Title
NKR-P1A is a target-specific receptor that activates natural killer cell cytotoxicity.

Journal
Journal of Experimental Medicine, 181(5)

ISSN
0022-1007

Authors
Ryan, JC
Niemi, EC
Nakamura, MC
et al.

Publication Date
1995-05-01

DOI
10.1084/jem.181.5.1911

Peer reviewed

Brief Definitive Report

NKR-P1A Is a Target-specific Receptor That Activates Natural Killer Cell Cytotoxicity

By James C. Ryan, Eréne C. Niemi, Mary C. Nakamura, and William E. Seaman

From the Department of Medicine, University of California, San Francisco, San Francisco, California 94143; and the Veterans Administration Medical Center, San Francisco, California 94121

Summary

NKR-P1A is a lectinlike surface molecule expressed on rat natural killer (NK) cells. NKR-P1A has structural and functional features of an activating NK cell receptor, but a requirement for NKR-P1A in target cell lysis has not been determined. To define the role of NKR-P1A in natural killing, we have generated a mutant of the rat NK cell line, RNK-16, lacking expression of all members of the NKR-P1 receptor family. Although these NKR-P1–deficient NK cells were able to kill many standard tumor targets, including YAC-1, they were selectively deficient in the lysis of IC-21 macrophage, B-16 melanoma, and C1498 lymphoma targets. Reexpression of a single member of the NKR-P1 family, NKR-P1A, on mutant cells restored lysis of IC-21, and killing of IC-21 targets through rat NKR-P1A was completely blocked by $F(ab')_2$ anti-NKR-P1A. Reexpression of NKR-P1A also restored transmembrane signaling to IC-21, as assessed by the generation of inositol-1,4,5-trisphosphate. The generation of inositol-1,4,5-trisphosphate was also restored in response to B-16 targets, but both B-16 and C1498 cells remained resistant to lysis, indicating that other NK cell molecules, perhaps within the NKR-P1 family, are required for the efficient killing of these tumors. These results are the first to demonstrate that NKR-P1A is a target-specific receptor that activates natural killing.

NK cells are large granular lymphocytes that spontaneously lyse certain tumors, virally infected cells, and blast cells. The receptors on NK cells that recognize target cells and thereby activate NK cells have not been defined (1). In rats, candidates include the lectinlike molecules NKR-P1A, which can activate NK cell cytotoxicity (2, 3). The NKR-P1 receptor family was defined by NKR-P1A, a 60-kD type II integral membrane homodimer with an extracellular calcium-dependent (C-type) lectin domain (2–4). NKR-P1A is one member of a family of receptors, at least three in number, identified by cDNA cross-hybridization (5, 6). mAb to NKR-P1A (3.2.3) binds to all rat NK cells and stimulates NK cytotoxicity, phosphoinositide turnover, calcium mobilization, and degranulation (2, 7). The importance of NKR-P1A in tumor lysis, however, is yet to be determined. A physiologic ligand for NKR-P1A has not been identified, and mAb to NKR-P1A does not block cytotoxicity against standard targets such as YAC-1 (2, 7).

To define the role of NKR-P1A in natural killing, we have used the rat NK cell line RNK-16, which expresses NKR-P1A (7). After treatment of RNK-16 cells with the mutagen ethylmethane sulfonate, we isolated a variant cell line that lacked surface expression of NKR-P1A and, by Northern blot analysis, lacked transcripts for all members of the NKR-P1 family. Functional studies using this NKR-P1A–deficient mutant line demonstrate that NKR-P1A is a target-specific receptor that activates natural killing.

Materials and Methods

Antibodies. mAb 3.2.3 (anti-NKR-P1A), 3G7 (anti-gp42), and B22.249 (anti-H-$2D^b$, α_1/α_2) were produced as ascites and were partially purified by precipitation in 50% saturated ammonium sulfate at 4°C for 18 h followed by dialysis against PBS. $F(ab')_2$ fragments were produced as previously described (7), and $F(ab')_2$ preparations were analyzed for completeness of digestion by SDS-PAGE.

Cell Lines. RNK-16, an NK cell leukemia line from F344 rats, was a gift from Craig Reynolds (National Cancer Institute, National Institutes of Health, Frederick, MD) (7). The MHC class I transfectants of C1498, termed C1498.D12, C1498.K18, C1498.D8, were reported previously (8) and were provided by Wayne Yokoyama and Franz Karlhofer (Mount Sinai Medical Center, New York), as were the tumor targets KF.9, RBL-5, and RMA-S. The 51B colon carcinoma line (9) was obtained from Robert Bresalier (Henry Ford Hospital, Detroit, MI). The B-16 melanoma line was from Klas Kärre (Karolinska Institute, Stockholm, Sweden). The B22.249 hybridoma was supplied by David Raulet (University of California, Berkeley, CA). The PA317 and the Ψ_2 retroviral packaging lines were from Dan Littman (University of California, San Francisco, CA). All other tumors were obtained from the American Type Culture Collection (Rockville, MD). All cells were grown in com-

plete RPMI (RPMI 1640, 10% fetal bovine serum, penicillin [100 U/ml], streptomycin [100 μg/ml], glutamine [2 mM], and 2-ME [50 μM]) at 37°C in a humidified 5% CO_2 atmosphere.

Preparation of Murine Blast Targets. Con A–stimulated blast cells were generated as described (10). Fresh murine splenocytes were adjusted to 2 × 10^6 cells/ml and cultured for 2–3 d in complete RPMI supplemented with 5 μg/ml of Con A. Before use as targets, cells were collected on Ficoll to remove dead cells.

Generation of NKR-P1A–deficient Mutants of RNK-16. RNK-16.M13 mutants were derived as previously described (11). RNK-16 cells were treated with ethylmethane sulfonate (200 μg/ml) in complete RPMI for 24 h. Cells were washed, and then allowed to grow for 3 d. They were then selected against the expression of the 3.2.3 (NKR-P1A) antigen by flow sorting. After five rounds of negative selection, NKR-P1A–negative cells were cloned and then subcloned to limiting dilution. The cell surface phenotype of each clone was determined, and several clones were analyzed for NKR-P1 mRNA expression.

Northern Blot Analysis. Poly(A)$^+$ RNA was prepared by a variation of the mRNA isolation protocol (Fast Track; Invitrogen, San Diego, CA) according to the manufacturer's instructions. 2 μg of poly(A)$^+$ RNA from each cell line was analyzed by Northern blot analysis according to standard methods (12). The RNA was resolved by gel electrophoresis and transferred to a nitrocellulose membrane. A ^{32}P-radiolabeled full-length rat NKR-P1A cDNA probe was generated using α-[^{32}P]dCTP (3,000 Ci/mmol, Amersham Corp., Arlington Heights, IL) by using the Random Hexanucleotide Primer Labeling kit (Boehringer Mannheim Corp., Indianapolis, IN) according to the manufacturer's instructions. After prehybridization, the membrane was incubated with the probe overnight at 42°C in hybridization solution (50% formamide, 6× SSC, 5× Denhardt's, 1% SDS, and 50 μg/ml salmon sperm DNA) as described (12). The membrane was then washed at progressively higher stringency, with the final wash in 0.25× SSC, 0.1% SDS, at 42°C. As a control, the blot was stripped by treatment with 1 mM EDTA at 95°C for 15 min and reprobed with the glyceraldehyde-phosphate dehydrogenase (GAPDH) cDNA.

Transfection of RNK-16.M13 with the Rat NKR-P1A cDNA. Reconstitution of NKR-P1A expression in RNK-16.M13 mutants was accomplished using the retroviral gene transfer system as described (13). Briefly, the full-length rat NKR-P1A cDNA was subcloned into the EcoRI site of the pMV7 expression vector, which was then transfected into the packaging cell line Ψ_2 by using calcium phosphate precipitation (12). After 48 h, culture supernatants were used to infect PA317 packaging cells in 100-mm dishes in the presence of polybrene (8 μg/ml) for 2 h. Stably transfected PA317 packaging lines were generated by selection in G418 (250 μg/ml in complete RPMI). Supernatants from NKR-P1A–transfected PA317 cells were incubated with RNK-16.M13 cells in the presence of polybrene. After 2 d, the RNK-16.M13 cells were selected in 300 μg/ml of G418 in complete RPMI. NKR-P1A–expressing cells were isolated and subcloned after ~20 d in selection. One clone, RNK-16.T18, exhibited a phenotype characteristic of all high expressing transfectants.

Cytotoxicity Assays. Cytotoxicity was measured by using ^{51}Cr-release microcytotoxicity assays as previously described in detail (7). For antibody-blocking studies, F(ab')$_2$ fragments (50 μg/10^6 effectors) were added to effectors at 20°C 15 min before assays.

Assays for Phosphoinositide Turnover. Assays for inositol-1,4,5-trisphosphate ($InsP_3$) were performed exactly as described (7). Briefly, duplicate samples of 5 × 10^6 [^{3}H]inositol-labeled NK cells were stimulated with 10^7 targets in a total volume of 1 ml. The cells were rapidly pelleted and resuspended in 1 ml of 10% TCA. The [^{3}H]$InsP_3$ from each sample was extracted with diethyl ether and resolved by ion exchange chromatography on Dowex Ag 1-X8 (Bio-Rad Laboratories, Hercules, CA).

Results and Discussion

RNK-16 cells were treated with the mutagen ethylmethane sulfonate and selected against the surface expression of rat NKR-P1A. Eight cloned cell lines lacked detectable surface expression of NKR-P1A. One line, RNK-16.M13, also lacked detectable mRNA transcripts for all members of the NKR-P1 family (Fig. 1). RNK-16.M13 expressed other surface molecules at normal levels, including CD2, CD45, and gp42 (not shown).

RNK-16.M13 lysed most tumor targets, including YAC-1, with approximately the same efficiency as the parent line (Table 1). Three targets that were susceptible to lysis by RNK-16, however, were not lysed by RNK-16.M13: IC-21 macrophage, C1498 lymphoma, and B-16 melanoma cells. Similar results were obtained with the seven other mutant RNK-16 clones that lacked NKR-P1A (not shown). Of note, IC-21, C1498, and B-16 were all derived from C57BL/6 mice (H-2^b), and they were the only H-2^b targets that were killed by wild-type RNK-16. Tumors from H-2^d or from (H-2^b × H-2^d)F_1 mice were lysed equally by the parent and mutant cell lines, and transfection of H-2^b C1498 lymphoma cells with the cDNAs encoding H-2D^d, H-2K^d, or H-2L^d did not alter their susceptibility to lysis by either line. The H-2^b tumor target RBL-5 and its MHC-deficient mutant, RMA-S (14), were uninformative, since neither was lysed by RNK-16 or RNK-16.M13 cells, nor were T cell blast targets from either C57BL/6 (H-2^b) or BALB/c (H-2^d) mice.

The expression of NKR-P1A, the most abundant member of the NKR-P1 family, was restored on RNK-16.M13 cells by retroviral gene transfer. A cloned line, RNK-16.T18, expressed NKR-P1A at a level similar to that on parental RNK-16 cells (Fig. 2). The killing of informative targets by RNK-16.T18, RNK-16.M13, and RNK-16 cells was exam-

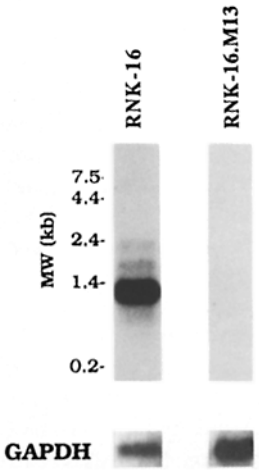

Figure 1. Northern blot analysis of RNA from wild-type RNA-16 and the NKR-P1–deficient mutant RNK-16.M13. When probed with the full-length (1.0 kb) rat NKR-P1A cDNA, wild-type RNA-16 expresses 1.1 kb, 1.7 kb, and 2.4 kb NKR-P1–related mRNA species. RNK-16.M13, however, expresses no NKR-P1 transcripts. Both cells express similar amounts of control GAPDH mRNA.

Table 1. *Lysis of Murine Target Cells by RNK-16 and RNK-16.M13*

Cell line	Type	H-2	RNK-16				RNK-16.M13			
			40	20	10	5	40	20	10	5
			E/T ratio				*E/T ratio*			
YAC-1	Lymphocytes	a	69	64	56	43	44	36	30	18
RLσ1	Lymphoma	d	69	64	51	38	65	64	49	30
P388.D1	Macrophage	d	71	58	51	37	27	18	12	8
51B	Carcinoma	d	46	41	36	29	42	38	34	25
RAW.309	Macrophage	b × d	20	19	17	12	13	6	4	3
IC-21	Macrophage	b	30	27	23	19	6	5	6	6
B-16	Melanoma	b	28	23	17	13	5	4	4	4
C1498	Lymphoma	b	31	30	24	17	0	0	0	0
C1498.D12	Lymphoma	b + D^d	49	43	34	21	0	0	0	0
C1498.K18	Lymphoma	b + K^d	38	33	25	17	0	0	0	0
C1498.L8	Lymphoma	b + L^d	39	37	28	18	0	0	0	0
KF.9	Lymphoma	b	6	5	3	2	0	0	0	0
TIMI.4	Lymphoma	b	1	1	1	1	2	2	2	1
RBL 5	Lymphoma	b	1	2	1	3	0	0	0	0
RMA-S	Lymphoma	b^-	1	1	1	0	0	0	0	0
P815	Mastocytoma	d	9	7	4	1	2	1	1	0
R1.1	T cell	k	2	3	3	1	0	0	1	0
BW5147.3	T cell	k	4	5	6	5	4	3	3	2
BALB/c	T blast*	d	5	3	1	1	0	0	0	0
C57BL/6	T blast*	b	4	3	3	1	0	0	0	0

Mutational loss of NKR-P1 by RNK-16 cells is accompanied by loss of cytotoxicity against selected targets. Lysis of most targets by RNK-16 and NKR-P1–deficient RNK-16.M13 was similar. NKR-P1–deficient RNK-16.M13, however, was selectively unable to kill IC-21, B-16, and C1498. Transfection of C1498 with the cDNAs encoding H-$2D^d$ (C1498.D12), H-$2K^d$ (C1498.K18), or H-$2L^d$ (C1498.L8) did not alter susceptibility to lysis by either effector. RBL-5 and its MHC class I–deficient mutant, RMA-S (8), were uninformative targets, since neither was killed. Lysis of all tumor targets was examined at E/T ratios of 40:1, 20:1, 10:1, and 5:1.

* For T cell blast targets, effectors were added at E/T ratios of 100:1, 50:1, 25:1, and 12.5:1.

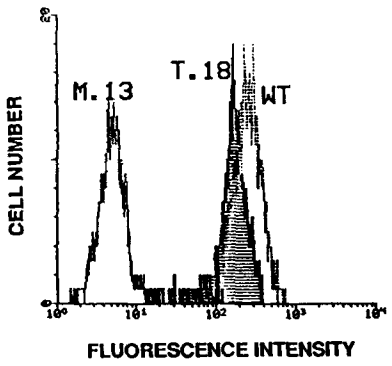

Figure 2. NKR-P1A–specific cell surface staining of wild-type RNK-16, RNK-16.M13, and RNK-16.T.18 by flow cytometry. RNK-16.M13 mutant cells (*M.13*) express no cell surface NKR-P1A as determined by staining with the 3.2.3 mAb. NKR-P1A–transfected mutants RNK-16.T18 (*T.18*, *shaded*) express NKR-P1A at levels comparable to those of wild-type RNK-16 (*WT*). Expression of CD45, CD2, and gp42 was similar in all lines (not shown). None of the cell lines stained with various control antibodies, including antibodies against rat CD3 (not shown).

ined (Fig. 3, *A–D*). Killing of YAC-1 targets by RNK-16.T18 was equal to killing by RNK-16.M13 cells. (In the experiment presented, killing of YAC-1 by both lines was less than by RNK-16, but this was not regularly the case.) Killing of IC-21 cells, not seen with RNK-16.M13 cells, was substantially restored by expression of NKR-P1A on RNK-16.T18 cells. Thus, NKR-P1A is necessary and sufficient for lysis of IC-21. Other members of the NKR-P1 family, however, might also have this capability. In support of this, $F(ab')_2$ antibody to NKR-P1A completely blocked killing of IC-21 by RNK-16.T18 cells, but it had no effect on killing of IC-21 by parent RNK-16 cells, which express all members of the

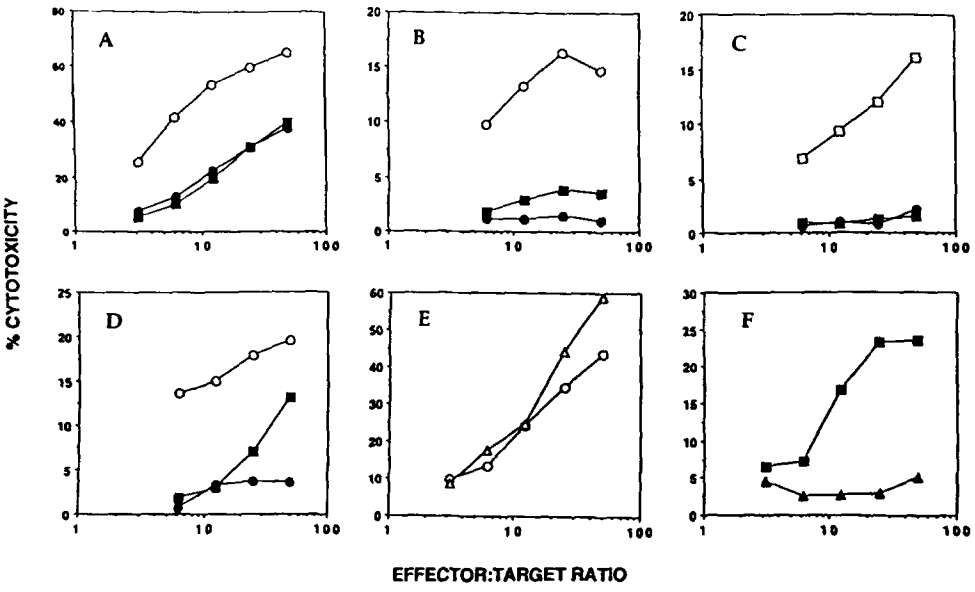

Figure 3. Cytotoxicity (*A–F*) by wild-type RNK-16, RNK-16.M13, and RNK-16.T18 cells. NK cell cytotoxicity was tested against YAC-1 (*A*), C1498 (*B*), B-16 (*C*), and IC-21 (*D–F*) tumors. YAC-1 (*A*) was killed by wild-type RNK-16 (○), RNK-16.M13 mutant cells (●), and by NKR-P1A–transfected RNK-16.T18 (■). C1498 (*B*) was lysed by RNK-16, not by RNK-16.M13, and weakly by RNK-16.T-18. B-16 (*C*) was lysed by RNK-16, not by RNK-16.M13, and not by RNK-16.T18. IC-21 (*D*) was killed by RNK-16, and not by RNK-16.M13, but was lysed by the RNK-16.T18 transfectants. Lysis of IC-21 by RNK-16 (○, *E*) was not blocked by F(ab')$_2$ 3.2.3 (△, *E*). Killing of IC-21 by RNK-16.T18 (■, *F*), however, was completely blocked by F(ab')$_2$ 3.2.3 (▲, *F*), but not by F(ab')$_2$ B22.249 (anti-H-2D^b, α_1/α_2) or F(ab')$_2$ 3G7 (anti-gp42) (not shown).

NKR-P1 family (Fig. 3, *E–F*). We conclude that NKR-P1A is a specific receptor for IC-21 but that other members of the NKR-P1 family can also serve this function.

Killing of C1498 or B-16 cells, in contrast to IC-21 cells, was not substantially restored by expression of NKR-P1A; in each of three experiments, killing was either absent or was only slightly above baseline (Fig. 3, *B* and *C*). Thus, NKR-P1A alone cannot significantly restore the lytic response to these two targets.

As another assay for the NK response to targets, we next examined polyphosphoinositide turnover, an early transmembrane signal in NK cell activation (7). The generation of $InsP_3$ by RNK-16 cells in response to target cells was compared to the generation of $InsP_3$ by RNK-16.M13 and RNK-16.T18 cells (Fig. 4, *A–C*). All three NK cell lines demonstrated a brisk response to YAC-1, consistent with the demonstration that NKR-P1A is not required for lysis of this target. Both IC-21 and B-16 cells stimulated a response in RNK-16 cells that was absent in RNK-16.M13 cells and was restored in RNK-16.T18 cells. The response to C1498 was too low in all cell lines to detect significant changes. The response to IC-21 is thus concordant with cytotoxicity, but NKR-P1A restores the inositol response to B-16 without restoring killing. Additional signals are apparently needed for lysis of B-16, perhaps supplied by other members of the NKR-P1 family.

The NKR-P1 gene family is part of a superfamily of lectinlike receptors that are genetically linked (15–18). In the mouse, these include the Ly-49 family, whose ligands include class I MHC antigens. Ly-49A binds to H-2D^d and, in contrast to NKR-P1A, inactivates cytotoxicity (8, 19, 20). The specificity of Ly-49A for class I MHC antigens and the inability of RNK-16.M13 cells to lyse H-2^b targets raised the possibility that NKR-P1A might specifically recognize class I MHC antigens of the H-2^b haplotype. Lysis of these targets, however, was not blocked by F(ab')$_2$ antibody to the

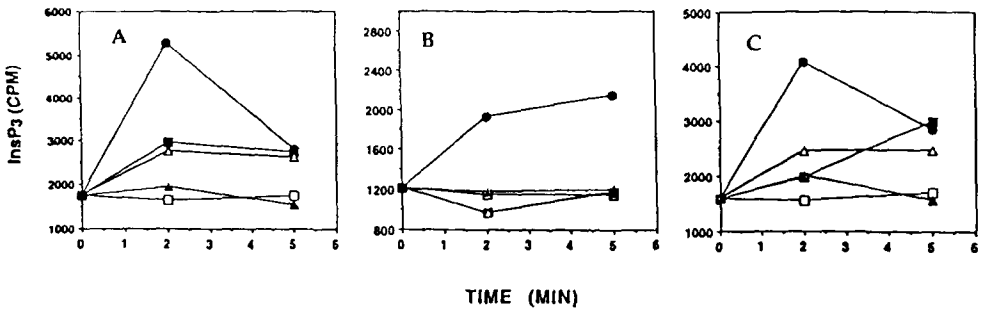

Figure 4. Tumor-induced $InsP_3$ generation by RNK-16 (*A*), RNK-16.M13 (*B*), and RNK-16.T18 (*C*). NK cell lines were stimulated with YAC-1 (●), IC-21 (■), B-16 (△), C1498 (▲), or medium alone (□). RNK-16 (*A*) responded briskly to YAC-1 (●), IC-21 (■), and B-16 (△), and responded weakly to C1498 (▲). RNK-16.M13 (*B*) was stimulated by YAC-1, but not by IC-21, B-16, or C1498. RNK-16.T18 (*C*) was stimulated by YAC-1, IC-21, and B-16, and was minimally stimulated by C1498. Thus, NKR-P1A restores inositol signaling in response to IC-21, and it restores inositol signaling but not killing in response to B-16. Signaling in response to C1498 by all three NK cell lines was too low to detect significant changes.

α_1/α_2 domain of the H-2D^b allele, and not all H-2^b targets were killed by NKR-1A$^+$ cells (Table 1). Also, recent studies of human NK clones have not shown a correlation between response to class I antigens and the expression of a human homologue of NKR-P1A (18). Resolution of this issue will require isolation of the ligands for NKR-P1A. Although a physiologic ligand for NKR-P1A has not yet been fully characterized, soluble preparations of the rat NKR-P1A lectin domain can bind to specific monosaccharides and neoglycoconjugates (4). IC-21 targets should be a fruitful source for characterization of a natural ligand for NKR-P1A.

Our studies are the first to demonstrate a target-specific receptor that activates natural killing. The superfamily of lectinlike receptors on NK cells thus includes specific receptors that can either activate (NKR-P1) or inhibit (Ly-49) lysis by NK cells in response to specific ligands on target cells. The NKR-P1 family appears to provide specificity for only a limited range of targets. It is likely that other specific receptors remain to be identified, perhaps within the superfamily of NK lectinlike receptors.

We thank Greg Bell for assistance with RNK-16 mutagenesis, and John Imboden and David Wofsy for their help in preparation of the manuscript.

This work was supported by the United States Veterans Administration and the National Institutes of Health (R29 CA-60944-02).

Address correspondence to Dr. James C. Ryan, Department of Medicine 111R, Veterans Administration Medical Center, 4150 Clement Street, San Francisco, CA 94121.

Received for publication 31 October 1994 and in revised form 23 January 1995.

References

1. Trinchieri, G. 1989. Biology of natural killer cells. *Adv. Immunol.* 47:187–376.
2. Chambers, W.H., N.L. Vujanovic, A.B. DeLeo, M.W. Olszowy, R.B. Herberman, and J.C. Hiserodt. 1989. Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine-activated killer cells. *J. Exp. Med.* 169:1373–1389.
3. Giorda, R., W.A. Rudert, C. Vavassori, W.H. Chambers, J.C. Hiserodt, and M. Trucco. 1990. NKR-P1, a signal transduction molecule on NK cells. *Science (Wash. DC).* 249:1298–1300.
4. Bezouska, K., G. Vlahas, O. Horvath, G. Jinochova, A. Fiserova, R. Giorda, W.H. Chambers, T. Feizi, and M. Pospisil. 1994. Rat natural killer cell antigen, NKR-P1, related to C-type animal lectins is a carbohydrate binding protein. *J. Biol. Chem.* 269:16945–16952.
5. Giorda, R., and M. Trucco. 1991. Mouse NKR-P1. A family of genes selectively co-expressed in adherent lymphokine-activated killer cells. *J. Immunol.* 147:1701–1708.
6. Yokoyama, W.M., and W.E. Seaman. 1993. The Ly-49 and NKR-P1 gene families encoding lectin-like receptors on natural killer cell: the NK gene complex. *Annu. Rev. Immunol.* 11:613–635.
7. Ryan, J.C., E.C. Niemi, R.D. Goldfien, J.C. Hiserodt, and W.E. Seaman. 1991. NKR-P1, an activating molecule on rat NK cells, stimulates phosphoinositide turnover and a rise in intracellular calcium. *J. Immunol.* 147:3244–3250.
8. Karlhofer, F.M., R.K. Ribaudo, and W.M. Yokoyama. 1992. MHC class I allospecificity of Ly-49$^+$ IL-2-activated natural killer cells. *Nature (Lond).* 358:66–70.
9. Bresalier, R.S., R.W. Rockwell, R. Dahiya, and Y.S. Kim. 1990. Cell surface sialoprotein alteration in metastatic murine colon cancer cell lines selected in an animal model for colon cancer metastasis. *Cancer Res.* 50:1299–1307.
10. Vaage, J.T., C. Naper, C. Løvik, D. Lambracht, A. Rehm, H.J. Hedrich, K. Wonegeit, and B. Rolstad. 1994. Control of rat natural killer cell mediated allorecognition by a major histocompatibility complex region encoding nonclassical class I antigens. *J. Exp. Med.* 180:641–651.
11. Bell, G.M., G.M. Dethloff, and J.B. Imboden. 1993. CD45-negative mutants of a rat natural killer cell line fail to lyse tumor target cells. *J. Immunol.* 151:3646–3653.
12. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
13. Glaichenhaus, N., N. Shastri, D.R. Littman, and J.M. Turner. 1991. Requirement for the association of p56^{lck} with CD4 in antigen-specific signal transduction in T cells. *Cell.* 64:511–520.
14. Ljunggren, H.-G., S. Pääbo, M. Cochet, G. Kling, P. Kourilsky, and K. Kärre. 1989. Molecular analysis of H-2 deficient lymphoma lines: distinct defects in biosynthesis and association of MHC class I heavy chains and β_2-microglobulin observed in cells with increased sensitivity to NK cell lysis. *J. Immunol.* 142:2911–2917.
15. Yokoyama, W.M., J.C. Ryan, J.J. Hunter, H.R.C. Smith, M.R. Stark, and W.E. Seaman. 1991. A gene complex encoding natural killer cell surface molecules resides on distal mouse chromosome 6. cDNA cloning of mouse NKR-P1 and genetic linkage with Ly-49. *J. Immunol.* 147:3229–3236.
16. Houchins, J.P., T. Yabe, C. McSherry, and F.H. Bach. 1991. 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.* 173:1017–1020.
17. Yabe, T., C. McSherry, F.H. Bach, P. Fich, R. Schall, P.M. Sondel, and J.P. Houchins. 1993. A multigene family on chromosome 12 encodes natural killer cell lectins. *Immunogenetics.* 36:455–460.
18. Lanier, L.L., C. Chang, and J.H. Phillips. 1994. Human NKR-P1A: a disulfide linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J. Immunol.* 153:2417–2428.
19. Kane, K.P. 1994. Ly-49 mediates EL4 lymphoma adhesion to isolated class I major histocompatibility complex molecules. *J. Exp. Med.* 179:1011–1015.
20. Daniels, B.F., F.M. Karlhofer, W.E. Seaman, and W.M. Yokoyama. 1994. A natural killer cell receptor specific for a major histocompatibility complex class I molecule. *J. Exp. Med.* 180:687–692.