lysates from COS-7 cells transiently transfected with the NKLAM-pcDNA3 expression vector or empty vector (lanes 5 and 6, respectively). Fig. 6 shows that several bands could be detected in IL-2-stimulated NK3.3 cells after immunoprecipitation with NKLAM-specific AbN (lane 3). These bands were absent from extracts precipitated with nonrelated Abs to thioredoxin and were barely detectable in extracts from unstimulated NK3.3 cells precipitated with AbN (Fig. 6, lanes 1 and 2, respectively). The apparent molecular masses of the two lower bands seen in IL-2-stimulated NK3.3 cells are approximately 65 and 75 kDa. The 65-kDa band migrates in a fashion consistent with the molecular mass of NKLAM determined by computer analysis, in vitro.

![FIGURE 4. Correlation between IFN-β-stimulated cytolytic activity and IL-2-stimulated induction of NKLAM mRNA in NK3.3 cells. IL-2-starved, NK3.3 cells were treated with IFN-β (10,000 U/ml) for 0–12 h. At the indicated time points, an aliquot of cells was assessed for NK cytolytic activity against 51Cr-labeled K562 cells. In parallel, a second aliquot of cells was used to prepare RNA for Northern blot analysis. A, Northern blot analysis indicating the kinetics of IFN-β induction of NKLAM mRNA in NK3.3 cells. Blots were probed with a 32P-labeled NKLAM cDNA probe (a 2-kb fragment corresponding to the entire 3’ end of NKLAM), an IFN-γ cDNA probe (ATCC no. 39042), and an RPS3 cDNA probe (ATCC no. 65825). B, Quantitative analysis of kinetics of IFN-β induction of NKLAM mRNA. The level of mRNA at each time point was calculated as a function of the volume of radioactivity in hybridizing bands. RNA values for NKLAM and IFN-γ at each time point were normalized to the levels of the noninducible, highly stable RPS3 transcripts. The relative amount of NKLAM mRNA at each point was determined by setting the level of NKLAM mRNA in IL-2-starved, unstimulated NK3.3 cells (time zero) to a value of 1. Quantitative analysis was performed using a PhosphorImager. C, Kinetics of IFN-β stimulation of cytolytic activity in NK3.3 cells. At each of the designated time points, an aliquot of NK3.3 cells was removed and assayed for cytolytic activity in 4-h 51Cr release assays using radiolabeled K562 cells as targets. Results are expressed as the percent specific lysis at an E:T cell ratio of 20:1.](http://www.jimmunol.org/DownloadedFrom/FIGURE_4.png)
compared with unstimulated NK3.3 cells. IFN-$\gamma$ (bar 3). These levels are increased 3- to 5-fold further by 24-h IFN-$\gamma$ constitutively express high levels of NKLAM RNA than the unstimulated cells. Peripheral blood A PBMC were fractionated into monocytes, T cells, and NK cells, and each subpopulation was examined for the presence of NKLAM mRNA. The results are presented in Fig. 7A. In this graphic representation, the baseline, low level of NKLAM in unstimulated, starved NK3.3 cells was set to a value of 1 (bar 10); 4-h IFN-$\beta$-stimulated NK3.3 cells (bar 11) have 6 times more NKLAM RNA than the unstimulated cells. Peripheral blood monocytes (bar 1) constitutively express high levels of NKLAM compared with unstimulated NK3.3 cells. IFN-$\beta$ stimulation significantly enhances their expression of NKLAM (bar 2). Unstimulated peripheral blood NK cells also express significant levels of NKLAM (equivalent to that of IFN-$\beta$-stimulated NK 3.3 cells; bar 3). These levels are increased 3- to 5-fold further by 24-h IFN-$\beta$

FIGURE 5. Correlation between IL-2-stimulated cytolytic activity and IL-2-stimulated induction of NKLAM mRNA in NK3.3 cells. A. Northern blot analysis of IL-2 induction of NKLAM mRNA in NK3.3 cells. Cells were stimulated with 200 U/ml of IL-2 for the times indicated and treated as described in Fig. 4A. B. Quantitative analysis of the kinetics of IL-2 induction of NKLAM mRNA was performed as described in Fig. 4B. C. Kinetics of IL-2 stimulation of cytolytic activity in NK3.3 cells. Lysis of K562 at an E:T cell ratio of 20:1 was assessed as described in Fig. 4C.

treatment (bar 4). In contrast, peripheral blood T cells express barely detectable levels of NKLAM RNA (bar 7). Neither IL-2 nor PHA stimulation significantly induced NKLAM expression in these purified T cells (bars 8 and 9). However, a CD8$^+$ alloreactive, cytotoxic T cell line generated from these purified T cells by multiple stimulation with irradiated, allogeneic B lymphoblastoid JY cells expressed significant levels of NKLAM RNA (comparable to unstimulated NK3.3 cells; bar 5). Upon stimulation of these CTL with their specific alloreactive target for 4 h, NKLAM RNA was strongly induced, increasing approximately 10-fold in expression compared with unstimulated CTL (bar 6). These results indicate that NKLAM expression is restricted to cells with cytolytic activity and further induced when cytolysis is triggered or enhanced.

To determine whether expression of the NKLAM transcript was limited to hemopoietic cells, a number of other cell types were screened (Fig. 7B). NKLAM was not strongly expressed in any of these cell types tested, including fibroblasts, breast cancer cells (ZR-75–1), T cell tumor lines (MOLT-4), and myeloma cell lines (ARH77). There was a low level of expression in liver and in the neuroglioma cell line (H4; bars 3 and 4). However, NKLAM was not induced in any of these cells following a 4-h stimulation with 10,000 U/ml IFN-$\beta$. Further analysis of NKLAM expression in a variety of human tissues using a commercially obtained tissue blot (Clontech multiple tissue Northern blot) indicated that NKLAM RNA levels lower than those in liver were seen in pancreas, muscle, and brain (data not shown).

Potential role of NKLAM in cytolysis

To attempt to establish a link between NKLAM expression and cytolytic function, a series of antisense oligonucleotide experiments were performed. NK3.3 cells were cultured for 24 h in IL-2-supplemented medium and then transferred to complete medium without IL-2. NKLAM S and AS phosphorothioate-modified ODN were introduced into NK3.3 cells by electroporation. As an additional control, a well-defined c-Myc antisense phosphorothioate ODN was used. This ODN corresponds to the region of c-myc mRNA at the AUG start codon and has been used successfully to suppress c-myc expression (44). All ODN were used at a final concentration of 15 $\mu$M. After electroporation without (E) or with...
ODN, cells were placed in IL-2-supplemented medium. One set of electroporated cells were incubated in medium without IL-2 to act as an unstimulated control (—). The results of cytotoxicity experiments using two representative NKLAM antisense ODN (designated AS and AS8) are presented in Fig. 8. NKLAM antisense treatment of NK3.3 reduced its cytotoxic activity against K562 by about 75%, while NKLAM S and Myc AS treatments had minimal effects. These data represent one of four experiments performed with identical results. Fig. 9 demonstrates the specificity and selectivity of the NKLAM antisense ODN for NKLAM. As shown in Fig. 9, A and B, NKLAM antisense treatment of NK3.3 dramatically reduced the levels of NKLAM mRNA (65% decrease) without significantly affecting RPS3 or IFN-γ mRNA expression. This illustrates the specificity of the interaction of the NKLAM AS ODN with NKLAM. Treatment of NK3.3 with Myc AS ODN only slightly reduced the levels of NKLAM mRNA and had no effect on RPS3 or IFN-γ mRNA expression. Fig. 9C shows the growth curves of NK3.3 after electroporation with 15 μM NKLAM AS ODN or Myc AS ODN compared with those of control, electroporated cells growing in the absence of ODN. The IL-2-dependent proliferation of NK3.3 was unaffected by the NKLAM AS ODN, confirming the selective role of NKLAM in cytokine-enhanced NK cytotoxic activity. As expected, the Myc AS ODN treatment induced growth arrest in NK3.3 cells during the first 24 h of culture. Cumulatively, these data, demonstrating a selective and dramatic reduction in NKLAM mRNA levels and cytotoxic activity in IL-2-activated NK3.3 cells treated with NKLAM AS ODN, with no effect on other gene expression or cell proliferation, strongly support the role of NKLAM in cytokine-activated NK cytolysis.

NKLAM antisense ODN experiments using CTL were performed with similar results. CTL were electroporated without oligonucleotides (E) or with NKLAM S, NKLAM AS, or Myc AS ODN. After incubation for 18 h, 51Cr release assays were performed as described using radiolabeled K562 target cells. Results are expressed as the percent specific lysis of K562. This represents one of four experiments with similar results. SDs of triplicate values in each experiment did not exceed 8%.

FIGURE 7. Expression of NKLAM mRNA in human PBMC subpopulations and other cell types. Graphs represent the quantitative analysis of hybridization of a 32P-labeled NKLAM cDNA probe to RNA from isolated subpopulations of peripheral blood cells (A) and different cell lines and tissues (B). The relative amount of NKLAM mRNA in each cell type was determined by setting the level of NKLAM mRNA in unstimulated NK3.3 cells (A, bar 10) to a value of 1 after normalizing RNA levels using the RPS3 probe. Quantitative analysis was performed using a PhosphorImager. A, Peripheral blood monocytes, NK cells, T cells, and CTL were obtained as described in Materials and Methods. By flow cytometric analysis, monocytes were >80–96% pure, T cells were >96% CD3+, NK cells were at least 85% CD16+ and/or CD56+ and <1% CD3+, and the CTL line was >99% CD8+. CTL were stimulated with irradiated JY cells at an E:T cell ratio of 5:1 for 4 h. Peripheral blood NK cells, monocytes, and NK3.3 were cultured in complete medium with or without 10,000 U/ml IFN-β for the times indicated. T cells were cultured for 24 or 48 h in complete medium with or without IL-2 (200 U/ml) or PHA (50 μg/ml). B, Cell lines were cultured with or without IFN-β (10,000 U/ml) for 4 h. These included the breast cancer cell line ZR-75–1, the T cell line MOLT-4, the erythroleukemia cell line K562, and the myeloma cell line ARH77. Other cells tested were the neuroglioma cell line H4, the fibroblast line A23, and normal liver obtained from autopsy.

FIGURE 8. NKLAM antisense oligonucleotide-mediated decrease in NK cytolysis activity. NK3.3 cells were transferred to complete medium without IL-2. NKLAM S, AS, and myc AS ODN were added to the medium at concentrations of 15 μM and introduced into the cells by electroporation, as described in Materials and Methods. After the cells rested in the electroporation chambers for 1 h, they were placed in medium supplemented with 200 U/ml IL-2. Control cells were electroporated without ODN. One set of control cells was placed in IL-2 medium to act as an electroporation control (E), and the other set was placed in medium without IL-2 to act as an unstimulated control (—). After incubation for 18 h, 51Cr release assays were performed as described using radiolabeled K562 target cells. Results are expressed as the percent specific lysis of K562. This represents one of four experiments with similar results. SDs of triplicate values in each experiment did not exceed 8%.
activity. A corresponding, selective decrease in NKLAM RNA levels was seen (Fig. 10).

Studies were performed to determine where NKLAM protein resides in cytolytic cells. Cellular organelles from IL-2-stimulated NK3.3 cells disrupted by nitrogen cavitation were separated by iodixanol (Optiprep) density gradient centrifugation. As shown in Fig. 11, both granzyme B and NKLAM were detected in fractions with densities ranging from 1.06 – 1.1 g/cm³. These fractions also contained the lysosomal enzyme β-glucuronidase (not shown).

NKLAM colocalizes with granzyme B to cytoplasmic granules

Studies were performed to determine where NKLAM protein resides in cytolytic cells. Cellular organelles from IL-2-stimulated NK3.3 cells disrupted by nitrogen cavitation were separated by iodixanol (Optiprep) density gradient centrifugation. As shown in Fig. 11, both granzyme B and NKLAM were detected in fractions with densities ranging from 1.06 – 1.1 g/cm³. These fractions also contained the lysosomal enzyme β-glucuronidase (not shown).

NKLAM colocalizes with granzyme B to cytoplasmic granules

Discussion

This report describes the initial characterization of a novel gene, designated NKLAM. This gene, isolated from a cDNA library made from IFN-β-stimulated NK3.3 cells, is highly expressed in activated cells with cytotoxic potential, including NK cells, CTL, and macrophages. Kinetic studies demonstrate that NKLAM RNA expression just precedes and strongly correlates with cytokine-enhanced NK cytolytic activity. The full-length NKLAM cDNA clone is 2874nt. The 5′-untranslated region is very GC rich (80% GC) and can potentially form strong secondary structures. This
The Journal of Immunology

Cytokine treatment often results in elevation of RNA levels by increasing message stability. For example, IL-2 dramatically enhances the half-life of IFN-γ mRNA in NK3.3 cells, resulting in a significant accumulation and corresponding increase in IFN-γ mRNA (43). However, the half-life of NKLAM mRNA, which is approximately 2.5 h in untreated NK cells, is identical in IL-2- and IFN-β-treated cells (data not shown). Therefore, the increased steady state levels of NKLAM mRNA in response to IL-2 and IFN-β are most likely due to cytokine-mediated transcriptional activation of NKLAM.

Data obtained with a combination of immunoprecipitation and Western blot techniques suggest that NKLAM protein is also overexpressed in cytokine-stimulated cells. The set of proteins in IL-2-stimulated NK3.3 cells detected by Western blotting using polyclonal Abs to NKLAM probably corresponds to NKLAM by the following criteria. First, these proteins are not recognized or precipitated by control Abs to thioredoxin purified from the same rabbit antisera. However, the same pattern of proteins is precipitated by Abs to the N- and C-terminal part of NKLAM and is recognized by both these Abs in immunoblotting (data not shown). Second, the 65-kDa protein recognized by NKLAM-specific Abs has an apparent molecular mass consistent with that predicted from the cDNA sequence of NKLAM and is the same size as the in vitro translation product and protein derived by transient transfection of COS-7 cells with NKLAM. The 75-kDa protein may be a product of post-translational modification. The nature of the large protein species migrating in the 110–130 kDa range in these Western blots is still uncertain. The size of the band suggests that it might represent a homodimer of NKLAM or a multiprotein complex. However, this seems unlikely, since all samples were boiled in SDS under reducing conditions before electrophoresis. Alternatively, this band may be another novel protein with homology to NKLAM that reacts with Abs directed against both the N- and C-terminal regions of NKLAM. However, if it is another protein, it is also found in NK cytoplasmic granules, comigrating with NKLAM and granzyme B. Another possibility is that this band represents the protein product of alternatively spliced NKLAM mRNA. This interpretation is consistent with our recent finding of a homologous murine NKLAM transcript with a significantly longer open reading frame than that of human NKLAM. These possibilities are currently being tested.

To further assess the potential functional involvement of NKLAM in the cytolytic process, CTL were stimulated with their specific alloreactive target, JY. NKLAM RNA levels increased >10-fold in CTL after 4 h of target stimulation. Under similar conditions of target stimulation using NK cells as effectors, Salcedo et al. saw a similar increase in granzyme B mRNA accumulation (16). These results lend additional support for the role of NKLAM in cytosis.

To more directly establish a link between NKLAM expression and cytolytic function, a series of antisense oligonucleotide experiments was performed. Antisense approaches have been successfully used to delineate functional roles for a number of gene products involved in cell-mediated cytosis, including the involvement of granzymes A, B, and NK-TR in NK lytic activity (47–50). We have tried to control for potential nonspecific, nonantisense-mediated effects in a variety of ways. The NKLAM antisense constructs used in these experiments do not contain a dG quartet, which has been associated with nonspecific binding of oligomer to proteins (51). Several antisense constructs were tested; data from two constructs showing the most dramatic effects on NKLAM expression with little or no toxicity are presented. Electroporation was used to introduce ODN into cells, which facilitates entry and allows lower ODN concentrations to be used (44). By monitoring the expression

FIGURE 11. NKLAM protein colocalizes with granzyme B to the cytoplasmic granules of IL-2-stimulated NK3.3 cells. Consecutive fractions from NK3.3 lysates centrifuged through a preformed 10–50% Optiprep gradient were collected and separated on a 12% polyacrylamide gel. Western blotting was performed using a mixture of Abs to granzyme B and NKLAM. The positions of the m.w. markers (Benchmark, Life Technologies) are shown on the right. The positions of granzyme B (Gr) and NKLAM proteins are indicated on the left. The fractions presented range in density from 1.013–1.139 g/cm³. NKLAM and granzyme B are detectable in fractions with densities between 1.06 and 1.1 g/cm³. The peak of both granzyme B and NKLAM expression is in the fraction with a density of 1.087 g/cm³.
of several RNA species over time, including IFN-γ and RPS3, we
were able to determine that NKLAM antisense oligonucleotide
treatment selectively decreased NKLAM RNA levels, while hav-
ing no apparent effect on other gene expression. This reduction in
NKLAM RNA expression was associated with a significant reduc-
tion in both NK and CTL cytolytic function. Further specificity of
the antisense NKLAM treatment for cytotoxicity was shown by its
lack of effect on NK cell growth or viability. Cumulatively, these
results support the thesis that NKLAM is associated with NK and
T cell lytic activity.

Subcellular fractions of NK3.3 cells were obtained by density
gradient centrifugation. Immunoblotting of fractions using Abs to
NKLAM and granyme B indicate that these two proteins pre-
cisely comigrate and peak at a density characteristic of cytoplas-
mic granules. These data plus the putative transmembrane domains
predicted for NKLAM suggest that NKLAM is present within
granule membranes. Further fractionation studies and immuno-
localization studies are in progress. We have shown that small
amounts of NKLAM protein are detectable in unstimulated NK3.3
cells; IL-2 stimulation increases NKLAM protein levels 10- to
20-fold (Fig. 6). Our working hypothesis is that preformed
NKLAM is responsible for basal NK activity, but higher levels
must be produced for cytokine-mediated, enhanced NK cytosis.
A similar scenario might be envisaged for CTL function, where Ag
stimulation would induce higher levels of NKLAM expression,
and therefore increase cytotoxicity. The antisense experiments
suggest that NKLAM protein is relatively short lived and can be
significantly depleted within 18–24 h. This would explain the dra-
matic reduction in NK and CTL function after antisense treatment.

High level expression of NKLAM mRNA is limited to activated
or actively killing NK cells, CTL, and macrophages. In freshly
isolated monocytes, the initially high level of NKLAM mRNA
(which may in part be due to the mechanical stimulation of these
cells by adherence, followed by scraping) can be further increased
by stimulation with IFN-β (Fig. 7A) or with IFN-γ and LPS (data
not shown). While NK cells, CTL, and macrophages have distinct
lineages and immunological properties, they share the ability to
kill. The major mechanism of NK- and CTL-mediated cytosis is
by the Ca2+-dependent release of perforin and granzymes from
cytoplasmic granules. A second lytic pathway functional in NK
cells and CTL is mediated by Fas-FasL interactions. It is highly
likely that NKLAM is at least involved in the perforin-dependent
mechanism of killing due to its location in the cytolytic granules
and since K562, the tumor target used in these studies, does not
express Fas. It remains to be determined whether NKLAM may
also play a role in Fas-dependent lysis.

Monocyte/macroage killing of tumor targets appears to be
mediated by TNF-α and/or nitric oxide production (52–55). TNF-
mediated killing results from interaction between insoluble TNF
on the surface of the macrophage and the TNF receptor on the
target cell. It is of interest that the TNF receptor (types I and II)
and Fas are members of the same family of molecules with three or
four modules of cysteine-rich domains in their N-termini (56, 57).
Although the cysteine arrangement in NKLAM is different from
that of the TNF receptor family, the suggestion of a distant simi-
larity in structure, and perhaps function, is nevertheless intriguing.
In summary, the restricted expression of NKLAM mRNA to
killing cells (NK cells, CTL, and macrophages), the strong corre-
lation of NKLAM RNA expression with cytolytic function and
up-regulation by cytokines that enhance cytosis, the localization
of NKLAM protein to NK cytolytic granules, and the selective
inhibition of cytokine-enhanced cytosis by NKLAM antisense
oligonucleotide-treated NK cells and CTL, strongly indicate an
important role for NKLAM in cell-mediated cytotoxicity. Further
functional characterization of NKLAM should increase our under-
standing of the killing mechanism used by NK and other cytolytic
cells, which is critical for designing better means of immunothera-
peutic intervention and perhaps, ultimately, the prevention of can-
cer and viral diseases.

Acknowledgments

We thank James Crouch, J. Catherine Cone, and Kathleen Hayes for their assistance and Dr. Richard Hoover for his constructive comments.

References

2. Trinchieri, G. 1989. Biology of natural killer cells. In Advances in Immunology,
cultures: effects of interleukin-2 and interferon on cell growth and cytokoric re-
and B. Perussia. 1984. Response of resting human peripheral blood natural killer
5. Kobayashi, M., L. Fitz, M. Ryan, R. M. Hewick, S. C. Clark, S. Chan, R. London,
of natural killer cell stimulatory factor (NKSF): a cytokine with multiple biologic
(IL) 15 is a novel cytokine that activates human natural killer cells via compo-
ments of the IL-2 receptor. J. Exp. Med. 180:1395.
killer phenomenon: relative contribution of peripheral blood natural killer cells
on the treatment of 157 patients with advanced cancer using lymphokine-
activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N. Engl.
J. Med. 316:809.
of 283 consecutive patients with metastatic melanoma or renal cell cancer using
high-dose bolus IL-2. JAMA 271:907.
of intravascular fluid mediated by the systemic administration of recombinant in-
11. Belldegerm, A., D. E. Webb, H. A. Austin, S. M. Steinberg, D. E. White,
Med. 106:817.
directions. Contemp. Oncol. 92:34.
of metabolic inhibitors on spontaneous and interferon-boosred human natural killer
with interleukin-2 and interferon-β-induced augmentation of natural killer activity.
In Natural Killer Cells: Biology and Clinical Application. R. E. Schmidt, ed.
Karger, Basel, p. 212.
perforin and granzyme messenger RNA expression in human natural killer cells.
Human natural killer adhesion molecules: differential expression after activation
19. Leiden, J. M., K. M. Gottedeiner, J. Quertierloum, L. Coursy, R. A. Bray,
1988. T-cell receptor gene rearrangement and expression in human natural killer
cells: natural killer activity is not dependent on the rearrangement and expression
of T-cell receptor α, β, or γ genes. Immunogenetics 27:231.
with IFN-β and IL-2-induced augmentation of human natural killer cell function.
Protocols in Immunology. J. E. Coligan, A. M. Kruisbeek, D. H. Margulies,


