2253.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin-phenol agent. J. Biol. Chem. 193:265.
19. Lewis, J. G., and S. V. Pizzo. 1981. Quantification of plasminogen activator. *In* Methods for Studying Mononuclear Phagocytes. Edited by D. O. Adams, P. J. Edelson, and H. S. Koren. Academic Press, New York. In press.
20. Finney, D. J. 1962. Probit Analysis. 2nd ed. Cambridge University Press, Cambridge.
21. Papermaster, B. W., J. H. Pincus, P. Lincoln, and R. A. Reisfeld. 1974. Probit analysis of cytotoxicity data. Transplantation 18:541.
22. Vassalli, J.-D., J. Hamilton, and E. Reich. 1977. Macrophage plasminogen activator: induction by concanavalin-A and phorbol myristate acetate. Cell 11:695.
23. Adams, D. O., W. J. Johnson, and P. A. Marino. 1981. Mechanisms of target recognition and destruction in macrophage-mediated tumor cytotoxicity. Fed. Proc. In press.
24. Chapman, H. A., Z. Vavrin, and J. B. Hibbs, Jr. 1979. Modulation of plasminogen activator secretion by activated macrophages: influence of serum factors and correlation with tumoricidal potential. Proc. Natl. Acad. Sci. 76:3899.
25. Pike, M. C., and R. Snyderman. 1980. Biochemical and biological aspects of leukocyte chemotactic factors. *In* The Reticuloendothelial System: A Comprehensive Treatise, Vol. II. Edited by A. J. Sbarra and R. R. Strauss. Plenum Press, New York. Pp. 1–20.
26. Edelson, P. J. Monocytes and macrophages: aspects of their cell biology. 1980. *In* The Cell Biology of Inflammation. Edited by G. Weissmann. Elsevier-North-Holland Biomedical Press, Amsterdam. Pp. 464–496.
27. Van Furth, Ralph, ed. Mononuclear Phagocytes: Functional Aspects. 1980. Martinus Nijhoff Publishers, The Haig, Netherlands.
28. Cabilly, S., and R. Gallily. 1981. Artificial binding of macrophages to syngeneic cells elicits cytostasis but not cytoysis. Immunology 42:149.
29. Kurisu, M., M. Yamazaki, and D. Mizuno. 1980. Induction of macrophage-mediated tumor lysis by the lectin wheat germ agglutinin. Cancer Res. 40: 3798.
30. Johnston, R. B., C. A. Godzik, and Z. A. Cohn. 1978. Increased superoxide anion production by immunologically activated macrophages. J. Exp. Med. 148:115.
31. Nathan, C. F., L. Brukner, G. Kaplan, J. Unkeless and Z. Cohn. 1980. Role of activated macrophages in antibody-dependent lysis of tumor cells. J. Exp. Med. 152:183.
32. Nathan, C. F., and Z. A. Cohn. 1980. Role of oxygen-dependent mechanisms in antibody-induced lysis of tumor cells by activated macrophages. J. Exp. Med. 152:198.
33. Adams, D. O., W. J. Johnson, E. Fiorito, and C. F. Nathan. 1981. Hydrogen peroxide and cytolytic factor can interact synergistically in effecting cytolysis of neoplastic targets. J. Immunol. 127:1973.

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NATURAL KILLER ACTIVITY IN THE PERITONEAL EXUDATES OF MICE INFECTED WITH *LISTERIA MONOCYTOGENES*: CHARACTERIZATION OF THE NATURAL KILLER CELLS BY USING A MONOCLONAL RAT ANTI-MURINE MACROPHAGE ANTIBODY (M1/70)[1]

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Exudates induced by i.p. injection of live *Listeria monocytogenes* (LM) constituted a rich source of CBA/J murine natural killer (NK) cells. Maximum expression of NK activity was seen from day 2 through day 6 after initial exposure to LM. When nylon wool nonadherent peritoneal exudate cells were examined by a single-cell cytotoxicity assay, the number of cells binding to YAC-1 target cells increased after infection as did their individual lytic capacity. A monoclonal rat anti-murine macrophage antibody (M1/70), previously shown by our group to recognize human NK cells, can also be used as a marker for murine NK cells. Utilizing M1/70 and the fluorescence-activated cell sorter, selection of M1/70-labeled mononuclear cells led to the enrichment of both NK and antibody-dependent cellular cytotoxicity. These M1/70-positive cells had a distinctive morphology and contained granules on Wright-Giemsa staining. They were not phagocytic, did not contain nonspecific esterase, and lacked surface I-A^k, IgM determinants, complement receptors, and high levels of Thy 1.2.

Natural killer (NK)[4] cells are mononuclear cells of disputed lineage that kill certain virus-infected cells, thymocytes, and tumor cells. A number of surface markers, such as NK-1, Ly 5, Thy 1, asialo-GM1, and Mph, have been described on NK cells (for a review, see Reference 1). With the exception of the NK-1 marker, all of the above surface molecules are also expressed on other cells. Some of these markers seem to place the NK cell in a developmental relationship to T lymphocytes (2–6), whereas others suggest a relationship to macrophages (7). Previous work in our laboratory has shown that M1/70, a rat anti-murine macrophage monoclonal antibody, cross-reactively recognizes both human phagocytic cells and the human NK cells that lyse K562 targets (8). The Mac-1 antigenic determinant, identified by M1/70, is present in large amounts on murine splenic macrophages and on unelicited and thioglycollate-elicited peritoneal exudate macrophages. It is present in lesser amounts on neutrophilic granulocytes, blood monocytes, and half of the nucleated bone marrow cells. Mac-1 is seen on less than 0.8% of murine thymus cells and on less than 1% of murine lymph node cells (9–11). In these studies, we have

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[4] Abbreviations used in this paper: ADCC, antibody-dependent cellular cytotoxicity; SRBC, sheep red blood cells; EA 7S, SRBC coated with IgG rabbit anti-SRBC antibody; EA 19S, SRBC coated with IgM rabbit anti-SRBC antibody; EAC, SRBC coated with IgM rabbit anti-SRBC antibody and complement; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; LM, *Listeria monocytogenes*; Mac-1, a cell-surface antigen recognized by M1/70 antibody; M1/70, a monoclonal rat anti-murine macrophage antibody; PEC, peritoneal exudate cells, NAPEC, nylonwool nonadherent PEC; NK, natural killer; p/s, penicillin (50 U/ml) and streptomycin (50 μg/ml); HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

shown that M1/70 recognizes and can be used to enrich for murine NK cells that lyse YAC-1 targets.

Early studies had demonstrated that the single injection of a mixture of *Listeria monocytogenes* (LM) and tumor cells led to a decrease in the growth of the tumor cells (12, 13) and that ^{89}strontium treatment of mice, which affects only the precursor cells contained within the bone marrow, led to a diminution in both NK activity and the early stages of resistance to LM infection (14). Other studies (15–17) have shown that NK activity increased after infection with Bacillus Calmette-Guerin. For these reasons, we felt that infecting mice with the intracellular pathogen, LM, would also lead to an increase in NK activity. We have found this to be the case since murine NK activity is dramatically enhanced in the nylon wool nonadherent peritoneal exudate cells (NAPEC) of mice after LM infection, and these exudates are a rich source of very active NK cells that label with M1/70 antibody. This LM system allows us to answer important questions about the lineage of a specific NK cell, the stimuli that enhance NK activity after infection, and the role of this NK cell in defense against LM. In this paper, we report on the characterization of these M1/70-labeled NAPEC with regard to surface markers and biologic activities.

MATERIALS AND METHODS

Mice. CBA/J (H-2^k) male mice were obtained from the Jackson Laboratories, Bar Harbor, ME. All mice were used at 6 to 8 wk of age, unless otherwise indicated.

Bacteria. LM was provided by Dr. Emil R. Unanue, Harvard Medical School. The preparation of the bacteria has been previously described (18). Aliquots of LM, stored at −20°C, were incubated overnight at 37°C in brain-heart infusion broth (Difco Laboratories, Detroit, MI). They were washed extensively in phosphate-buffered saline (PBS) and the number of bacteria was measured by turbidity and confirmed by colony enumeration.

Preparation of murine cells. PBS or 1.25 to 5 × 10^4 live LM organisms suspended in PBS were injected i.p. into CBA/J mice. The harvest of the PEC was accomplished by peritoneal washing with cold Hanks' balanced salt solution (HBSS) (Microbiological Associates, Walkersville, MD), containing 5% heat-inactivated fetal calf serum (FCS) (Microbiological Associates), penicillin (50 U/ml), streptomycin (50 μg/ml (p/s), and heparin (10 U/ml) (Scientific Products, McGaw Park, IL). The cells were washed 1 time and resuspended in HBSS containing 0.5 mg/ml bovine serum albumin (BSA), p/s, and 10 mM HEPES buffer (Microbiological Associates). The cells were then layered on top of a cushion of Ficoll-Hypaque, density 1.0780 g to 1.0805 g (Pharmacia, Piscataway, NJ), and were centrifuged for 12 min at 1200 rpm. The interface area was collected and the cells were washed 3 times in the HBSS/BSA medium. Cells (1 to 2 × 10^8) in HBSS with 5% FCS, p/s, and HEPES were run into a 12-ml syringe that was packed with 600 mg of nylon wool and previously preincubated at 37°C. The cells were left on the column for 45 min at 37°C and then 20 ml of warm HBSS/5% FCS medium were slowly run through the column. The cells were washed 1 time and then were either labeled for analysis or sorting or were left untreated, resuspended in RPMI 1640 (Grand Island Biological Co., Grand Island, NY), 10% FCS, p/s, and 2 mM L-glutamine (Difco Laboratories), and assayed for killing activity. Only 1 to 2% of the NAPEC were macrophages, determined by phagocytosis of human γ-globulin coated latex beads, fluoresceinated bacteria, and by nonspecific esterase staining. The injection of 1.25 to 5 × 10^4 live LM gave similar amounts of NK activity. The largest dose of LM was used in all the experiments reported in this paper. Murine spleens, when studied, were teased with forceps, Ficolled, and run over nylon wool columns like the PEC.

Measurement of natural killing and antibody-dependent cellular cytotoxicity (ADCC). Natural killing was measured by a standard 3½ hr ^{51}Cr-release assay with YAC-1, an A/Sn T cell lymphoma line, originally provided by Dr. Mark Greene, Harvard Medical School, used as the target. Target cells (2 to 2.5 × 10^6) in 500 μl of RPMI/10% FCS medium were labeled with 500 μl of ^{51}Cr (1 mCi/ml) (New England Nuclear, Boston, MA) for 60 min in a 37°C, 5% CO_2 incubator. The cells were then washed 4 times before being resuspended at a concentration of 2.5 × 10^5 cells/ml. Fifty microliters of the targets were added to the round-bottom wells of a multiwell tissue culture plate (Linbro Chemical Co., Hamden, CT). Effector cells in 100 μl of RPMI/10% FCS medium at various effector to target ratios were then added to the wells.

ADCC activity was evaluated by measuring the killing of ascites-(BALB/c × DBA/2)F_1 carried P815 (H-2^d) targets in the absence or presence of anti-H-2^d antiserum (C57/b6 anti-DBA/2). The antibody (50 μl) at various dilutions was added to the P815 targets in the Linbro well plates. The P815 cells were a gift of Dr. Eric Martz, Harvard Medical School.

After the addition of the effector cells, all of the plates were centrifuged for 5 min at 1000 rpm and then left for 3½ hr in a 37°C, 5% CO_2 incubator. The supernatants in the wells were harvested by the Titertek system (Flow Laboratories, Rockville, MD). Triplicate wells were done for each effector to target ratio. Spontaneous release of the labeled YAC-1 was 3 to 5% and was 8 to 17% of the labeled P815. A 2% dilution of the nonionic detergent, IGEPAL CA-630 (GAF, New York, NY), released 80 to 92% of the total counts added to a well. The killing results are expressed as percent specific release as described previously (19).

Single-cell cytotoxicity assay. The procedure that we used was basically that reported by Silva *et al.* (20). A 10% agarose stock made up in distilled water was melted in a boiling water bath and diluted to agarose with 44°C-heated RPMI with HEPES medium. Day 3 LM (0.5 × 10^6) or PBS NAPEC and YAC-1 cells (0.5 × 10^6) in 1 ml of RPMI/10% FCS medium were added to a 3-ml, V-shaped centrifuge tube. Control tubes contained only 1 × 10^6 YAC-1 cells. The cells were left in a 37°C water bath for 5 min and then were centrifuged at 1500 rpm for 5 min. All but 0.200 ml of medium was drawn off and the cells were gently resuspended by a pasteur pipette. Melted, cooled agarose (1.5 ml) was added and the cells and agarose were mixed by inverting the paraffin-covered tubes 4 times. Agarose and cells (500 μl) were added to a Falcon 35 x 10 mm tissue culture dish (Becton, Dickinson, Oxnard, CA) and the agarose was spread thinly over the bottom of the plate with a syringe plunger. After solidification of the agarose, 1 ml of RPMI/10% FCS medium was layered on top. Plates were put in a 37°C, 5% CO_2 incubator and were removed at various times. Upon removal, the medium was gently taken off a plate with a pasteur pipette and 1 ml of 0.1% trypan blue in PBS was added for 5 min. The trypan blue was removed and 4 ml of RPMI/10% FCS medium were added for 5 min and then removed. One milliliter of 0.5% paraformaldehyde solution was added for 15 min to fix the cells. It was then removed and a 2nd 1 ml of paraformaldehyde was added. The plates were then scored. Only 2-cell conjugates made up of a single NAPEC and a single YAC-1 were counted. Size was used as the criterion to distinguish the large YAC-1 from small NAPEC. One hundred small NAPEC were examined to determine the percent of NAPEC bound to a single YAC-1 cell. Fifty 2-cell conjugates were inspected and the percent of bound cells cytotoxic at each time point was defined as the (percent of dead targets in a 2-cell conjugate)—(fraction of spontaneous dead targets) × (percent of dead targets in a 2-cell conjugate). On the average, at 0 hr, 1 to 2%; at 1 ½ hr, 2%; and at 3 ½ hr, 4 to 5% of the YAC-1 cells in the control plates were dead.

Labeling protocol for analysis or sorting. For optimal labeling for analysis or sorting on the fluorescence-activated cell sorter (FACS), 1 to 5 × 10^6 pelleted NAPEC or nylon wool nonadherent spleen cells were incubated first with 50 μl of Protein-A absorbed (21) F(ab')$_2$ fragments of IgG rabbit anti-mouse Ig (9.5 mg/ml). The cells were left for 10 min on ice and then were incubated for 20 min at room temperature in 0.5 ml of HBSS/5% FCS, p/s, and HEPES medium. After 3 washings in the cold (4°C) at 1500 rpm for 6 min, the pelleted cells were placed on ice and then exposed for 30 min to either: 50 μl of PBS; an optimal amount of supernatant from the M1/70.15.1 HL (M1/70) clone (i.e., 1/10 dilution); normal rat IgG (10 μg/ml); purified M1/70 IgG2b (10 μg/ml); F(ab')$_2$ M1/70 IgG2b (7 μg/ml); or F(ab')$_2$ rat IgG (7 μg/ml). These later antibodies were prepared as previously described (8). The cells were then washed 5 times in the cold and then incubated another 30 min on ice with 50 μl of fluoresceinated (fluoroscein isothiocyanate, (FITC)) F(ab')$_2$ fragments of IgG rabbit anti-rat globulin (2.5 mg/ml) F/P = 11.5, previously absorbed on glutaraldehyde-gelled normal mouse serum. After 2 more cold washes, the cells, if not to be used in functional studies, were fixed in 1% paraformaldehyde.

Monoclonal anti-I-A^k clone 10-2.16 from Dr. Leonard Herzenberg, Stanford University (described in Reference 22), alloanti-Thy 1.2 (AKR anti-CBA/J thymocytes), and FITC-F(ab')$_2$ rabbit anti-mouse IgM were also used to label pelleted NAPEC. All antibodies were used at previously determined saturating concentrations for B, T, or macrophage detection. Pelleted NAPEC were exposed to either the monoclonal anti-I-A^k (control: IgG2b myeloma protein) or anti-Thy 1.2 (control: normal mouse serum), washed, and then exposed to FITC-F(ab')$_2$ rabbit anti-mouse Ig (500 μg/ml). In the case of labeling for surface IgM, the cells were exposed only to FITC-F(ab')$_2$ rabbit anti-mouse IgM. The control was to expose the cells to the same antibody after it was absorbed on adherent spleen cells removed from a nylon wool column.

Antibody and complement (C) lysis of cells. Pelleted NAPEC (4 to 1.5 × 10^6) were labeled with 50 μl of the appropriate antibody for 30 min on ice and then washed 2 times. HBSS medium (450 μl) with 2.5% FCS, 5 mM HEPES, and 25 μl each of rabbit and guinea pig serum (Becton, Dickinson, Mountain View, CA) was added to the labeled cells. Both sera were sources of C and were previously absorbed on normal mouse cells. The NAPEC were then placed in a 37°C, 5% CO_2 incubator for 30 min and finally washed 2 times. When the treated cells were tested for their ability to lyse YAC-1 targets, the dead cells were included in establishing the various effector to target ratios.

Cell sorting and analysis. A Becton-Dickinson FACS II was used to analyze and to separate labeled, NAPEC from LM-infected mice (LM NA-

PEC) or labeled, nylon wool nonadherent normal spleen cells. Fluorescence analysis was restricted to viable cells that displayed the light scatter properties of lymphocytes. By combining size restriction and macrophage depletion on nylon wool columns, it was possible to exclude macrophages from the analysis. In sorting experiments, the M1/70-labeled cells were separated from the unlabeled cells by selecting the 10 to 12% most fluorescent cells. About 10% of the cells of the intermediate fluorescence were discarded to reduce cross-contamination. Analysis of the sorted labeled and unlabeled cells showed 80 to 90% purity. Mock sorting did not affect the NK activity of the cells.

Rosette formation with antibody- or C-coated sheep red blood cells (SRBC). SRBC were coated with IgM (EA 19S) by adding equal volumes of 2% washed SRBC and an optimal dilution of 19S rabbit anti-SRBC serum (Cordis, Miami, FL). The cells were allowed to react with the antibody for 30 min in a 37°C water bath and were then washed 3 times in HBSS, resuspended, and counted. EA 19S cells were coated with C (EAC) by incubating equal volumes of the above EA 19S cells with a 1/5 dilution of freshly obtained A/St normal mouse serum. IgG-coated SRBC (EA 7S) were made by incubating equal volumes of washed 2% SRBC with an optimal dilution of 7S rabbit anti-SRBC serum.

One hundred coated or noncoated SRBC were added for each NAPEC. The mixed cells were left for 15 min at room temperature in a total volume of 200 μl of HBSS in a V-shaped glass tube. The cells were centrifuged for 5 min in the cold at 1000 rpm and placed for an additional hour on ice. After gentle resuspension with a pasteur pipette, the cells were examined under a microscope for rosette formation. One hundred to 200 murine cells were scored. In this paper, the data are expressed either as the percentage of murine cells specifically rosetted with 2 or more coated SRBC or the percentage specifically rosetted with 3 or more coated SRBC.

C3b-FITC Salmonella typhi binding. Equal volumes of heat-inactivated or nonheat-inactivated human serum were mixed with FITC heat-killed *Salmonella typhi* (23) and were left to incubate for 15 min in a 37°C water bath. The cells were then washed 3 times in cold PBS at 3000 rpm for 8 min. PEC (1 to 4 × 10^6) or NAPEC (1 to 4 × 10^6) were added to 0.5 ml of treated bacteria and were allowed to interact for 30 min at room temperature. One hundred to 200 murine cells were examined microscopically for binding to the 2 types of FITC-bacteria.

Nonspecific esterase activity. Nonspecific esterase staining of cytocentrifuge slides was done according to published protocol (24). One hundred to 200 cells were examined.

Uptake of human γ-globulin coated latex particles. One drop of a 1/4 dilution of human γ-globulin coated latex beads (25) (W/V 6.47) was added to 1 to 0.8 × 10^5 sorted or not sorted cells suspended in 300 μl of HBSS/50% FCS. The cells were left for 1 hr at 37°C and then a portion of the mixture (100 μl) was cytocentrifuged. The slides were stained with Wright-Giemsa staining. Cells with 5 or more latex particles were considered positive. One hundred to 200 cells were examined.

Target-binding cell assay. Briefly, 0.3 to 0.5 × 10^6 sorted or not sorted LM NAPEC suspended in 400 to 500 μl of RPMI/10% FCS medium, were placed in a 3-ml, V-shaped glass tube and allowed to interact at 37°C for 7 min with equal amounts of either YAC-1 or P815 cells. All of the samples were then centrifuged at 24°C at 1500 rpm for 5 min. The medium was gently removed from the cells and the cells were resuspended in 300 μl of FCS by pipetting, at most, 5 times. The samples were placed on ice while wet mounts were made and were examined under a Zeiss fluorescent microscope. Two hundred LM NAPEC and 100 conjugates were scored. The percentage of LM NAPEC that were fluorescent was calculated. The percentage of NAPEC that were conjugated with either YAC-1 or P815 was established by using size as the criterion to distinguish between the larger tumor cells and the smaller NAPEC. Finally, the percentage of NAPEC that were bound to a tumor cell and were also fluorescent was determined. The control for specific staining was to expose the NAPEC to rat IgG instead of M1/70.

About 3 ml of FCS were added to the tubes containing YAC-1 cells and sorted or not sorted LM NAPEC. One hundred microliters of the mixture were cytocentrifuged and the slides were stained with Wright-Giemsa. One hundred to 200 NAPEC and 100 conjugates were examined. The percentage of NAPEC that were conjugated to a YAC-1 tumor cell, the percentage of NAPEC that contained 3 or more cytoplasmic, azurophilic granules, and the percentage of conjugated NAPEC that bore such granules were all calculated.

RESULTS

Time course of appearance of NK activity after exposure to LM. On various days after the i.p. injection of live LM or PBS, NAPEC were tested for their ability to lyse YAC-1 targets. In 2 different experiments, primary exposure to LM first produced a maximum expression of NK activity above PBS control levels on day 2. This level of activity remained high through day 6. A 2nd exposure to LM did not give as augmented a NK response and, in fact, seemed to cause a decrease in the amount of NK activity when compared with the amount of activity seen after a single exposure to LM (Fig. 1).

Reasons for the increased NK activity in LM-infected mice. In the single-cell cytotoxicity assay, more day 3 LM NAPEC bound to YAC-1 targets than did PBS NAPEC (by the T test, $p < 0.02$). The lytic capacity of the individual LM NAPEC was also increased judged by the trypan blue uptake of YAC-1 cells contained in a single NAPEC-single YAC-1 conjugate. In 2 experiments, it was found that, after 3½ hr of incubation, 52 and 57% of the bound LM NAPEC and 28 and 14% of the bound PBS NAPEC displayed cytotoxic activity (Table I) (by the *t* test, $p < 0.05$). That there was no suppressive influence in the PBS cells was tested by mixing equal numbers of LM NAPEC with PBS NAPEC (data not shown). It seems then that after LM infection, the NK cells are both more numerous and more active than in control exudates.

Binding of M1/70 to LM or PBS NAPEC. Our group has previously shown that M1/70 antibody cross-reacts with human NK cells (8). Consequently, the binding of M1/70 to day 3 LM or PBS NAPEC was tested by using an indirect labeling protocol and the FACS. At first, high background levels of staining seen after exposure to only the fluoresceinated $F(ab')_2$ rabbit anti-rat Ig obscured our ability to see any specific labeling by M1/70. Because this high background level of staining might have been caused by the reacting of our FITC-antibody with mouse Ig bound to cells through their Fc receptors, we decided to pre-expose (as described in *Material and Methods*) the NAPEC to $F(ab')_2$ fragments of IgG rabbit anti-mouse Ig. This pre-exposure before specific labeling was successful in overcoming our high background problem as it allowed distinct differences to be seen in the staining of the size-selected portion of the NAPEC population that had labeled brightest in our initial experiments (Fig. 2).

The ranges for specifically stained cells were established in 4 experiments (Table II). All data in Table II are expressed in terms of the percent of total NAPEC population.

Expression of other surface markers on LM NAPEC. We then analyzed for the expression of other surface markers on those LM NAPEC that were similar in size to the cells that stained specifically with M1/70. Both day 3 and day 4 LM NAPEC bore the same markers. No cells bore I-A^k or IgM on their

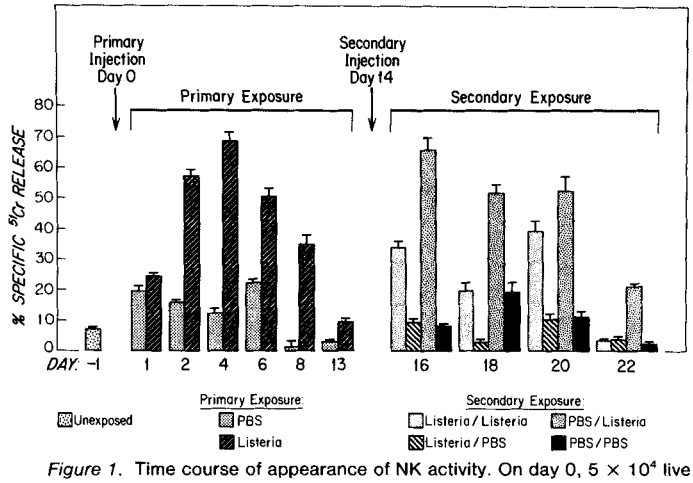

Figure 1. Time course of appearance of NK activity. On day 0, 5 × 10^4 live LM bacteria suspended in 1 ml of PBS or 1 ml of PBS was injected into 7-wk-old CBA/J mice. Five mice/group were sacrificed on the indicated days and their NAPEC were assayed for NK activity. Fourteen days after the initial injection, the remaining mice in each group were divided into 2 groups: one group was injected with LM as above, the other group was injected with PBS. The killing of YAC-1 targets at a 10:1 effector-to-target ratio is graphed here.

TABLE I

Single-cell cytotoxicity assay

Time	% of NAPEC in Conjugates[a]			
	Listeria		PBS	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
hr				
0	21	19	8	8
½	21	N.T.[b]	11	N.T.
1½	19	18	10	9
3½	16	17	9	7

Time	% of Bound NAPEC Cytotoxic at the Indicated Time Point[a]			
	Listeria		PBS	
	Expt. 1	Expt. 2[c]	Expt. 1	Expt. 2[c]
hr				
0	0	0	0	0
½	5	N.T.	0	N.T.
1½	5	4	10	2
3½	52	57	28	14

[a] Based on 2-cell conjugates between one YAC-1 and one NAPEC.

[b] N.T., not tested.

[c] Percent specific ^{51}Cr released by the cells at a 10:1 effector-to-target ratio: (1) after a 90-min incubation, *Listeria* NAPEC:53.1 ± 2.7; PBS NAPEC:11.6 ± 1.3 and (2) after a 3½ hr incubation, *Listeria* NAPEC:66.3 ± 3.4; PBS NAPEC: 25.2 ± 1.6.

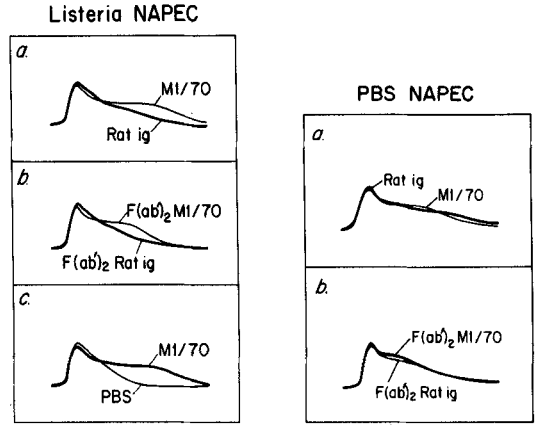

Figure 2. Fluorescent staining of size-selected *Listeria* (*left*) or PBS (*right*) NAPEC. Each curve is a tracing of the fluorescence distribution analysis of 50,000 cells. Fluorescence intensity is plotted on the horizontal axis and the number of cells having any degree of fluorescence is plotted on the vertical axis. The vertical axis is logarithmic. By using the standard labeling protocol described in the *Material and Methods* section, the NAPEC were exposed either to purified M1/70 or rat IgG or to $F(ab')_2$ fragments of purified M1/70 or $F(ab')_2$ fragments of rat IgG.

TABLE II

% of total NAPEC specifically labeled

Specific Label	Control Label	Source of Cells	% Specifically Labeled
Whole purified M1/70 IgG	Rat IgG	Listeria	5.4 to 12.4
$F(ab')_2$ M1/70	$F(ab')_2$ rat IgG	Listeria	3.4 to 13.5
Whole purified M1/70 IgG	Rat IgG	PBS	0 to 2.2
$F(ab')_2$ M1/70	$F(ab')_2$ rat IgG	PBS	0 to 2.2

surface. Whereas 29% of the cells stained specifically with M1/70, 58% of the cells labeled specifically with the polyclonal alloanti-Thy 1.2. The number of cells stained was such that we could not rule out that some of the M1/70-labeled cells were contained within the Thy 1.2-labeled population of cells. Because our labeling protocol does not allow us to label simultaneously the NAPEC with M1/70 and anti-Thy 1.2, we tried to deplete NK activity with anti-Thy 1.2 and C treatment. We found that our alloantiserum, even undiluted, in the presence of C had approximately a 20% inhibitory effect on the expression of NK activity (data not shown). We then killed the bulk of the T cells with undiluted anti-Thy 1.2 and C treatment, removed the dead cells on a Ficoll gradient, and labeled the remaining live cells with either M1/70 or the anti-Thy 1.2. Under these circumstances, 65% of the size-selected cells labeled specifically with M1/70 and 25% of the cells labeled specifically with the anti-Thy 1.2. Clearly, the M1/70-labeled cells were enriched by this procedure and many of the M1/70-labeled cells could not also be labeled with the polyclonal alloanti-Thy 1.2 serum.

In other experiments, we have used a murine IgM monoclonal anti-Thy 1.2 (New England Nuclear, Boston, MA). Although this antibody in the presence of C kills T cells at dilutions of greater than 1/1000, a higher concentration of the monoclonal antibody (i.e., 1/10 dilution) causes, in the presence of C, at most a 55% depletion in NK activity (data not shown).

NK activity displayed by sorted M1/70-positive LM NAPEC or normal spleen cells. Because analysis on the FACS showed that M1/70 recognized a population of day 4 LM NAPEC, we tested the NK activity of the sorted M1/70-positive cells. We also used M1/70 to select for NK activity in nylon wool nonadherent normal spleen cells. All cells were labeled according to the established protocol. Four cell populations (unlabeled, not sorted; labeled, not sorted; M1/70-positive; and M1/70-negative) were compared for NK activity (Fig. 3*A* and *B*). The labeling protocol itself did not affect the lytic capacity of the NK cells. NK activity was dramatically enriched in the M1/70-positive LM NAPEC and normal spleen cells.

Because we normally enriched for NK activity by selecting for the 10 to 12% most fluorescent cells within the M1/70-labeled cell population and because 5% of the LM NAPEC fell within the same fluorescence region when labeled only by the rat Ig (control) staining protocol, we decided to compare the NK activity of cells selected by a more stringent criterion. Cells were either selected on the FACS by the standard method or were selected by gating only for cells that were clearly more brightly labeled by the M1/70-labeling protocol than by the rat Ig-labeling protocol. Although the more rigid selection yielded approximately 40% fewer cells, both of the selection processes led to a similar enrichment of NK activity (data not shown).

Ability of M1/70-positive day 4 LM NAPEC to mediate ADCC. M1/70-positive sorted cells were then examined for their ability to mediate ADCC. ADCC activity was dramatically enriched in the M1/70-positive cell population (Fig. 4). Again, the labeling protocol did not affect the expression of ADCC.

Characterization of M1/70-positive sorted LM NAPEC. The M1/70-positive sorted LM NAPEC were tested for their capacity to bind to C3bi-coated, IgM-coated, or IgG-coated SRBC, their expression of nonspecific esterase activity, their phagocytic activity, their morphology under Wright-Giemsa staining, and for their ability to form conjugates with P815 or YAC-1 cells. After the sort, all cells being characterized were also simultaneously tested for enrichment of NK activity in the M1/70-positive cells. M1/70-positive sorted cells did not rosette with C-coated or IgM-coated SRBC (Table III). Because the C-coated SRBC bear predominantly C3bi on their surface, whole PEC and NAPEC were tested for their ability to bind C- (C3b) coated, fluoresceinated *Salmonella typhi*. Thirty-four percent of the whole PEC bound elaborately and specifically the C3b-coated FITC-bacteria, whereas 1% of the NAPEC bound the bacteria. Because M1/70-positive cells were enriched for ADCC activity, we were not surprised to find that they were also enriched for cells that rosetted with IgG-coated SRBC. Mock sorting had no effect on the rosetting capacity of LM NAPEC. The M1/70-positive cells also did not contain nonspe-

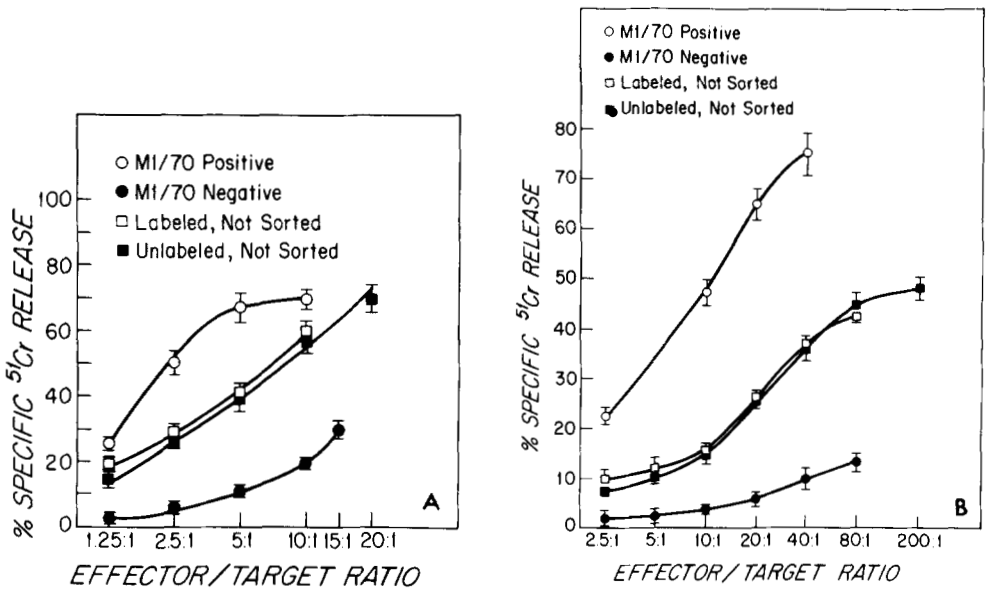

Figure 3. Natural killing of YAC-1 targets by M1/70 sorted day 4 *Listeria* NAPEC (*A*) or M1/70 sorted nylon wool nonadherent normal spleen cells (*B*). The data presented is a representative example of 6 different sorts. *A*: 12% of the cells were sorted as positive, 79% as negative. *B*: 11% of the cells were sorted as positive, 75% as negative.

TABLE III

Markers expressed by day 4 Listeria peritoneal exudate cells[a]

	EAC Binding (2 or more coated-SRBC)	EA 19S Binding (2 or more coated-SRBC)	Nonspecific Esterase Staining (Red color: positive)	Ingestion of Human γ-Globulin-Coated Latex Beads (5 or more)	Containing Azurophilic Granules (3 or more)	EA 7S Binding (3 or more coated-SRBC)
Whole PEC	10%	28%	62%	71%	2%	46%
Unlabeled, not sorted NAPEC	0%	1%	2%	1%	10%	16%
Labeled, not sorted NAPEC	1%	1%	2%	1%	15%	14%
M1/70, positive NAPEC	3%	1%	2%	3%	55%	24%
M1/70, negative NAPEC	2%	1%	2%	1%	3%	3%

[a] Data is presented as the percentage of cells expressing the marker and is representative of the results obtained from at least 3 sorting experiments for each marker study.

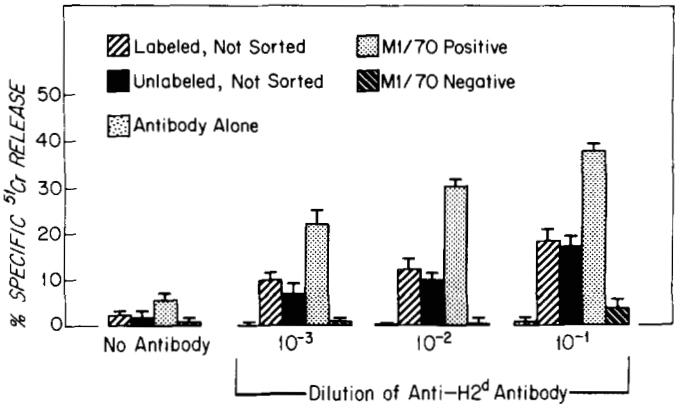

Figure 4. ADCC activity of M1/70 sorted day 4 *Listeria* NAPEC. Ten percent of the cells were selected as positive, 82% as negative. The target cells were anti-H-2^d-coated P815 cells. Killing at a 10:1 effector-to-target ratio is reported here.

cific esterase and did not phagocytize human γ-globulin coated latex beads. With Wright-Giemsa staining, the M1/70-positive NAPEC were enriched for cells that displayed a distinctive morphology (Fig. 5*A* and *B*); 55% of the M1/70-positive cells contained 3 or more azurophilic granules in their cytoplasm (Table III). M1/70-positive spleen cells, on the other hand, had much less cytoplasm and it was difficult to score them for granules. The results of the target-binding cell assay showed that the M1/70-positive cells were enriched only in their capacity to bind to YAC-1 and not in their ability to bind to P815 cells (Table IV). Eighty-one percent of the M1/70-positive cells and 4% of the M1/70-negative cells that were conjugated with a YAC-1 cell were fluorescent. Fifty-seven percent of the M1/70-positive cells and 9% of the M1/70-negative cells that bound YAC-1 bore 3 or more cytoplasmic, auzophilic granules (Table V). These same M1/70-positive cells, when placed in a ^{51}Cr-release assay with YAC-1, gave 31.8 ± 1.6% specific ^{51}Cr-release, whereas the M1/70-negative cells gave only 3.2 ± 1.6% specific ^{51}Cr release.

DISCUSSION

Infection with LM leads to the generation of NK activity in peritoneal exudates. Maximum expression of NK activity first occurs on day 2 and remains high until day 6. Not only is there an increase in the number of cells that bind YAC-1 targets, there is also an increase in their individual lytic capacity when compared with the PBS control cells. M1/70, a rat anti-murine macrophage monoclonal antibody, not only selects human NK cells (8) but also can be used to select murine NK cells from nylon wool nonadherent normal spleen cells and LM PEC. The M1/70-selected LM NAPEC can also mediate ADCC activity. They have a distinctive morphology—containing azurophilic granules on Wright-Giemsa staining. They are not phagocytic, do not contain nonspecific esterase, and lack surface I-A^k, IgM determinants, C receptors, and high levels of Thy 1.2.

The NK cell probably displays on its surface less of the M1/

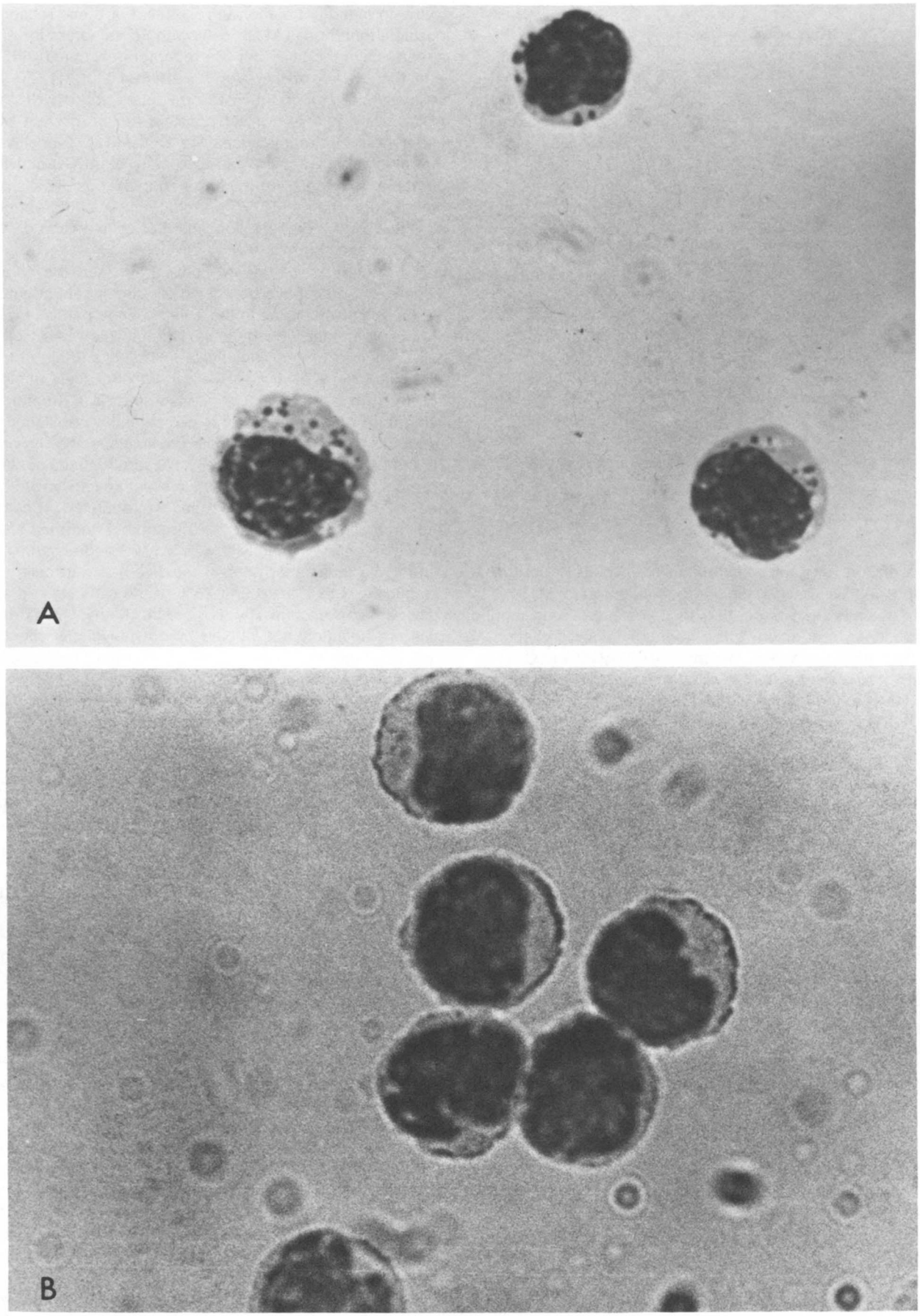

Figure 5. Morphology of M1/70 positive or negative selected day 4 *Listeria* NAPEC. In the M1/70 positive selected *Listeria* NAPEC population (*A*), about half of the cells display upon Wright-Giemsa staining 3 or more cytoplasmic azurophilic granules. In the M1/70 negative selected population (*B*), these granule-bearing cells are rarely seen.

TABLE IV

Target binding by day 4 Listeria NAPEC

	% of NAPEC that were:			
	Fluorescent labeled	In conjugate with YAC-1	In conjugate with P815	In conjugate with YAC-1 and fluorescent labeled
M1/70[a], labeled, not sorted	19	14	10	36
M1/70[a], positive	69	33	12	81
M1/70[a], negative	2	13	6	4
Rat IgG, labeled, not sorted	1	19	8	0

[a] Percent specific ^{51}Cr release by the cells at 2.5:1 effector-to-target ratio against YAC-1: M1/70 labeled, not sorted: 14.7 ± 1.1; M1/70 positive: 31.8 ± 1.6; and M1/70 negative: 3.2 ± 1.6, and against P815: M1/70 labeled, not sorted: 1.2 ± 1.0; M1/70 positive 4.0 ± 0.6; and M1/70 negative: 0.1 ± 0.6.

TABLE V

Presence of auzophilic granules in day 4 Listeria NAPEC

	% of NAPEC[a]:		
	With granules	In conjugate with YAC-1	In conjugate with YAC-1 and containing granules
M1/70, labeled not sorted	12	19	26
M1/70, positive	47	47	57
M1/70, negative	6	15	9

[a] The data was obtained by using the same cells as those in Table IV.

70 recognized antigenic determinant than does a peritoneal macrophage. We have used more concentrated M1/70 to detect NK cells than is needed to detect Mac-1 on murine macrophages. In addition, there is only minimal depletion of NK activity after treatment with undiluted M1/70 and C. Thus, in terms of reactivity with M1/70, the murine NK cells appear to be similar to the murine blood monocytes (9).

Our sorting experiments have demonstrated that the NK population is contained within the most fluorescent 10 to 12% of the M1/70-labeled cells. When only the brightest of these M1/70-labeled cells were chosen on the FACS by using a more stringent selection criterion, the NK activity of the cells was not significantly increased. This finding implies that the distribution on NK cells of the M1/70-recognized antigenic determinant is quite heterogeneous. Some cells appear to be brightly labeled by M1/70, whereas others appear to be dimly labeled.

Using the target-binding cell assay in parallel with the ^{51}Cr-release assay, we have found that the M1/70-positive cells are enriched in their ability to bind and kill YAC-1 cells and not in their ability to bind and kill P815 cells. About 60% of the M1/70-positive cells that are conjugated to a YAC-1 cell possess 3 or more cytoplasmic, azurophilic granules, although about 80% of the M1/70-positive cells that were conjugated to a YAC-1 were fluorescent. The lower percentage of granule-bearing cells may be due simply to the fact that it is difficult to assess a cell as granule bearing or not if too little cytoplasm is visible. Alternatively, the presence of cytoplasmic, azurophilic granules may vary with the degree of activation of the NK cell. However, the antigenic determinant recognized by M1/70 may be present regardless of the state of activation of the NK.

The murine NK cell, like the human peripheral blood NK cell (8), may bear receptors for the Fc region of IgG. We have found it necessary to pre-expose the LM NAPEC to $F(ab')_2$ fragments of IgG rabbit anti-mouse Ig in order to overcome the problem of high background labeling that is seen after exposure to only FITC-$F(ab')_2$ rabbit anti-rat Ig. In addition, rosetting of the M1/70-labeled cells with IgG-coated SRBC never leads to the formation of elaborate rosettes. For these reasons, it is possible that those LM NK cells with Fc receptors for IgG may already have the bulk of these receptors occupied with Ig.

In the course of this work, we noted that M1/70-labeled cells, selected for on the FACS, mediated not only NK but also ADCC activity. It is not yet possible for us to state whether under certain circumstances the same M1/70-positive cells that mediate NK activity can also mediate ADCC activity. We do not know if there are 2 different populations of cells that are both recognized by M1/70 and that express different biologic activities, or if there is 1 population of cells that binds M1/70 and expresses both cytolytic activities.

The lineage of murine NK cells has been the subject of dispute. NK cells have been said to arise independently from the bone marrow, to be in the T cell lineage pathway, or to be premonocyte-macrophage cells. Normal splenic NK cells have been reported to bear low levels of Thy 1 on their surface (2, 3). Like Pollack *et al.* (4), we cannot effectively deplete NK activity with our Thy 1.2 alloantiserum and C treatment. By using the FACS, analysis of a size-selected population of LM NAPEC leads to 29% of the cells labeling specifically with M1/70 and 58% with our alloanti-Thy 1.2 serum. Since our labeling protocol does not allow us to stain simultaneously with M1/70 and our alloanti-Thy 1.2 serum, we depleted T cells with undiluted alloanti-Thy 1.2 and C treatment and then analyzed the viable cells for reactivity with M1/70 and alloanti-Thy 1.2 serum. We found that 65% of the size-selected cells stained specifically with M1/70 and 25% of the cells stained specifically with the alloanti-Thy 1.2 serum. Many M1/70-bearing cells are definitely not labeled by our polyclonal alloanti-thymocyte serum. When we examined the effect of exposing our NK cells to a murine IgM monoclonal anti-Thy 1.2, we found that although the antibody in the presence of C killed T cells at dilutions of greater than 1/1000, a higher concentration of the monoclonal antibody (i.e., 1/10 dilution) caused, in the presence of C, only a 55% depletion in NK activity.

Chun *et al.* (26) have reported that NK cells may become activated as a consequence of reacting with immune interferon secreted by activated lymphocytes. It is possible then that our results are due to the fact that the polyclonal alloanti-Thy 1.2 serum is less efficient than the monoclonal anti-Thy 1.2 in depleting, in the presence of C, T cells whose products can activate NK cells. Alternatively, we cannot rule out the possibilities that some of our NK cells display a very low level of Thy 1.2 on their surface or display a cross-reacting antigenic determinant that can be recognized by high concentrations of the monoclonal anti-Thy 1.2

In general, however, we believe that Thy 1, even if present on NK cells, is itself not a reliable marker of immunologic, ontogenic relatedness because it is also on brain and epithelial cells. We feel that an antibody like M1/70 that recognizes only bone marrow-derived cells offers a better means to study the question of NK ontogeny.

In 1979, Lohmann-Matthes *et al.* (7) reported that promonocytes that grew out of murine bone marrow cultures displayed NK activity and were anti-Mph and C sensitive, nonadherent, nonphagocytic, and nonspecific esterase negative. Recently, Reinherz *et al.* (27) have shown that human cells bearing receptors for the Fc region of IgG and that mediate NK activity react with a monoclonal antibody OKM1 that is said to be specific for human monocytes. Our group has shown that murine NK, like human NK cells (8), bear antigenic determinants that can be recognized by M1/70, a rat anti-murine macrophage monoclonal antibody. Selection on the FACS of

M1/70-labeled, nylon wool nonadherent cells from LM-infected mice or normal mice leads to the enrichment of NK activity.

M1/70 is known to react with less than 0.8% of murine thymus cells and less than 1% of murine lymph node cells. It binds to murine-unelicited and -elicited peritoneal exudate macrophages, neutrophilic granulocytes, blood monocytes, splenic macrophages, 50% of the nucleated bone marrow cells (9–11), and now NK cells. All of the cells recognized by M1/70 have 2 things in common: they are all bone-marrow-derived and all bear receptors for the Fc region of IgG. Although we have not specifically defined the surface polypeptides precipitated by M1/70 with respect to function, we believe that the antigenic determinant reconized by M1/70 is definitely not the receptor for the Fc region of IgG. M1/70 precipitates 2 non-disulfide-bonded polypeptides of m.w. 190,000 and 105,000 (9). The receptors for IgG on murine macrophages are reported to be 67,000 and 52,000 (28). Experiments in our laboratory have shown that M1/70 labeling does not interfere with the ability of heat-aggregated Ig to bind to cells and M1/70 labeling does not affect the ability of cells to mediate ADCC activity.

By using the M1/70-labeled NK cells, it will be possible for us to study the importance of natural killing in the establishment of a primary defense mechanism against an intracellular bacteria. Because treatment of mice with ^{89}strontium, which affects only precursor cells in the bone marrow, led to a diminution in both NK activity and the early resistance of mice to LM (14) and because we have shown that infection with LM led to an early increase in NK activity, the appearance in mice of NK activity may not be just a fortuitous occurrence due to high levels of interferon; it may point to the importance of NK in establishing an initial defense against LM. Preliminary experiments in our laboratory have shown that M1/70-sorted LM NAPEC did not decrease the colony formation capacity of live LM and that LM NAPEC did not bind rhodamine-labeled, heat-killed LM. We are now examining the question of whether the NK cell that appears early in LM infection can kill LM-infected cells.

Because selection for M1/70-labeled cells allows us to obtain an enriched and very active NK population, we have initiated experiments to trace with time changes in the cellular activity and surface markers of these selected cells. It is now possible to select M1/70-labeled bone marrow cells and to examine the effect of various stimuli on the ability of these selected cells to express NK activity. We hope that through these approaches we will be able to clarify the differentiation scheme and lineage of NK cells.

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REFERENCES

1. Herberman, R. B., ed. 1980. Natural Cell-Mediated Immunity Against Tumors. Academic Press, Section I, parts A, B, and D.
2. Herberman, R. B., M. E. Nunn, and H. T. Holden. 1978. Low density of Thy-1 antigen on murine effector cells mediating natural cytotoxicity against tumor cells. J. Immunol. 121:304.
3. Mattes, M. J., S. O. Sharrow, R. B. Herberman, and H. T. Holden. 1979. Identification and separation of Thy-1 positive murine spleen cells active in natural cytotoxicity and ADCC cytotoxicity. J. Immunol. 123:2851.
4. Pollack, S. B., M. R. Tam, R. C. Nowinski, and S. L. Emmons. 1979. Presence of T cell-associated surface antigens on murine NK cells. J. Immunol. 123:1818.
5. Kasai, M., M. Iwamori, Y. Nagai, K. Okumura, And T. Tada. 1980. A glycolipid on the surface of mouse natural killer cells. Eur. J. Immunol. 10: 175.
6. Durdik, J. M., B. N. Beck, and C. S. Henney. 1980. Asialo GM1 and Thy 1 as cell surface markers of murine NK cells. *In* Natural Cell-Mediated Immunity Against Tumors. Edited by R. B. Herberman. Academic Press, New York. P. 37.
7. Lohmann-Matthes, M.-L., W. Domzig, and J. Roder. 1979. Promonocytes have the functional characterization of natural killer cells. J. Immunol. 123: 1883.
8. Ault, K. A., and T. Springer. 1981. Cross-reaction of a rat-anti-mouse phagocyte specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. J. Immunol. 126:359.
9. Springer, T. 1980. Cell surface differentiation in the mouse: characterization of "jumping" and "lineage" antigens using xenogeneic rat monoclonal antibodies. *In* Monoclonal Antibodies-Hybridomas: A New Dimension in Biological Analysis. Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum Press. P. 185.
10. Ho, M-K., and T. A. Springer. 1981. Rat anti-mouse macrophage monoclonal antibodies and their use in immunofluorescent studies of macrophages in tissue sections. *In* Monoclonal Antibodies and T Cell Hybridomas. Edited by U. Hämmerling, G. Hämmerling, and J. Kearney. Elsevier. Pp. 53–61.
11. Springer, T., G. Galfré, D. S. Secher, and C. Milstein. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. 9:301.
12. Bast, Jr., R. C., B. Zbar, G. B. Mackaness, and H. J. Rapp. 1975. Effect of *Listeria monocytogenes* on growth of a murine fibrosarcoma. J. Natl. Cancer Inst. 54:749.
13. Bast, Jr., R. C., B. Zbar, T. E. Miller, G. B. Mackaness, and H. J. Rapp. 1975. Antitumor activity of bacteria infection: effect of *Listeria monocytogenes* on growth of guinea pig hepatoma. J. Natl. Cancer Inst. 54:757.
14. Bennett, M., and E. E. Becker. 1977. Marrow-dependent cell function in early stages of infection with *Listeria monocytogenes*. Cell. Immunol. 33: 203.
15. Wolfe, S. A., D. E. Tracey, and C. S. Henney. 1976. Induction of NK cells. Scand. J. Immunol. 8:215.
16. Tracey, D. E., S. E. Wolfe, J. M. Durdik, and C. S. Henney. 1977. BCG-induced murine effector cells. I. Cytolytic activity in peritoneal exudates: an early response to BCG. J. Immunol. 119:1145.
17. Wolfe, S. E., D. E. Tracey, and C. S. Henney. 1977. BCG-induced murine effector cells. II. Characterization of natural killer cells in peritoneal exudates. J. Immunol. 119:1154.
18. Ziegler, K., and E. Unanue. 1979. The specific binding of *Listeria monocytogenes*-immune T lymphocytes to macrophages. I. Quantitation and role of H-2 gene products. J. Exp. Med. 150:1143.
19. Ault, K. A., and H. L. Weiner. 1979. Natural killing of measles-infected cells by human lymphocytes. J. Immunol. 122:2611.
20. Silva, A. B., B. Bonavida, and S. Targan. 1980. Mode of action of interferon-mediated modulation of natural killer cytotoxic activity: recruitment of pre-NK and enhanced kinetics of lysis. J. Immunol. 125:479.
21. Kessler, S. W. 1976. Cell membrane antigen isolation with the staphylococcal protein A-antibody adsorbent. J. Immunol. 117:1482.
22. Scher, M. G., D. I. Beller, and E. R. Unanue. 1980. Demonstration of a soluble mediator that induces exudates rich in I-A-positive macrophage. J. Exp. Med. 152:1684.
23. Gelfand, J. A., A. S. Fauci, I. Green, and M. M. Frank. 1976. A simple method for determination of complement receptor bearing mononuclear cells. J. Immunol. 116:595.
24. Koski, R., D. G. Poplack, and R. M. Blaese. 1976. A nonspecific esterase stain for the identification of monocytes and macrophages. *In In vitro* Methods in Cell Mediated and Tumor Immunity. Edited by B. R. Bloom and J. R. David. Academic Press, New York. P. 359.
25. Ault, K. A., and H. L. Weiner. 1978. Isolation and characterization of the class of human blood lymphocytes responsible for antibody-dependent cellular cytotoxicity. Clin. Immunol. Immunopathol. 11:60.
26. Chun, M., G. Fernandes, and M. K. Hoffman. 1981. Mechanism of NK activation: relationship between Qa5$^+$ NK cells and lymphocytes. J. Immunol. 126:331.
27. Reinherz, E. L., L. Moretta, M. Roper, J. M. Breard, M. C. Mingari, M. D. Cooper, and S. F. Schlossman. 1980. Human T lymphocyte subpopulation defined by Fc receptors and monoclonal antibodies: a comparison. J. Exp. Med. 151:969.
28. Lane, B. C., J. Kan-Mitchell, M. Mitchell, and S. M. Cooper. 1980. Structural evidence for distinct IgG subclass-specific Fc receptors on mouse peritoneal macrophages. J. Exp. Med. 152:1147.