0022-1767/81/1261-0359$02.00/0
THE JOURNAL OF IMMUNOLOGY
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Vol. 126, No. 1, January 1981
Printed in U.S.A.

CROSS-REACTION OF A RAT-ANTI-MOUSE PHAGOCYTE-SPECIFIC MONOCLONAL ANTIBODY (ANTI-Mac-1) WITH HUMAN MONOCYTES AND NATURAL KILLER CELLS[1]

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A monoclonal antibody produced by a hybridoma cell line that has previously been shown to recognize an antigen present on murine macrophages and granulocytes (Mac-1) has now been shown to bind to human monocytes and polymorphonuclear leukocytes. Human monocytes bind about 40,000 M1/70 (anti-Mac-1) F(ab')$_2$ or IgG molecules per cell in saturating conditions. In addition, M1/70 antibody recognizes a small population (less than 10%) of human blood lymphocytes. These cells express approximately 3-fold fewer Mac-1 antigenic determinants than monocytes. Separation of this lymphocyte subset on a fluorescence-activated cell sorter has shown that all the natural killing activity in human blood can be found among these cells. Similarly, separation of the natural killer cells by an independent method based on their surface Fc receptor has shown that nearly all of them can be labeled by the hybridoma antibody. The same results are obtained when an F(ab')$_2$ fragment of the M1/70 hybridoma antibody is used. The anti-Mac-1 antibody does not interfere with binding to the Fc receptor, nor does it interfere with either natural killing or antibody-dependent cellular cytotoxicity mediated by these cells. We conclude that there is a similar antigenic structure on the surface of murine and human monocytes and granulocytes and that this structure is also found on human natural killer cells.

A hybridoma rat anti-mouse cell surface antibody (M1/70)[3] recognizing the phagocyte-specific antigenic determinant Mac-1 has previously been described (1, 2). M1/70 is a stable, subcloned hybrid cell line derived by fusion between immune rat spleen cells and the mouse NSI myeloma line. Grown in tissue culture, the line yields about 100 μg of rat IgG2b/ml of culture medium (3). This line secretes a truly monoclonal antibody containing only specific H and L chains, apparently having lost myeloma kappa chain secretion at an early stage (1). The Mac-1 antigenic determinant defined by M1/70 antibody is expressed in large quantities on thioglycollate-induced peritoneal exudate macrophages and in lesser amounts on neutrophilic granulocytes, blood monocytes, 8% of spleen cells, 44% of bone marrow cells, and less than 0.3% of thymus cells. The monoclonal antibody to Mac-1 precipitates 2 polypeptides of 190,000 and 105,000 daltons from peritoneal exudate cells and the same polypeptides in much lesser amounts from spleen cells. Because of the possibility that such a molecule might be shared on phagocytic cells of other species, we have investigated the binding of the antibody to human blood cells and have found that it labels human monocytes and polymorphonuclear leukocytes. The number of antibodies binding to human monocytes has been quantitated.

In the course of these studies it was noted that a small population of human lymphocytes was also labeled by the antibody. Studies of these lymphocytes show they are the same as those previously described as having an avid Fc receptor (4-10) and that they are capable of carrying out both natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC).

MATERIALS AND METHODS

Monoclonal antibodies. The M1/70.15.1 HL (M1/70) subcloned line (1) was grown to maximal density in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS). This line may be obtained from the Cell Distribution Center, Salk Institute, P.O. Box 1809, San Diego, CA. The yield was 110 μg of rat IgG per ml as determined by Mancini single radial immunodiffusion (11) against rabbit anti-rat Fab. Unless otherwise specified, this spent culture supernatant was used in labeling experiments. As a control, normal rat IgG at the same final concentration was added to nonsecretor NSI spent culture supernatants. To purify M1/70 IgG, solid $(NH_4)_2SO_4$ was added to culture supernatants to 2.2 M. The precipitate was dialyzed vs 0.1 M Tris-HCl, pH 7.8, and applied at 8 mg protein per ml bed vol to a DEAE cellulose column (DE-52, Whatman), equilibrated with the same buffer, and immediately eluted with 8 column vol of 0.1 M Tris-HCl, pH 7.8, containing a linear gradient of 0 to 0.05 M NaCl. Fractions enriched for M1/70 IgG were pooled, concentrated using an Amicon PM-10 membrane, and applied to a 4 × 120 cm Sephadex G-200 column (Pharmacia, Piscataway, NJ). Purification was monitored by Mancini diffusion against rabbit anti-rat Fab. The G-200 pool was homogenous as shown by SDS-PAGE (Fig. 3) and by the rat IgG concentration estimated according to Mancini (11), which was very close to the protein estimated spectrophotometrically (a value of E280, 1% = 14.6, was taken as standard; 12). F(ab')$_2$ fragments were prepared by pepsin digestion in 0.1 M acetate buffer, pH 4.0, and separated from peptides by gel filtration (13).

Site number estimation. Purified M1/70 IgG or F(ab')$_2$ were iodinated using 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril to 0.5 to 1 mole carrier-free ^{125}I per mole protein as described by Fraker and Speck (14), except the reaction was stopped by transfer to fresh tubes and addition of 0.4 mg/ml L-tyrosine to avoid reduction of F(ab')$_2$. Cells (2×10^7/ml, in 20 μl) in DMEM-HEPES,[2] 10% FCS, and 4 mg/ml human IgG were mixed with varying concentrations of ^{125}I antibody in 80 μl of 8 mg/ml human IgG, 100 mg/ml BSA, 0.01 M Tris-HCl, pH 7.4, 0.14 M NaCl in microtiter plates and shaken 1 hr at 4°C. Cells were washed 3 times with ice-cold 1% BSA in 0.01 M Tris-HCl, pH 7.4, 0.14 M NaCl and gamma-counted.

Preparation of human blood cell populations. Blood from normal donors was defibrinated. In experiments requiring depletion of monocytes, the blood was then incubated for 1 hr at 37° with 1/5 volume of lymphocyte separator reagent (Technicon Instruments, Tarrytown, NY). The erythrocytes were then sedimented in 1.5% dextran (Pharmacia Fine Chemicals, Piscataway, NJ), and the leukocyte-rich supernatant was centrifuged over a Ficoll-Hypaque cushion (15). In some experiments T cells were depleted by forming rosettes with normal sheep erythrocytes and centrifuging again over a Ficoll-Hypaque cushion (16).

Labeling of cells. 5×10^6 cells were incubated with 50 μl of various dilutions of the anti-Mac-1 or control supernatants for 1/2 hr at 0°C. They were then washed 4 times at 4°C and incubated with 50 μl of 1 mg/ml fluoresceinated F(ab')$_2$ fragment of rabbit anti-rat globulin IgG (F-Fab$_2$RaRG) (Cappel Laboratories, Cochranville PA) for 1/2 hr at 0°C. The

Received for publication July 14, 1980.
Accepted for publication September 25, 1980.
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[1] This work was supported by National Institutes of Health Grants AI14732 and CA27547.
[2] Dr. Ault is a recipient of Research Career Development Award CA00605 from the National Institutes of Health.
[3] Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; DMEM-HEPES, Dulbecco modified Eagle's medium with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer; FACS, fluorescence-activated cell sorter; F-Fab$_2$RaRG, fluoresceinated F(ab')$_2$ fragment of rabbit anti-rat immunoglobulin; F-Fab$_2$RaHuIgG, fluoresceinated F(ab')$_2$ fragment of rabbit anti-human IgG; HPBM, human peripheral blood mononuclear cells; Mac-1, a cell surface antigen recognized by M1/70 antibody; MPEC, murine peritoneal exudate cells; M1/70, a monoclonal rat anti-mouse phagocyte-specific antibody; NK, natural killer.

cells were then washed twice at 4°C and, if not being used in functional studies, they were fixed in 1% paraformaldehyde.

The specificity of the anti-rat globulin was confirmed by its inability to react in agar double diffusion with human globulins. In addition, absorption of the antibody by glutaraldehyde-gelled normal human globulin (17) did not affect the labeling.

Labeling of lymphocytes binding non-aggregated IgG was done exactly as described previously (4). Briefly, the cells were incubated first in fresh human serum, washed extensively in the cold, and then labeled with fluoresceinated $F(ab')_2$ fragment of rabbit anti-human IgG immunoglobulin ($F\text{-}Fab_2RaHuIgG$).

Cell sorting. A Becton-Dickinson (Mountainview, CA) FACS II cell sorter was used to analyze and separate the labeled cell populations. Analysis could be done by recording the distribution of fluorescence intensity on large cells (monocytes) or by limiting the analysis to small cells (lymphocytes). Thus, by combining size restriction with monocyte depletion, it was possible to exclude contaminating monocytes from the analysis. The recognition of monocytes by size criteria on the FACS is justified by extensive experience in our laboratory showing that phagocytic cells of monocyte morphology fall into a distinctly larger size range when analyzed on the FACS (16).

Cell separation was done by the FACS. In each case the labeled cells were separated from the unlabeled cells, and about 10% of the cells of intermediate fluorescence were discarded to reduce cross-contamination.

Measurement of NK and ADCC activity. Natural killing (NK) was measured by a standard 6-hr ^{51}Cr release assay using the cell line K-562 as target. ADCC was evaluated either by measuring enhanced killing of K-562 after the addition of a rabbit anti-K-562 antiserum prepared in our laboratory, or by measuring the killing of the murine cell line P-815 in the presence of a rabbit anti-P-815 antiserum kindly supplied by Dr. Kirk Ziegler (Harvard Medical School). The anti-sera were used at an optimal dilution of 1:1000. The results are expressed as percent specific release as described previously (5).

RESULTS

Mac-1 antibody cross-reacts with human monocytes and granulocytes. Binding of the rat anti-mouse M1/70 antibody to human blood cells was examined using an indirect FITC-rabbit $F(ab')_2$ anti-rat IgG reagent and the fluorescence-activated cell sorter (FACS). Two-dimensional plots of cell size (light scatter) *vs* cell fluorescence demonstrated clear labeling of cells in the size region previously shown to contain the monocytes (4) (Fig. 1). In preparations of whole blood mononuclear cells that had not been monocyte depleted there was also less intense labeling of a population of cells in the size range of lymphocytes (Fig. 1).

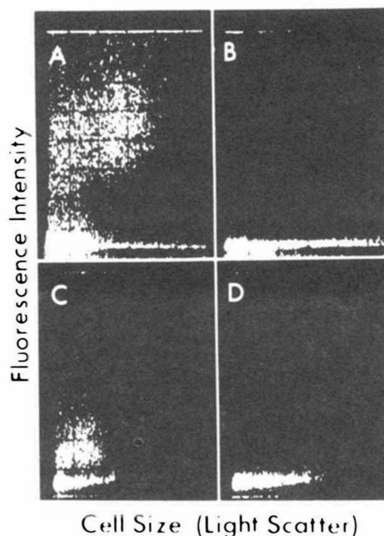

Figure 1. M1/70 Labeling of human monocytes and a lymphocyte subpopulation. Two-dimensional dot-plot analyses of Mac-1 labeling are shown. Each cell is represented by a dot whose position on the horizontal axis is determined by the size of the cell and whose position on the vertical axis is determined by the degree of fluorescence. Each panel contains data from 40,000 cells. Panel A is an analysis of whole blood mononuclear cells labeled with M1/70 (10 μg per ml) and shows labeling of large cells corresponding to monocytes. Panel B is the same cells labeled with 10 μg per ml normal rat IgG as a control. Panel C is an analysis of blood lymphocytes after depletion of monocytes and T cells. In this panel, a population of small lymphocytes is labeled by the M1/70 antibody. The corresponding control is shown in Panel D.

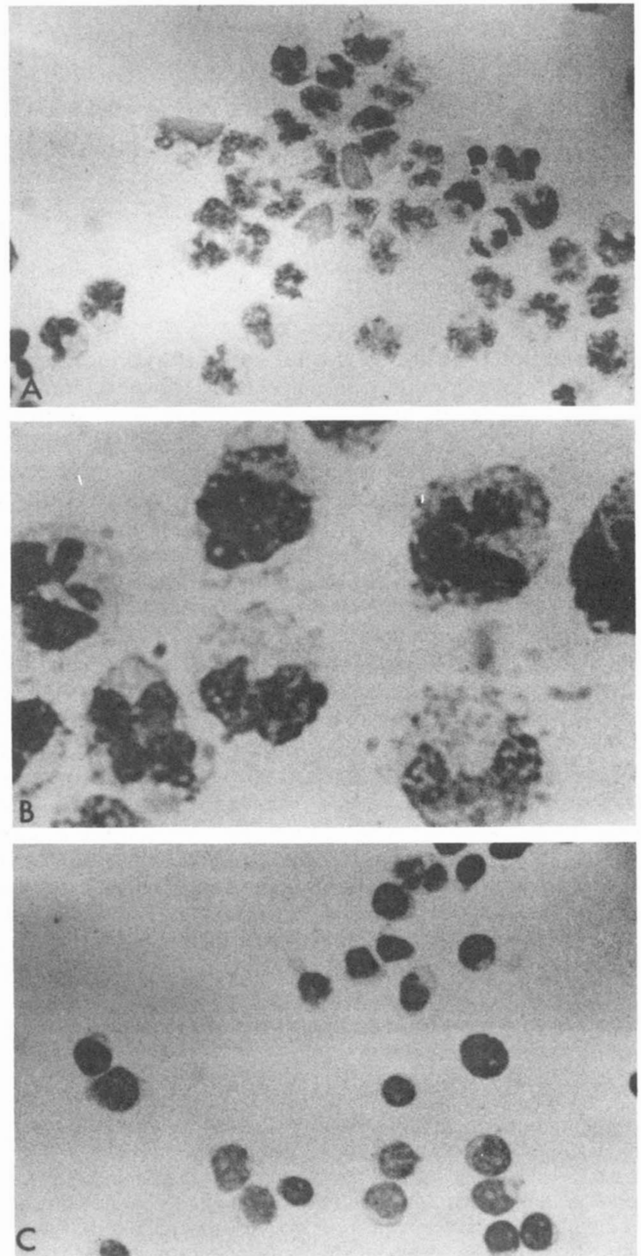

Figure 2. Morphology of sorted Mac-2-positive and -negative cells. Views of the cells obtained by sorting the large Mac-1-positive cell population. Wright-Giemsa staining shows monocytes and polymorphonuclear leukocyte morphology. *A*, low-power view of Mac-1-positive cells; *B*, high-power view of Mac-1-positive cells; *C*, Mac-1-negative cells.

When the large, brightly labeled cells were purified by sorting on the FACS, Wright-Giemsa staining showed them to consist of monocytes and polymorphonuclear leukocytes. Sorting of the negative cells showed them to consist predominantly of lymphocytes (Fig. 2).

In some studies done on blood cells that were not depleted of polymorphonuclear leukocytes by the Ficoll-Hypaque technique, we have also seen labeling of granulocytes in addition to monocytes (data not shown).

Labeling by either M1/70 IgG or $F(ab')_2$ is equally effective. The above experiments strongly suggested that M1/70 antibody bound in a specific fashion to human monocytes. Also in the mouse, M1/70 binding to macrophages appeared completely specific, since it was not inhibitable by heat-aggregated or normal IgG (2), and [^3H]M1/70 antibody bound to mouse but not rat peritoneal exudate cells (1). However, to definitely test the possibility that the Fc portion of M1/70 could mediate binding to human cells, the $F(ab')_2$ fragment was prepared. M1/70 antibody secreted into tissue

culture medium containing 5% FCS was purified and pepsin cleaved as described in *Methods*. SDS-PAGE under nonreducing conditions of purified M1/70 IgG and its $F(ab')_2$ fragment (Fig. 3) revealed that the preparations were homogeneous and that digestion to the 105,000 dalton $F(ab')_2$ fragment was complete. SDS-PAGE after reduction of IgG or $F(ab')_2$ revealed a single H or H(Fab') chain band, respectively, and a single light chain band. This is consistent with other data showing that M1/70 secretes specific heavy and light chains in the absence of any myeloma chain components (1).

The purified M1/70 $F(ab')_2$ fragments, M1/70 IgG in spent culture supernatants, and purified M1/70 IgG (data not shown) were equally active on a molar basis in labeling human monocytes (Fig. 4). Labeling by both antibodies was maximal at 0.3 pmole/50 μl, corresponding to a 100-fold dilution of spent culture supernatant, and fell off below this concentration. The fluorescence plateaued at a higher level for the M1/70 IgG than $F(ab')_2$, suggesting that fewer sites are available for binding of FITC anti-rat IgG on the digested antibody.

In all subsequent studies, saturating doses of anti-Mac-1 and $F(ab')_2$ anti-Mac-1 were used for labeling.

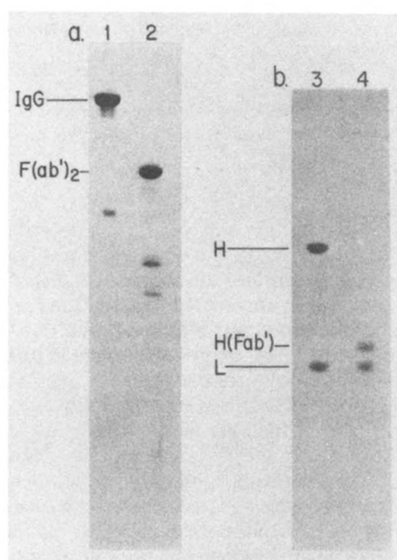

Figure 3. Analysis of M1/70 IgG and $F(ab')_2$. SDS-PAGE of purified M1/70 IgG, before and after pepsin digestion. *A*, nonreduced: 1, M1/70 IgG (20 μg); 2, M1/70 ($F(ab')_2$ (20 μg). *B*, reduced: 3, M1/70 IgG (10 μg); 4, M1/70 $F(ab')_2$ (8 μg). Samples were prepared in SDS sample buffer containing 50 mM iodoacetamide (nonreduced) or 5% 2-mercaptoethanol (reduced), subjected to SDS 5 to 15% PAGE (13), and stained with Coomassie Blue. The 2 gels in A and B were run on separate occasions. The positions of calibration proteins established the identity of bands noted in the figure.

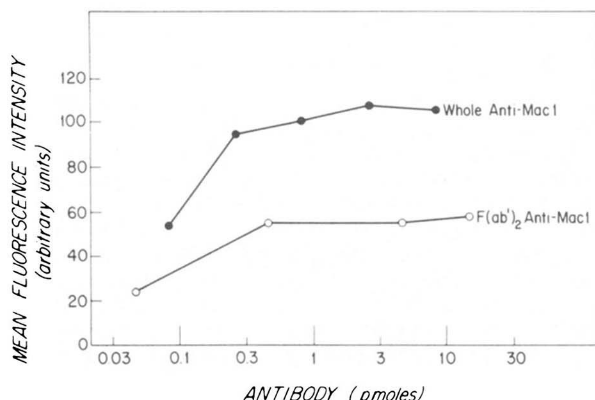

Figure 4. Saturable binding of M1/70 to human monocytes. Human mononuclear cells were labeled with either intact M1/70 IgG or $F(ab')_2$ fragments, by using the indicated amount of antibody in a volume of 0.1 ml for labeling 1×10^6 cells. This was followed by 50 μg of fluoresceinated $F(ab')_2$ fragment of rabbit-anti-rat IgG. The fluorescence distribution of the monocytes was measured on the FACS. The weighted average channel number is plotted.

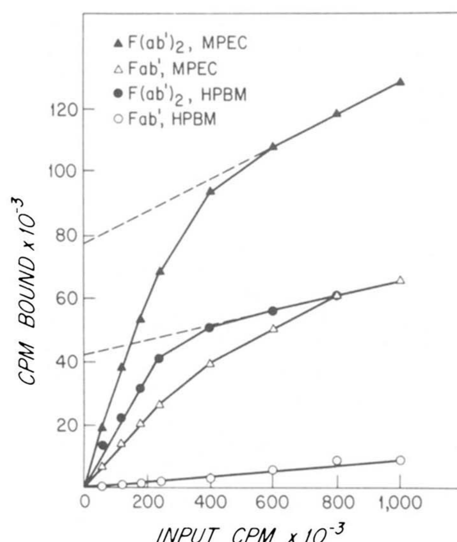

Figure 5. Saturation binding of ^{125}I-M1/70 $F(ab')_2$ and Fab' to human blood mononuclear cells and murine peritoneal exudate cells. Saturation binding of ^{125}I-M1/70 $F(ab')_2$ or the same preparation after reduction and alkylation (^{125}I-M1/70 Fab') was carried out on 2.3×10^6 fresh human peripheral blood mononuclear cells (HPBM) or 2×10^5 glutaraldehyde-fixed murine thioglycollate-induced peritoneal exudate cells (MPEC) as described in *Materials and Methods*. Symbols: $F(ab')_2$ on MPEC, ▲; Fab' on MPEC, △; $F(ab')_2$ on HPBM, ●; Fab' on HPBM, ○. Monocytes constituted 25% of the mononuclear cell preparation as determined by nonspecific esterase staining.

Site number estimation. The question of how many Mac-1 antigenic sites are expressed per human monocyte was addressed using saturation binding of ^{125}I labeled M1/70 IgG (not shown) and M1/70 $F(ab')_2$ and Fab' fragments to human blood mononuclear cells (Fig. 5). Site numbers were determined by extrapolation to the ordinate of the amount of ^{125}I bound in saturation. In 3 different experiments using 2 different mononuclear cell preparations (25% monocytes), the number of M1/70 antibodies bound per monocyte was 36,000 (^{125}I-IgG), 31,000 and 48,000 (^{125}I-F9ab'$)_2$). It was concluded that blood monocytes bind 40,000 ± 20,000 molecules of M1/70 IgG or $F(ab')_2$ per cell. Since M1/70 positive lymphocytes constitute a smaller population and bear less antigen per cell then monocytes, the contribution by these cells to the site number calculations was considered negligible. While M1/70 ($F(ab')_2$ bound both to human mononuclear cells and to mouse peritoneal exudate cells, it was noteworthy that M1/70 Fab' gave saturable binding only to mouse peritoneal exudate cells (Fig. 5). This suggests that the affinity of the antibody is lower for the cross-reacting human antigen. Glutaraldehyde-fixed mouse peritoneal exudate cells bind about 5-fold more M1/70 $F(ab')_2$ antibody than human monocytes (Fig. 5), whereas fresh mouse peritoneal exudate cells bind about 10-fold more (M.-K. Ho, unpublished observations).

Labeling of Human Lymphocytes by anti-Mac-1. As suggested above, there was some low-level labeling of small lymphocytes with anti-Mac-1. Generally the labeling was about 2- to 5-fold less bright than that of monocytes. After monocyte depletion a well-defined population of small lymphocytes could be labeled. Depletion of T lymphocytes by rosetting with normal sheep erythrocytes further enriched for the Mac-1 positive lymphocyte subpopulation. In such preparations as many as 30 to 50% of the cells could be so labeled (Fig. 1). Use of the $F(ab')_2$ anti-Mac-1 gave less intense labeling of the same proportion of cells.

Sorting of Mac-1 positive lymphocytes for NK activity. Preliminary experiments were done to test for any effect of the Mac-1 labeling procedure on the ability of lymphocytes to mediate NK and ADCC. When effector cells were pretreated with anti-Mac-1 or control supernatant followed by F-Fab$_2$RaRG there was no difference in their ability to mediate either NK or ADCC as compared with untreated cells (data not shown).

The Mac-1 positive lymphocyte population was purified using the FACS. Beginning with monocyte and T cell-depleted lymphocytes, the cells were labeled with anti-Mac-1 and sorted. Three cell populations (labeled but not sorted, Mac-1 positive, and Mac-1

negative) were compared in NK and ADCC assays. Essentially all of the NK activity and most of the ADCC activity was found in the Mac-1 positive population (Fig. 6). The same result was obtained in 2 experiments using intact anti-Mac-1 and in 3 experiments using $F(ab')_2$ fragment of anti-Mac-1. A total of 4 different individuals were tested (Table I). ADCC activity of the sorted cells was tested twice after sorting with intact antibody and twice after sorting with $F(ab')_2$ antibody. This involved 3 individuals. There was always some residual ADCC activity in the Mac-1 negative population in these experiments (Table 2).

Relationship between the Mac-1 positive lymphocytes and the IgG binding lymphocytes. Previous work (4–10) had shown that a population of lymphocytes that could be labeled by treatment with fresh human serum followed by F-Fab$_2$RaHuIgG (i.e., labeling of the Fc receptor) also contained all of the NK and ADCC activity in human blood. In order to determine the relationship between these 2 cell populations, we labeled with 1 marker, sorted for positive cells, then relabeled for the second marker and analyzed. Such relabeling after sorting was possible because both the serum-anti-IgG labeling and the anti-Mac-1 labeling were almost completely lost after incubation for 1 hr at 37°C. In these experiments, therefore, the sorted cells were held at 37°C for 1 hr before relabeling, and the per cent positive cells was determined by FACS analysis as compared with the same cells not relabeled.

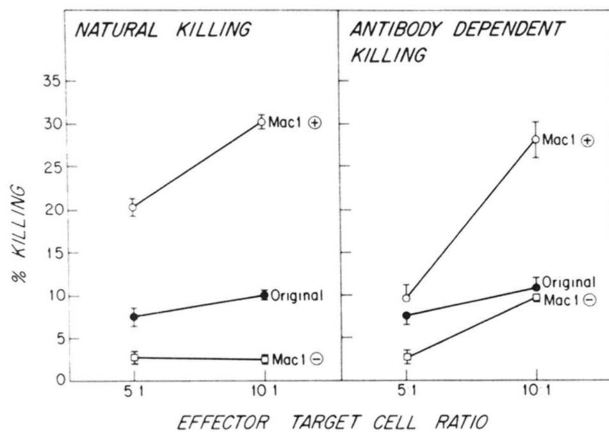

Figure 6. Natural and antibody-dependent killing by sorted human blood lymphocyte populations. Natural killing and antibody-dependent killing of K-562 target cells by sorted cell populations: labeled but not sorted (●——●); Mac-1-positive (○——○); and Mac-1-negative (□——□). In this experiment, monocytes and T cell-depleted lymphocytes were labeled with whole anti-Mac-1 and sorted 40% positive, 50% negative, with the intermediate 10% discarded. Other experiments using $F(ab')_2$ anti-Mac-1 gave the same results.

TABLE I

Summary of sorting data for NK activity[a]

Experiment	Lytic Units per 10^7 Cells		
	Unsorted cells	Macl-positive cells	Macl-negative cells
I	0.2	41.0	<0.001
II	1.7	87.0	<0.001
III	22.0	55.0	0.003
IV	0.2	5.1	<0.001

[a] Data from 4 experiments with 4 individuals showing recovery of all NK activity in the Macl-positive population. Lytic units are defined as the reciprocal of the number of effector cells required to achieve 30% lysis of the K-562 target cells. The data are calculated from at least 3 effector-to-target ratios. The labeling and sorting were done as described in Figure 6. Experiment I was done by using intact antibody; the other 3 experiments used $F(ab')_2$ fragments of anti-Macl.

TABLE II

Summary of sorting data for ADCC activity

Experiment	Lytic Units per 10^7 Cells[a]		
	Unsorted cells	Macl-positive cells	Macl-negative cells
I	33	91	13
II	29	53	18
III	170	200	33

[a] Data from 3 experiments with 3 individuals showing recovery of most ADCC activity in the Macl-positive population. The experiment numbers are the same as those used in Table I. In Experiments I and II, the ADCC target cells were K-562, and these results were calculated after subtraction of the NK activity. In Experiment III, the target was P815 which has no background NK activity.

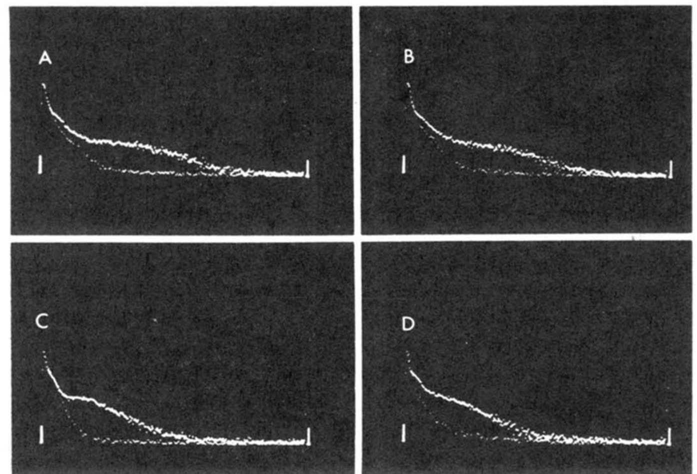

Figure 7. Lack of interaction between Mac-1 and Fc receptor sites and human lymphocytes. Each curve is a fluorescence distribution analysis of 50,000 cells. Fluorescence intensity is plotted on the horizontal axis, and the number of cells having any given degree of fluorescence is plotted on the vertical scale. The analysis was done on lymphocytes depleted of both monocytes and T cells. *A*, lymphocytes labeled with serum, followed by F-Fab$_2$RāHuIgG (*bright curve*) or with F-Fab$_2$RāHuIgG alone (*dim curve*). *B*, as above, but with lymphocytes were pretreated with a saturating dose of anti-Mac-1 antibody. *C*, lymphocytes labeled with anti-Mac-1 and F-Fab$_2$RāRg (*bright curve*) or with control supernatant and F-Fab$_2$RāRg (*dim curve*). *D*, as above, but the lymphocytes were pretreated with fresh serum.

When lymphocytes were first incubated with $F(ab')_2$ anti-Mac-1 and then labeled by the serum anti-IgG technique for Fc receptors, there was no difference in either the proportion of cells labeled or the intensity of labeling as compared to cells pretreated with control supernatant or not pretreated. This experiment was extended to doses of $F(ab')_2$ anti-Mac-1 as high as 100 times the saturating dose with no dimunition of the number of cells subsequently labeled by serum and F-Fab$_2$RaHuIgG. Conversely, when cells were first incubated with human serum followed by labeling with anti-Mac-1 and F-$F(ab')_2$RaRG, there was no difference as compared to cells not incubated in serum (Fig. 7). Thus, under the conditions employed here, there was no interaction between the Mac-1 antigenic site and the Fc receptor on these cells.

When the IgG binding cells were sorted as described previously (4) and the resulting positive cells were labeled by $F(ab')_2$ anti-Mac-1, 90% could be labeled. Conversely, sorting of the Mac-1 positive lymphocytes (using $F(ab')_2$ anti-Mac-1) followed by labeling by the serum anti-IgG technique showed that 76% of the Mac-1 positive cells could be labeled. Thus, there was extensive overlap between the populations labeled by these 2 independent methods.

DISCUSSION

The myeloma hybrid technique has revolutionized our ability to analyze complex antigenic systems, such as cell surfaces, by resolving polyspecific immune responses into series of cloned lines secreting single antibody species (3, 18). This technique was used to obtain an antibody to Mac-1, a murine phagocyte-specific antigen expressed on granulocytes and macrophages. More recently, antigens of 32,000 daltons (Mac-2) and 110,000 daltons (Mac-3) were identified, which are expressed on macrophages but not on granulocytes or lymphocytes (3). Because of their restricted expression, these have been called "lineage" differentiation antigens. The highly restricted nature of expression of these "lineage" differentiation antigens suggests them to be important in the unique functions of the cells on which they are expressed (3). However, the function of Mac-1 remains unknown at present.

These studies showed that M1/70 rat anti-mouse antibody cross-reacts with an antigenic determinant in humans with a very similar tissue distribution. In both humans and mice the antigen is present on granulocytes and blood monocytes but absent from

erythrocytes and B and T lymphocytes. In the present studies, labeling of a human lymphocyte subpopulation containing natural killer and antibody-dependent killer activity was also observed. Labeling of a small percentage of blood lymphocytes was not noticed in the previous murine studies, but labeling of the monocytes was weak and therefore the even weaker labeling of lymphocytes could have been undetectable. Also, with human blood mononuclear cell preparations, labeling of the lymphocyte subpopulation is difficult to detect without monocyte and T cell depletion (Fig. 1). Studies to determine whether M1/70 labels murine natural killer cells suggest that this is the case (L. Holmberg, and K. Ault, unpublished observations).

Monocytes bind 40,000 ± 20,000 molecules of M1/70 IgG or $F(ab')_2$ per cell, while the fluorescence data suggest that natural killer cells bind about 3-fold less. Murine peritoneal exudate macrophages bind considerably more M1/70 antibody than murine blood monocytes (2) and also bind 5- to 10-fold more M1/70 antibody than human monocytes. Murine peritoneal exudate cells bind monovalent M1/70 Fab' fragments, whereas human monocytes do not. This suggests that the affinity of M1/70 for the cross-reacting human antigen is lower than for the homologous antigen and that the multiplication of affinities brought about by bivalent binding to human cells compensates for the lowered monovalent affinity. This also suggests that the number of antigen sites is twice the number of antibodies bound, i.e., about 80,000 per monocyte. Several attempts to immunoprecipitate the human monocyte antigen after ^{125}I labeling have failed, whereas the murine antigen is readily precipitated (2). We believe this also reflects the requirement of a bivalent interaction for stable binding to the human antigen, since the detergent-solubilized antigen is probably monovalent.

The subsets of human lymphocytes mediating NK and ADCC as defined now by the presence of Mac-1 and as defined previously by the presence of a particularly avid Fc receptor for IgG (4–10) appear to be the same. Our relabeling experiments suggest that there is extensive overlap between these 2 populations and strengthen, but do not prove, this possibility that NK and ADCC are mediated by the same cells. Our results suggest that there are non-Mac-1 bearing cells capable of ADCC activity. Others have described ADCC activity by activated T cells (19), and this may explain the residual ADCC activity in the Mac-1 negative population. The enrichment of NK activity in the Mac-1 positive population is frequently higher than would be expected on the basis of cell numbers. Our data do not rule out cellular interactions regulating NK activity that might explain this observation.

The presence of an avid Fc receptor for IgG on the NK cell makes it necessary to exercise extreme caution in labeling the cells with antibody. We have shown that these cells can be labeled by very low concentrations (1 μg/ml) of M1/70 IgG antibody. One-hundred-fold higher concentrations of normal rat IgG give no labeling. We have also obtained the pepsin digested fragment ($F(ab')_2$) of the anti-Mac-1 antibody and have shown that the cells are equally well labeled by it. Because all the cells so far described as bearing Mac-1 also have a prominent Fc receptor for IgG, we carefully examined the possibility that the Mac-1 determinant was the same as, or associated with, the Fc receptor. We found no reduction of Fc receptor labeling of the NK cells when they were pretreated with up to 100 times the saturating dose of $F(ab')_2$ anti-Mac-1, and conversely we found no diminution of Mac-1 labeling when the cells were treated with human serum under conditions that brightly label the Fc receptors. We conclude that Mac-1 is distinct from the Fc receptor and that these 2 markers constitute 2 independent labels for the same population of cells.

In addition to providing a useful and well-defined marker for the human NK cell, the presence of Mac-1 raises important questions concerning the origin of this cell. The lineage of the human NK cell has been the subject of considerable debate for some time. There are three possibilities at present: 1) they may represent an independent cell line arising in the bone marrow; 2) they may be immature T cells; or 3) they may be immature cells of the monocyte-macrophage series. There is evidence in favor of each of these 3 possibilities. The majority of the work done to characterize the NK cell has been done in mice and in man. Numerous workers have contributed to the definition of this cell as a small lymphocyte lacking intrinsic surface immunoglobulin that has a very avid Fc receptor for IgG. In general, there is good agreement in both systems that the cell is not a typical B cell nor a typical T cell, that it is nonadherent, nonphagocytic, and has the morphology of a lymphocyte (4–10). Although it is clearly distinct from the monocyte, it has recently been proposed that the cell may be a "promonocyte" (20, 21). In addition, recent reports of cross-reaction of another monoclonal anti-monocyte antibody with human non-B, non-T lymphocytes having Fc receptors for IgG (22) support both our results and the suggestion that these cells are indeed members of the monocyte-macrophage lineage. It has also been suggested that human NK cells may be in the T lymphocyte lineage because of the observation that under certain conditions they can be made to rosette with specially treated sheep erythrocytes (10). In the mouse they have weak reactivity with anti-Thy-1 antisera (23), and recently they have been shown to react with alloantisera prepared against T cells (24, 25). However, Thy-1 antigen is also expressed on brain and epithelial cells and is therefore not a reliable marker of ontogenetic relatedness. The fact that NK activity can be ablated by ^{89}strontium irradiation of the bone marrow (26) strongly suggests that the cell arises from a precursor that may ultimately be shared by other lymphoid cells.

The studies reported here demonstrate an antigen on NK cells that is strongly associated with the granulocyte-monocyte cell lineage. We believe that sharing of antigens with highly restricted distributions such as Mac-1 is more suggestive of ontogenetic relatedness than sharing of antigens such as Thy-1, which are expressed on disparate tissues such as brain and thymus (reviewed in 3). However, the sharing of a single surface antigenic determinant between different cells does not necessarily imply that they are developmentally related. Ideally, several independent highly restricted shared markers should be demonstrated before conclusions are drawn. Further studies of this type will be necessary to resolve the question of the NK cell lineage satisfactorily.

Acknowledgments. The expert technical assistance of Murray Towle, Evelyn Reilly, Earl Adams, Jon Davis, and Edgar Luther is gratefully acknowledged.

REFERENCES

1. Springer, T., G. Galfré, D. S. Secher, and C. Milstein. 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur. J. Immunol. 8:539.
2. 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.
3. Springer, T. 1980. Cell surface differentiation in the mouse: characterization of "jumping" and "lineage" antigens by using xenogeneic rat monoclonal antibodies. Monoclonal antibodies. Edited by R. Kennett, T. McKearn, and K. Bechtol. Plenum Press, New York. In press.
4. 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.
5. Ault, K. A., and H. L. Weiner. 1979. Natural killing of measles-infected cells by human lymphocytes. J. Immunol. 122:2611.
6. Horowitz, D. A., and P. I. Lobo. 1975. Characterization of two populations of human lymphocytes bearing easily detectable surface Ig. J. Clin. Invest. 56:1464.
7. Winchester, R. J., S. M. Fu, T. Hoffman, and H. G. Kunkel. 1975. IgG on lymphocyte surfaces: technical problems and the significance of a third cell population. J. Immunol. 114:1210.
8. Kurnick, J. T., and H. M. Grey. 1975. Relationship between Ig-bearing lymphocytes and cells reactive with sensitized human erythrocytes. J. Immunol. 115:305.
9. Kay, H. D., G. D. Bonnard, W. H. West, and R. B. Herberman. 1977. A functional comparison of human Fc-receptor bearing lymphocytes active in natural cytotoxicity and antibody dependent cellular cytotoxicity. J. Immunol. 118:2058.
10. West, W. H., G. B. Cannon, H. D. Kay, G. D. Bonnard, and R. B. Herberman. 1977. Natural cytotoxicity reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J. Immunol. 118:355.
11. Ouchterlony, O., and L.-A. Nilsson. 1978. Immunodiffusion and immunoelectrophoresis. Handbook of experimental immunology. Edited by D. M. Weir. Blackwell, Oxford, P. 19.10.
12. Binaghi, R., and R. Oriol. 1968. Anticorps purifie's de type macroglobuline. Bull. Soc. Chim. Biol. 50:1035.
13. Stanworth, D. R., and M. W. Turner. 1978. Immunochemical analysis. Handbook of experimental immunology, Edited by D. M. Weir. Blackwell, Oxford, P. 6.25.

14. Fraker, P. J., and J. C. Speck, Jr. 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide 1,3,4,6-tetrachloro3a,6a-diphenylglycouril. Biochem. Biophys. Res. Commun. 80:849.
15. Perper, R. J., T. W. Zee, and M. M. Mickelson. 1968. Purification of lymphocytes and platelets by gradient centrifugation. J. Lab. Clin. Med. 72: 842.
16. Ault, K. A., and E. R. Unanue. 1977. Comparison of the two types of immunoglobulin-bearing lymphocytes in human blood with regard to capping and motility. Clin. Immunol. Immunopathol. 7:394.
17. Aurameas, S., and T. Terynynck. 1969. The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry 6:53.
18. Milstein, C., and E. Lennox. 1979. The use of monoclonal antibody techniques in the study of developing cell surfaces. Developmental immunology. Curr. Top. Dev. Biol. In press.
19. Lamon, E. W., H. D. Whitten, B. Lidin, and H. H. Fudenberg. 1975. IgM-induced tumor cell cytotoxicity mediated by normal thymocytes. J. Exp. Med. 142:542.
20. Roder, J. C., M.-L. Lohmann-Matthes, W. Domzig, R. Kiessling, and O. Haller. 1979. A functional comparison of tumor cell killing by activated macrophages and natural killer cells. Eur. J. Immunol. 9:283.
21. Lohmann-Matthes, M-L., W. Domzig, and J. Roder. 1979. Promonocytes have the functional characteristics of natural killer cells. J. Immunol. 123: 1883.
22. Breard, J., E. L. Reinherz, P. C. Kung, G. Goldstein, and S. F. Schlossman. 1980. A monoclonal antibody reactive with human peripheral blood monocytes. J. Immunol. 124:1943.
23. Herberman, R. B., M. E. Nunn, and H. T. Holden. 1978. Low density of Thy1 antigen on mouse effector cells mediating natural cytotoxicity against tumor cells. J. Immunol. 121:304.
24. Kasai, M., J. C. Leclerc, L. McVey-Boudreau, F. W. Shen, and H. Cantor. 1979. Direct evidence that natural killer cells in nonimmune spleen cell populations prevent tumor growth *in vivo*. J. Exp. Med. 149:1260.
25. 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.
26. Kumar, V., J. Ben-Ezra, M Bennett, and G. Sonnenfeld. 1979. Natural killer cells in mice treated with ^{89}Strontium: normal target binding cell numbers but inability to kill even after interferon administration. J. Immunol. 123: 1832.