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HUMAN MONONUCLEAR PHAGOCYTE DIFFERENTIATION ANTIGENS

I. Patterns of Antigenic Expression on the Surface of Human Monocytes and Macrophages Defined by Monoclonal Antibodies[1]

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Six newly developed monoclonal antibodies recognizing antigenic determinants expressed on the surface of human mononuclear phagocytes (MoPh) were applied to the antigenic analysis of this cell lineage. Antibody binding was detected by indirect immunofluorescence in conventional microscopy and flow microfluorometry. Through additive experiments, competitive blocking experiments and differences in the distribution histograms of immunofluorescence, evidence was obtained that each antibody detected a distinct antigenic determinant. Different increases in the amount of each antigen and in the frequency of positive cells were found on fluid or tissue macrophages when compared to blood monocytes. The six reagents could be placed in three general groups based on certain similar characteristics in their patterns of antigen expression. The reagents MϕP-9, MϕS-1, and MϕS-39 reacted with antigens found at high densities on up to 94% of phagocyte cells in purified blood monocyte preparations and on the vast majority of fluid or tissue macrophages. The reagent MϕP-15 recognized an antigen expressed at intermediate density on an average of 70% of blood monocytes and at greater density on almost all fluid and tissue macrophages. The reagents MϕP-7 and MϕR-17 detected antigens that were present at very low densities on 36% or less of blood monocytes but in larger quantities on almost all fluid or tissue macrophages; small amounts of these two antigens were also identified on a subpopulation of T cell blasts but not on resting T cells. The distribution and characteristics of the antigens recognized by the latter three reagents suggest they appear in sequence on the more mature members of the lineage MoPh and that their pattern of expression on blood monocytes defines three subsets.

Since their discovery by Metchnikoff almost a century ago (1), considerable information has accumulated about the morphologic, metabolic, and functional states of mononuclear phagocytes (MoPh),[4] including their participation in the afferent and efferent arms of the immune response (2–10). Because MoPh originate from a common bone marrow precursor (11–13), their phenotypic diversity suggests the existence of an intricate differentiation program associated with the expression of various gene products. The principal immunologic approach to the study of gene products on the surface membrane of human MoPh was initiated by the use of heteroantisera (14) and, more recently, it was advanced through the application of monoclonal antibody methodology (15–24).

The present study originated from an attempt to understand further MoPh lineage diversity by defining additional antigenic determinants on the surface of these cells. The questions approached were whether various members of human MoPh lineage were characterized by few or numerous differentiation antigens, as well as whether there was a change in the expression of these antigens during maturation. In preliminary experiments monoclonal reagents were identified that reacted with a variety of antigenic determinants expressed on the MoPh cell surface. This paper initiates the analysis of six distinct cell surface antigenic determinants and their respective patterns of expression on human monocytes and macrophages.

MATERIALS AND METHODS

Preparation of various cell populations. Mononuclear cell suspensions were obtained from the peripheral blood of healthy volunteers or leukapheresed rheumatoid arthritis patients by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NY) density gradient centrifugation (25). All cell suspensions were prepared in RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% FCS (Reheis Chemical Co., Phoenix, AZ; lots U41806 and U51105). Mononuclear cells were fractionated in lymphocyte-enriched and monocyte-enriched suspensions by centrifugation upon discontinuous Percoll (Pharmacia) density gradients performed as described (26). Monocytes recovered in the low density fraction of Percoll gradients were further purified by plastic adherence. In brief, 1×10^7 viable cells were incubated in 100 × 20 mm tissue culture petri dishes (Falcon Plastics, Oxnard, CA) in 10 ml RPMI medium supplemented with 10% FCS for 60 min at 37°C. Nonadherent cells were removed by five successive washings and adherent cells were recovered with a rubber policeman. Purified monocyte preparations contained 92% monocytes (range: 83 to 97%) as defined by morphology, nonspecific esterase staining (27), and latex particle endocytosis. Lymphocytes, recovered in the high density fraction of Percoll gradients, were further depleted of contaminating monocytes by plastic adherence and were divided in T cell-enriched and B cell-enriched populations by E-rosetting with neuraminidase-treated sheep red blood cells before centrifugation upon Ficoll-Hypaque density gradients (28).

Activated T cell blasts were obtained by culturing T cell-enriched populations in the presence of 1/100 phytohemaglutinin (PHA) or equal numbers of allogeneic mononuclear cells for 3 to 5 days in RPMI 1640 media supplemented with 10% FCS. Granulocytes were recovered from the pellet of peripheral blood Ficoll-Hypaque gradients by unit gravity sedimentation in 3% dextran (Macrodex, Pharmacia) as described (29). Noninflammatory fluid MoPh were obtained from two patients with pleural effusions secondary to cardiac failure and one patient with transudative ascites secondary to tumoral obstruction. The cells were isolated by centrifugation upon Ficoll-

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[4] Abbreviations used in this paper: MoPh, mononuclear phagocytes.

Hypaque density gradients followed by enrichment in adherent cells on plastic petri dishes in RPMI 1640 media supplemented with 10% FCS. These preparations contained from 80 to 92% esterase-positive and latex-positive mononuclear cells.

Synovial MoPh were prepared from synovial lining membranes of osteoarthritic patients according to a described method (30). In brief, finely minced synovial membrane fragments were treated with 1 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO) and 0.15 mg/ml DNAse (Sigma) for 60 min at 37°C with constant stirring in spinner flasks. Single cells were filtered through nylon sieves (Tetco, Elmsford, NY) and were washed twice in RPMI 1640 media. Mononuclear synovial cells were purified by centrifugation upon Ficoll-Hypaque density gradients. Further fractionation of synovial cells was performed by centrifugation for 10 min at 400 × G upon discontinuous Percoll density gradients consisting of 100, 60, 50, 20, and 0% Percoll. The layer between 50 and 20% Percoll is highly enriched in synovial lining cells and contained from 30 to 43% cells identified as MoPh by their morphology, positive reaction for nonspecific esterase, and latex phagocytic ability.

Hematopoietic and tumor cells. The following human hematopoietic cell lines were used in this study: six B cell lymphoblastoid lines, i.e., GM numbers 3103, 3104, 3105, 3161, 3163, and Daudi; three T cell lymphoblastoid lines: CEM 1, KE37, and 1301; U937, a human histiocytic lymphoma cell line with phenotypic features of an immature monocytoid cell (31); HL-60, a promyelocytic cell line capable of myeloid or monocytoid differentiation (32), and the erythroblastoid cell line K562. In addition, the tumor cell lines Hep 2, a laryngeal carcinoma; Cal 1, a bladder carcinoma; SKov, an ovarian sarcoma, and PLC, a human hepatoma, were used. All these cells were cultured for at least 48 hr in RPMI 1640 supplemented with 10% FCS before being used for cell surface staining with various monoclonal antibodies.

Production and selection of monoclonal antibodies. Three different hybridomas were produced by polyethylene glycol-induced fusion of Sp2/0 myeloma cells with splenocytes from BALB/c mice previously immunized with one of the following cell populations: a) purified monocytes from the peripheral blood of a normal individual (hybridoma R); b) purified monocytes from a leukapheresed rheumatoid arthritis patient (hybridomas P); and c) MoPh isolated from the pleural transudate of a patient with heart failure (hybridoma S). The immunization was performed by two i.p. administrations of 1×10^7 viable cells at 28 days of interval. Three days after the second immunization, the cell fusions were performed according to Kohler and Milstein (33) with minimal modifications (34). A total of 714 growing colonies were obtained. They were distributed in three fusions: 215 for the hybridoma R, 256 for the hybridoma P, and 243 for the hybridoma S. Preliminary screening performed by immunofluorescent surface staining on blood lymphocytes and monocytes revealed that 112 colonies produced antibodies that bound to various proportions of monocytes but not lymphocytes. The hybridomas with apparent specificity for antigens expressed on monocytes represent 15% of all colonies and were distributed as follows: 24 for the hybridoma R, 49 for the hybridoma P, and 39 for the hybridoma S; sixty-three of them grew vigorously and were cloned twice on thymocyte feeder layers. The selected clones were cultured in large volumes. Six established hybrids were selected for study. They were distributed in the three fusions and were designated MϕP-7, MϕP-9, MϕP-15, MϕR-17, MϕS-1, and MϕS-39. Ascites were produced in pristane-primed BALB/c mice by i.p. injection of 2×10^6 viable cells. Antibody subclasses were determined in a solid-phase radioimmunoassay on PVC microtiter plates using ^{3}H-labeled isotype-specific goat anti-mouse Ig class reagents kindly provided by Drs. P. Mongini and C. Bona. The monoclonal antibodies selected in this study were demonstrated to be IgG1 κ (MϕP-15 and MϕP-7), IgG2b κ (MϕP-9), and IgG2a κ (MϕS-1 and MϕS-39). In addition, the monoclonal antibody 63D3, directed to human monocytes antigens (15), was kindly provided by Dr. J. D. Capra.

Immunofluorescence. Binding of monoclonal antibodies to the surface of various cell populations was determined by indirect immunofluorescence as previously described (35, 36). In brief, 1×10^5 cells were incubated for 30 min at room temperature with 0.05 ml monoclonal antibody culture supernatants. In titration experiments on blood monocytes evaluated by cytofluorometric analysis, this volume of the reagents could be diluted from four to eight times before a detectable decrease in staining intensity was observed. The cells were washed four times, resuspended, and stained with affinity-purified tetramethylrhodamine isothiocyanate-conjugated $F(ab')_2$ fragments of rabbit anti-mouse Ig antibodies. After 30 min of incubation, the cells were washed three times and were processed for examination. For negative controls, monoclonal antibody supernatants of irrelevant specificities but appropriate isotypes were used in all experiments. Among them, the supernatants of monoclonal antibodies of the IgG1, IgG2a, and IgM class directed to dinitrophenol epitopes were kindly provided by Drs. D. Katz and N. Chiorazzi. Other murine myeloma proteins were provided by Dr. C. Bona. The blockage of Fc receptors before staining with monoclonal antibodies was performed by using human aggregated IgG-fraction 11 according to the previously described method (35). Positive cells were evaluated in fluorescent microscopy by incident illumination in a fluorescence microscope (Leitz, Wetzlar, West Germany). In parallel, quantitative cytofluorometry analysis was performed by using an FACS IV (Becton Dickinson, Mountain View, CA) fluorescence-activated cell sorter with sheep red blood cells as standards. At least 2×10^4 cells were examined by using 488 nm illumination under described conditions (37, 38).

Competitive blocking experiments were performed using fluorescein-labeled $F(ab')_2$ fragments of MϕS-1 and MϕS-39 at a concentration in which the monocytes were stained at 75 to 80% of the plateau intensity of saturation. In the first step, 1×10^5 monocytes were preincubated with 0.1 ml of inhibiting unlabeled monoclonal antibody for 30 min. This concentration contained a quantity of antibody eight to 16-fold greater than necessary for saturation. A second sample was incubated with 0.02 ml of the antibody. In parallel, samples were either washed four times or were incubated an additional 10 min without washing. In the second step, appropriate dilution of fluorescein-labeled $F(ab')_2$ fragments of monoclonal antibody were added, incubated 30 min, and washed four times. The samples were examined by cytofluorometry as described above.

RESULTS

Definition of multiple differentiation antigens on the surface of monocytes by monoclonal antibodies. The frequency of positive cells as well as the intensity of staining in immunofluorescent microscopy and flow cytofluorometry suggested that multiple antigenic determinants were detected on the surface of blood monocytes by the six monoclonal reagents (Table I). Preincubation of monocytes with aggregated human IgG before reacting with various antibodies did not result in any significant modification of surface immunofluorescence, indicating that the monoclonal reagents did not significantly bind on the cell surface via Fc receptors. The reagents MϕP-9, MϕS-1, and MϕS-39 gave generally similar intensity of staining on averages of 86 to 89%, (range 70 to 94%) of cells by fluorescence microscopy (Table I). In three experiments, purified monocytes were incubated with latex particles before staining for the purpose of defining the relationship between the expression of these antigens and the phagocytic ability of these cells. An average of 94% of the cells that ingested latex particles expressed the MϕP-9, MϕS-1, or MϕS-39 antigens, and reciprocally, only from 1 to 4% of cells that did not ingest latex were

TABLE I

Differentiation antigens on the surface of blood monocytes demonstrable by monoclonal antibodies

		Monoclonal Antibodies						Control
		MøP-9	MøS-1	MøS-39	MøP-15	MøP-7	MøR-17	
Conventional microscopy[a]	Positive cells (%)[b]	86 (70–93)	88 (74–95)	89 (74–95)	66 (38–89)	24 (10–36)	15 (5–34)	3
	Intensity[c]	2–3+	2–3+	2–3+	1+	1+	1+	1+
FACS analysis[d]	Positive Cells (%)[b]	84 (76–89)	83 (77–86)	87 (77–90)	70 (50–85)	37 (23–40)	33 (10–35)	5
	Intensity[e]	87	86	91	35	15	15	1

[a] Data obtained on 20 normal individuals.
[b] Median and range.
[c] Fluorescence scored from 1 to 4.
[d] Data obtained on six normal individuals.
[e] Median channel of fluorescence.

stained. Flow cytofluorometric analysis of six individuals revealed that in five MϕP-9, MϕS-1, and MϕS-39 gave similar broad distributions of positive cells with the median of fluorescence intensity at channels 87, 86, and 91, respectively (Table I, Fig. 1 *A–C*). The reagent 63D3 (15) was studied in parallel with these reagents and gave a comparable staining pattern in that 84% of monocytes were stained with a median fluorescence of channel 83. Repeat examination of these reagents on the same individual from two to four times yielded results that varied only by a very small number of channels. Among the six individuals, however, variations in the median fluorescence intensity of as much as 15 channels of the distribution histograms were observed from one individual to another when the same reagents were used. Moreover, in the case of one individual (PG), significant differences were observed in the shape of each of the distribution histograms given by reagents MϕP-9, MϕS-1, and MϕS-39, with the median fluorescence at channels 89, 80, and 99, respectively. For example, the distribution pattern of MϕS-39 resembled more closely that illustrated in Figure 2 for fluid macrophages. This evidence suggested that these three reagents reacted with distinct determinants.

A different pattern of antigen expression was encountered with the reagent MϕP-15; from 38 to 89% of purified monocyte preparation were stained at moderate intensity (Table I). Repeated staining in the same person gave reproducible values, but considerable variations in the percentage of cells that expressed this antigen were observed among different individuals. Flow cytometric analysis revealed an average of 70% positive cells with a distribution range of fluorescence intensity between channels 1 and 65, with the median of fluorescence intensity at channel 35 (Table I; Fig. 1*D*). Increasing the fluorescence gain up to eightfold resulted in a small increase in the percentage of cells that stained with this reagent. The differences in the frequency of positive cells among individuals were also demonstrated by flow cytofluorometric analysis.

The pattern of antigen expression given by the reagents MϕP-7 and MϕR-17 consisted of very weak staining on low percentages of blood monocytes (Table I; Fig. 1*E* and *F*). The reagent MϕP-7 detected an antigen present at low density on 10 to 36% of purified monocytes. The average median channel of fluorescence was 15 by cytofluorometric analysis. Reagent MϕR-17 weakly stained from 5 to 34% of blood monocytes using conventional microscopy. Cytofluorometry indicated a similar percent of positive cells and the median fluorescence intensity was at channel 15. For these last two reagents, the percent of positive cells was a reproducible characteristic of the same individual. No evidence for an additional population of cells with very weak antigen expression was obtained by increasing the fluorescent gain.

Independence of monocyte differentiation antigens on the surface of blood monocytes. Experiments performed by simultaneous addition of various combinations of the monoclonal antibodies MϕP-9, MϕS-1, MϕS-39, and MϕP-15 revealed each reagent detected an independent differentiation antigen (Table II). The expected values represent the median channel number calculated by averaging the number of median channel numbers obtained with equal quantities of the two monoclonal antibodies in separate experiments and assuming that both reagents equivalently bond to the same determinant. In each combination of monoclonal reagents the observed median channel number was considerably greater than that expected. Entirely comparable results were obtained when the antibodies were added in different sequences. In addition, in simultaneous and sequential additive experiments, the reagent 63d3 was examined with the reagents MϕP-9, MϕS-1, MϕS-39, and MϕP-15; in each instance a significant augmentation in median fluorescence channels was observed, but was not found with the addition of more 63d3. In similar additive experiments, the antigens defined by the reagents MϕR-17 and MϕP-7 were shown to be distinct.

To provide a more sensitive assay, competitive blocking experiments were performed with multiple combinations of monoclonal antibodies. Different concentrations of inhibiting

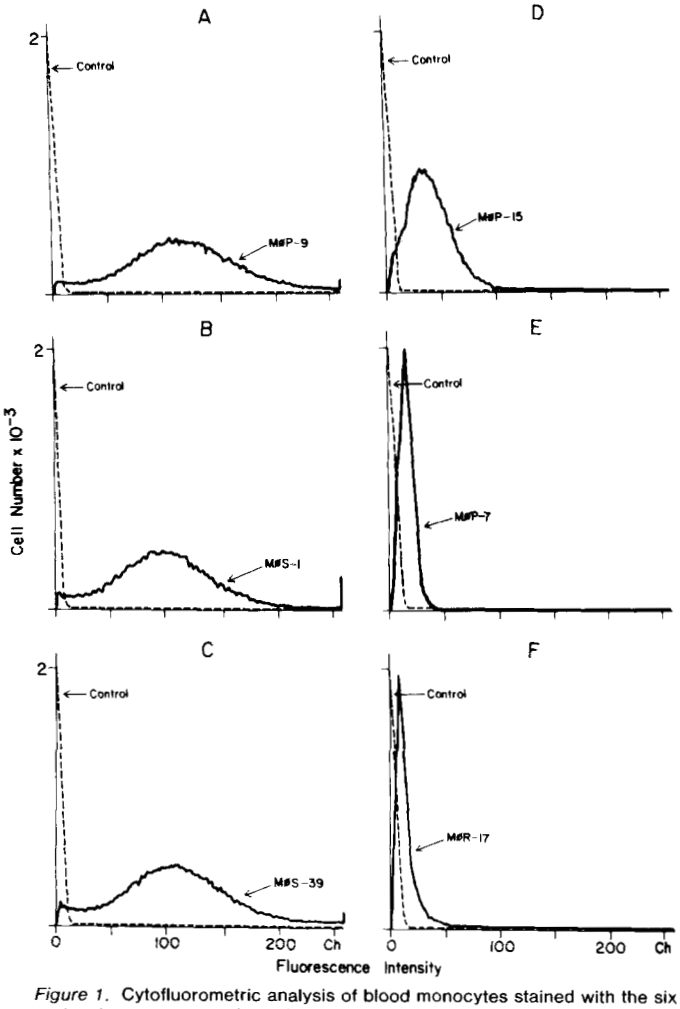

Figure 1. Cytofluorometric analysis of blood monocytes stained with the six monoclonal reagents in indirect immunofluorescence. The developing reagent was fluorescein-conjugated F(ab')$_2$ fragment. One hundred thousand cells were examined at 488 nm, 500 mW, gain of 1.

TABLE II

Independence of monocyte differentiation antigens as demonstrated by monoclonal antibodies in simultaneous addition experiments: Cytofluorometric analysis

Monoclonal Antibodies	Percent of Positive Cells	Median Channel of Fluorescence		
		Observed	Expected[a]	Obs-Exp
MøP-9 + MøP-9	85	63	—	—
MøS-1 + MøS-I	87	54	—	—
MøS-39 + MøS-39	88	69	—	—
MøP-15 + MøP-15	58	17	—	—
MøP-9 + MøS-1	87	90	59	+31
MøP-9 + MøS-39	88	110	66	+44
MøP-9 + MøP-15	85	81	40	+41
MøS-1 + MøS-39	87	92	62	+30
MøS-1 + MøP-15	85	70	36	+34
MøS-39 + MøP-15	88	77	43	+34

[a] Average of the two observed values obtained in independent staining based on the assumption that both reagents bind to the same determinant.

reagents were used with nonsaturating subplateau concentrations of directly fluoresceinated antibodies. In four of five experiments, no significant diminution in staining of the fluoresceinated antibody was observed with any of the monoclonal antibodies, indicating the reagents MϕP-9, MϕP-15, MϕS-1, MϕS-39, and 63D3 react with distinct antigenic determinants. Table III contains a representative experiment in which a washing step was employed after binding of the inhibiting reagent. Comparable results were obtained in three additional paired experiments performed without the washing step. In one experiment, however, the mean binding of fluorescein-labeled MϕS-1 was decreased by preincubation with MϕP-9 reagent.

Distribution of antigens detected by monoclonal antibodies on fluid and tissue MoPh. Analyses of MoPH derived from noninflammatory pleural or peritoneal fluids demonstrated considerable differences in the frequency and degree of expression of the six antigens compared to their expression on blood monocytes. All monoclonal antibodies stained from 79 to 86% of fluid MoPh (Table IV), but the cytofluorometric analysis revealed certain differences in their fluorescent profiles (Fig. 2*A–F*). The fluorescence intensity of the reagents MϕP-9, MϕS-1, and MϕS-39 was significantly higher with median fluorescence at channels 135, 108, and 145, respectively. The reagent MϕP-15 gave intermediate staining with median fluorescence at channel 70. The reagents MϕP-7 and MϕR-17 gave similar moderate staining on fluid MoPh with median fluorescence at channels 37 and 42, respectively. The staining pattern given by the same reagent on pleural or peritoneal macrophages was not distinguishable from one another. When fluid MoPh were compared with blood monocytes (Table V), the greatest increase was observed for the antigens detected by reagents MϕP-15, MϕP-7, and MϕR-17 in both the percentages of cells expressing the antigens as well as the intensity of the staining. The cytofluorometric profiles of fluid MoPh stained with these reagents revealed a broader distribution of positive cells compared with blood monocytes with a shift of 35, 22, and 27 median channels, respectively. The ratios of increase were 2.0, 2.3, and 2.8, respectively. Similar data were obtained in two additional experiments. In the case of reagents MϕP-9 and MϕS-39, significant enhancement of the staining intensity was also observed with a shift of median fluorescence intensity of 48 and 54 channels; the ratios of increase were 1.6 and 1.5, respectively. The staining observed on macrophages with the antibody MϕS-1 was less enhanced compared to that observed on blood monocytes; the ratio of the increase was 1.2 (Table VI).

Studies on tissue MoPh of the synovial lining membrane from six individuals with osteoarthritis revealed a high percentage of intensely stained cells given by all six monoclonal reagents.

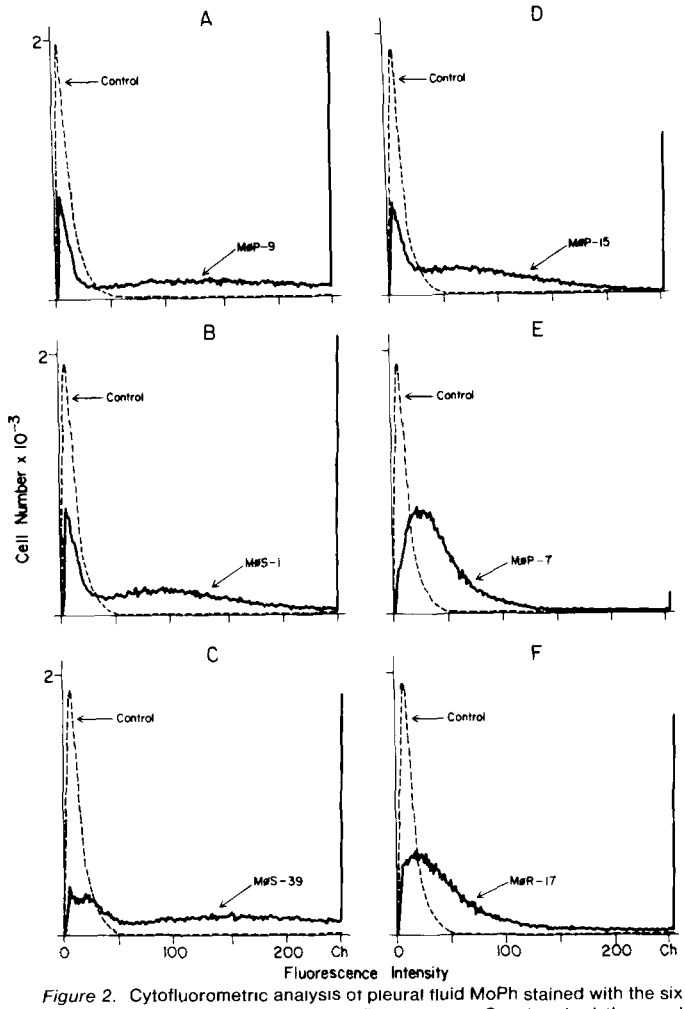

Figure 2. Cytofluorometric analysis of pleural fluid MoPh stained with the six monoclonal reagents in indirect immunofluorescence. One hundred thousand cells were examined at 488 nm, 500 mW, gain of 1.

TABLE III

Independence of various differentiation antigens as demonstrated by competitive blocking experiment: cytofluorometric analysis

Unlabeled Inhibiting Reagents	Intensity of Staining With Reagents Directly Labeled with Fluorescein	
	MoS-1 Fluorescein	MoS-39 Fluorescein
None	80[a]	122
IgG2a myeloma	86	124
MøP-9	82	129
MøP-15	79	115
MøS-1	11	110
MøS-39	87	10
63d3	76	120

[a] Median channel of fluorescence distribution.

TABLE IV

Expression of antigens defined by the monoclonal antibodies on the surface of fluid mononuclear phagocytes[a]

		Monoclonal Antibodies						Control
		MøP-9	MøS-1	MøS-39	MøP-15	MøP-7	MøR-17	
Conventional microscopy	Positive cells (%)[b]	86 (77–90)	85 (80–92)	87 (85–90)	80 (75–90)	79 (75–86)	81 (76–86)	5
	Intensity[c]	3+	3+	3+	2+	1–2+	1+	1
FACS analysis	Positive cells (%)[b]	78 (75–82)	80 (77–85)	85 (79–89)	79 (69–85)	87 (85–90)	86 (72–90)	5
	Intensity[d]	135	108	145	70	37	42	5

[a] Data representative of five experiments.
[b] Median and range.
[c] Fluorescence scored from 1 to 4+.
[d] Median channel of fluorescence.

TABLE V

Comparison of cell surface density of MoPh differentiation antigens on blood monocytes and fluid MoPh: cytofluorometric analysis[a]

	Monoclonal Antibodies					
	MøP-9	MøS-1	MøS-39	MøP-15	MøP-7	MøR-17
Median channel of:						
Blood monocytes	87	86	91	35	15	15
Fluid macrophages	135	108	145	70	37	42
Comparison monocytes/fluid MoPh:						
Number of shifted channels	48	22	54	35	22	27
Ratio of median channels fluid MoPh/blood monocytes	1.5	1.2	1.6	2.0	2.3	2.8

[a] Data representative of five experiments.

TABLE VI

Expression of antigens defined by the monoclonal antibodies on the surface of synovial mononuclear phagocytes[a]

		Monoclonal Antibodies						Control
		MøP-9	MøS-1	MøS-39	MøP-15	MøP-7	MøR-17	
Conventional microscopy	Positive cells (%)[b]	90 (86–92)	95 (88–96)	94 (90–95)	91 (90–94)	88 (85–89)	89 (95–92)	1
	Intensity[c]	3+	3+	3+	3–4+	3–4+	3–4+	1+

[a] Data obtained on six osteoarthritic patients.
[b] Median and range.
[c] Fluorescence scored from 1 to 4+.

Because of the presence of contaminating fibroblastoid synovial cells, the MoPh were identified by latex ingestion. The reagents MϕP-9, MϕS-1, and MϕS-39 stained 90 to 95% of the synovial phagocytic cells with an intensity of 3+, and the reagents MϕP-15, MϕP-7 and MϕR-17 stained 88 to 91 of the phagocytic cells with an intensity of 3 to 4+. Flow cytofluorometry (not illustrated) demonstrated an intense expression of all antigens at levels equal or greater than that found on fluid MoPh.

Staining of the long-term monocytoid cell line U-937 was negative with all reagents by both conventional microscopy and flow cytofluorometry performed at high gain. The promyelocytic leukemia cell line HI-60 gave extremely weak and equivocal staining with the reagents MϕS-1 and MϕS-39. In cytofluorometry, at a gain eight times that used for blood monocytes, staining with the reagent MϕS-39 resulted in an increase of eight channels in the peak fluorescent intensity compared to the control; 17% of the cells were weakly positive. For reagent MϕS-1 under the same conditions, there was a reproducible two to three channel shift in peak fluorescent intensity for 5% of the cells. Analysis of the staining with the monoclonal reagents MϕP-15, MϕP-7, and MϕR-17 did not indicate any shift in the fluorescence compared with the controls. Similarly, the erythroblastoid cell line K562 was not stained with any of these reagents.

Expression of MoPh lineage antigens on lymphocytes, granulocytes, and various cell lines. In B cell-enriched preparations depleted of adherent monocytes and rosette-positive T cells, 5 to 10% of the cells were stained with MϕP-9, MϕS-1, MϕS-39, and MϕP-15 reagents. Double label experiments in which rhodamine-tagged anti-Ig reagents (specific for IgD and IgM) and fluorescence-labeled anti-monocyte lineage monoclonal antibodies were used simultaneously revealed that all cells with monocyte antigens lacked surface IgD and IgM staining. Reciprocally, after incubation with fluorescein-conjugated latex particles, 5 to 10% of cells ingested latex and only these bound the antimonocyte reagents, indicating that the positive cells observed in the B cell-enriched preparation belong to the MoPh lineage.

The reagents MϕP-9, MϕS-1, MϕS-39, and MϕP-15 did not detectably react with different B or T cell lymphoblastoid lines, blood T cell-enriched preparations, as well as mitogen or alloantigen-stimulated T cell blasts by optical or by cytofluorometric techniques at an eightfold increased gain. In contrast, MϕP-7 and MϕR-17 reacted weakly with a small and variable (10 to 30%) percentage of PHA or alloantigen-stimulated T cell-blasts. This reaction ranged from 8 to 24 channels over controls when examined at high gain. Peripheral blood granulocytes staining with MϕP-15, MϕP-7, and MϕR-17 were indistinguishable from the controls; however, from 20 to 36% of granulocytes bound very small amounts of the reagents MϕP-9, MϕS-1, and MϕS-39 with median channel numbers of 7, 18, and 10, respectively. In all instances, these reagents were negative on human red blood cells and platelets. Table VII summarizes the reactivity of the six reagents on various hematopoietic cell populations. In addition, all six monoclonal reagents did not significantly react with any of the tumor cell lines examined and were not detected on fibroblasts.

TABLE VII

Reactivity of the six monoclonal reagents on various hematopoietic cell populations: cytofluorometric data

Cell Population	Monoclonal Antibodies Staining (Percent Positive Cells and Intensity)[a]					
	MøP-9	MøS-1	MøS-39	MøP-15	MøP-7	MøR-17
Monocytes	84 (2+)[b]	83 (2+)	87 (2+)	70 (1+)	37 (1+)	33 (1+)
Macrophages	78 (3+)	80 (3+)	85 (3+)	79 (2+)	87 (1+)	86 (1+)
T cells	0	0	0	0	0	0
B cell line	0	0	0	0	0	0
T cell blasts	0	0	0	0	25 (<)	16 (<)
Granulocytes	25 (<)	20 (<)	36 (<)	0	0	0
Red blood cells	0	0	0	0	0	0
Platelets	0	0	0	0	0	0

[a] Staining intensity expressed in terms of median channel of fluorescence.
[b] (<) less than 20 channels above control; (1+) 20 to 60 channels above control; (2+) 60 to 90 channels above control; (3+) more than 90 channels above control.

DISCUSSION

The analysis of monocyte and macrophage cell surface antigens with six new monoclonal reagents revealed: 1) The cell surface phenotype of human MoPh is characterized by the co-expression of multiple, distinct differentiation antigens. 2) Each antigen defined in this study has a characteristic cell surface expression on various MoPh in terms of percentage of positive cells as well as cell surface density. Certain of these antigens can also be demonstrated, at low densities, on cells outside the MoPh lineage. 3) The maturational transition from blood monocyte to fluid or tissue macrophages is associated

with independent rearrangements in the pattern of expression of each antigen, resulting in a different phenotype. 4) The fact that these antigens are not expressed on early monocytoid or myeloid cells as well as on early undifferentiated myeloid leukemias indicates the appearance of the antigen occurs in more advanced maturational stages of the MoPh lineage (41). 5) The sequential expression of these antigens on blood monocytes defines subsets that relate to successive stages of maturation. The various characteristics defining the antigens described in this study, i.e., their frequency and amount on blood monocytes, the nature of their modulation upon the maturational evolution from monocytes to macrophages, as well as their occurrence on MoPh only or MoPh and other cell lineages, provides a basis for the delineation of three general patterns of antigenic distribution.

The antigens defined by the reagents MφP-9, MφS-1, and MφS-39 shared the characteristics of an intense expression on almost all monocytes and fluid and tissue macrophages. Because cytofluorometric analysis revealed that a fraction of blood granulocytes bind small amounts of these antibodies, the question of whether these antigens actually are expressed on granulocytes or whether this staining is due to Fc receptor interactions of the monoclonal antibodies because of their IgG2 isotype was approached. The blockage of granulocyte Fc receptors with aggregated IgG before the staining with the monoclonal reagents diminished but did not entirely abolish this staining. It cannot be excluded that the antigens MφP-9, MφS-1, and MφS-39 may be present at very low concentrations on a minimal fraction of granulocytes. This finding, although it still requires further study, could be explained by the common origin of the two cell lineages from the same committed progenitor. Previous reports demonstrated the existence of antigens shared by both granulocytes and monocytes, although in general, the quantity of antigens demonstrable on the granulocytes with other reagents have been greater than the amounts observed in this study and suggest that different antigens are involved (39, 40). Despite the overall similarity of the cell distribution of MφP-9, MφS-1, and MφS-39, the observations that: 1) differences in their cytofluorometric profiles were observed on monocytes from some individuals, 2) additive costaining was obtained in simultaneous and sequential additive experiments, 3) no reproducible blocking was demonstrated in competitive blocking experiments, and 4) independent changes in their surface expression was demonstrated in association with the transition from blood monocytes to fluid or tissue macrophages, clearly indicate each antibody in this group binds to a different antigenic determinant. The finding that in one of five experiments antibody MφP-9 gave a moderate inhibition of the binding of antibody MφS-1 raises the possibility that these two antibodies recognize distinct epitopes on distant domains of one molecule or that they are present on two molecules that are in close physical association. The definitive answer to the molecular basis of these antigens will, however, be determined by biochemical studies that are currently in progress. The monoclonal antibody 63D3 described by Ugolini *et al.* (15) that exhibits a similar pattern of reaction was studied in parallel with the reagents of this report. It was also shown to react with an antigen distinct from the antigens MφP-9, MφS-1, and MφS-39 by blocking and additive experiments. Other previously reported monoclonal antibodies reacting with human monocytes, OKM1 (16) and Mo1 (21), have certain similarities to these three reagents but differ in that OKM1 and Mo1 are expressed on the surface of null cells, whereas MφP-9, MφS-1, and MφS-39 were absent from the surface of E rosette-depleted, surface immunoglobulin-negative, nonphagocytic mononuclear cells.

The antigen detected by MφP-15 was expressed in a distinctive pattern. This antigen was well represented on most fluid and tissue MoPh and was found at moderate concentrations on a major but variable fraction of blood monocytes. The restriction of the binding of this IgG1 class antibody to MoPh was confirmed by cytofluorometric analysis. Even with the use of cytofluorometry at high levels of amplification, it was not possible to demonstrate its presence on cell populations outside the MoPh lineage. The complete restriction of antigens such as MφP-15 to the MoPh lineage has been reported for certain other monoclonal reagents—Mo2 (21), Mo3 (24), and MAC120 (18). The distribution of the antigen detected by Mo2 and Mo3 is relatively similar but is not identical to that obtained with MφP-15, and further direct comparisons are necessary to determine if different or the same antigenic determinants are detected. Mo3 shares with MφP-15 an enhanced expression on cultured monocytes. The Mo3 antigen is reported to increase within 24 hr of culture whereas MφP-15 usually requires 3 days of culture to manifest a significant increase in concentration. In the case of MAC120, differences in the average percentage of blood monocytes or tissue macrophages provide evidence that these reagents recognize antigenic determinants different from MφP-15.

The reagents MφP-7 and MφR-17 define antigens distributed in a pattern principally expressed on fluid and tissue macrophages and that is weakly expressed on one-third or less of blood monocytes. Of interest, these antigens are also detectable on a fraction of mitogen or alloantigen-induced T cell blasts, but not on resting T cells or on other hematopoietic or tumor cells. A somewhat similar common expression on T lineage cells was reported for the monoclonal antibodies 4F2 (17, 20) and TA1 (19), with the major differences that the 4F2 determinants are present on numerous lymphoid and nonlymphoid cell lines and the TA1 determinants are found on unstimulated peripheral blood T lymphocytes. The sharing of antigens between monocytes and subsets of activated T lymphocytes, although not explicable in terms of classical developmental concepts, suggests such antigens might facilitate cell-to-cell contact during cooperative interreactions. In contrast to the increase upon culture demonstrated for MφP-15, both MφR-17 and MφP-7 did not alter in percent of positive cells or in intensity of expression upon activated cultures carried out for periods of up to 3 wk.

The question of the criteria used for the precise definition of an MoPh cell arises in several situations. For example, in the "B cell" preparations obtained upon elimination of adherent and E rosette cells, 5 to 10% of the cells bore antigens defined by certain reagents described in this study. The demonstration that virtually all of these positive cells phagocytized latex particles provided the interpretation for this observation. Reciprocally, in the case of purified blood monocytes, whether the small percentage of cells that are negative with the reagents MφP-9, MφS-1, and MφS-39 represent a small subset of monocytes lacking these particular antigens or a contaminating lymphoid population, still remains a question. By using fluorescent microscopy and anti-T cell reagents in double staining, it was possible to identify the occasional lymphoid cells contaminating monocyte-enriched preparations; they were always negative with these three reagents. On the contrary, the use of latex ingestion for the identification of phagocytic cells increases the average proportion of stained cells to 94%. In spite of these efforts, the resolution of whether the remaining nega-

tive cells belong to the MoPh lineage or not awaits further investigations. The recent development of monoclonal antibodies MφU-28 and MφU-48, however, which detect antigens expressed on the immunizing monocytoid cell line U-937 and on a small fraction of blood monocytes (42), provides reagents for the study of antigens found on early maturational stages of the human MoPh lineage. Preliminary data obtained in double-labeled fluorescent experiments suggest the fraction of monocytes stained by the MoU reagents includes the few cells lacking MφP-9, MφS-1, and MφS-39 antigens (unpublished data). This observation supports the possibility that at least some of the small fraction of cells negative with the reagents MφP-9, MφS-1, and MφS-39 represents more primitive monocytes that have not acquired these antigens. Myelomonocytic leukemias have not been found to express detectable amounts of any of the antigens studied in this paper (41, 42).

In conclusion, the detailed analysis of the distribution of the six antigenic determinants delineated in this study supports the hypothesis that the antigens are expressed in patterns associated with different maturational stages of the human MoPh lineage. The group of antigens defined by the reagents MφP-9, MφS-1, and MφS-39 have the broadest distribution including the vast majority of circulating monocytes and essentially all fluid or tissue macrophages. The antigen defined by the MφP-15 reagent appears to recognize a more advanced stage in monocyte maturation because it is found at low to moderate levels on only approximately two-thirds of blood monocytes, but is intensively expressed by virtually all fluid and tissue macrophages. Finally, the group of antigens defined by the reagents MφP-7 and MφR-17 represent markers of a later stage of maturation, demonstrated by their predominant expression on fluid and tissue mononuclear phagocytes with a limited representation on approximately one-third of blood monocytes. Based on this sequential appearance of the six antigens on blood monocytes, three stages of increasingly mature cells can be delineated. The first and presumably least mature one comprising approximately one-third of monocytes is defined by the co-expression of the antigens MφP-9, MφS-1, and MφS-39, and the absence of MφP-15, MφP-7, and MφR-17 antigens. The second stage, accounting for another one-third of monocytes, can be recognized by, in addition to the MφP-9, MφS-1, and MφS-39 antigens, progressively greater densities of MφP-15 antigen. The remaining small fraction of monocytes is characterized by the acquisition of MφR-17 and MφP-7 antigens in addition to the other four antigens; it can be considered to be the most mature subset. The subsequent transition to macrophage is associated with a progressive increase in the cell surface density of all these six antigens, which is most pronounced in the case of the antigens related to more advanced stages of maturation, i.e., MφP-15 and particularly MφP-7 and MφR-17.

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