Analysis of Antigenic Determinants on Human Monocytes and Macrophages

By Robert F. Todd III and Stuart F. Schlossman

Mo1, 2, 3, and 4, and Pt1-1 are a series of five distinct antigens detected on the surface of human peripheral blood monocytes by mouse monoclonal antibodies. Mo2 and 3 are restricted to the monocyte-macrophage series, while Mo1, as previously reported, is also expressed by human granulocytes and null cells. Mo3, as distinguished from Mo1 and Mo2, is weakly expressed by virgin peripheral blood monocytes but becomes well expressed if monocytes are cultured overnight at 37°C. Mo4 is coexpressed by monocytes and platelets, while Pt1-1 appears to be a platelet-specific antigen whose detection on monocytes reflects adherence of platelets to monocyte membranes. That Mo2-4 are true monocyte antigens is demonstrated by their resynthesis following protease treatment of monocytes (Mo1 expression is resistant to proteolytic digestion). During myeloid-monocyte differentiation, the Mo antigens are infrequently expressed by immature myeloid cells but are found at higher frequency on leukemic mononuclear forms. Macrophages from cultured peripheral blood monocytes and HL-60 cells exposed to lymphokines or phorbol diester express Mo1-4, but noncirculating peritoneal macrophages lack Mo3. The Mo antigens are differentiation markers whose expression reflects membrane heterogeneity during myeloid-monocyte-macrophage maturation.

We have recently demonstrated the expression of two distinct membrane antigens Mo1 and Mo2 on human peripheral blood (PB) monocytes. Mo1 is shared by monocytes, null cells, and granulocytes, whereas among circulating cells, Mo2 is uniquely expressed by monocytes. Likewise, other investigators using hybridoma monoclonal technology have discovered the existence of additional membrane markers that distinguish this class of cells. Given the significance of the monocyte-macrophage lineage in the functional integrity of the immune response as well as the existence of clinical disease states arising from abnormalities within the reticuloendothelial system, the characterization of immunologic markers that define members within the macrophage lineage is important.

In this article, we describe our experience with three monoclonal reagents derived from the same fusion that produced anti-Mo1 and anti-Mo2. These antibodies define three antigens that are distinct from Mo1 and Mo2: one antigen, Mo3, undergoes a change in antigen density as a function of time in culture, being weakly expressed on virgin PB monocytes but strongly evident on cultured cells; a second antigen, Mo4, is shared by both PB monocytes and platelets; while a third antigen, Pt1-1, is specific for platelets alone, which may adhere to the monocyte surface during cell isolation. Mo1-4 are borne by macrophages derived from 7-day cultures of monocytes, but only Mo1, 2, and 4 are expressed by peritoneal macrophages. The possible role of these Mo antigens as differentiation markers within the monocyte-macrophage lineage is discussed.

MATERIALS AND METHODS

Production of Monoclonal Antibodies: Immunization and Somatic Cell Hybridization

A 6-wk-old female BALB/c mouse was immunized i.p. with 5 x 10^6 plastic-adherent PB mononuclear cells (PBM). Six months later, the mouse was boosted i.v. with 8 x 10^6 adherent cells from the same donor, and somatic cell hybridization was carried out as described in our earlier report.

Selection and Growth of Hybridomas

After fusion, cells were cultured in hypoxanthine, aminopterin, and thymidine (HAT) medium as described previously. Supernatants from cultures exhibiting cell growth were tested for the presence of antibodies reactive with the large cell fraction of adherent mononuclear cells by indirect immunofluorescence as previously reported. In brief, 10^6 adherent cells were incubated with culture supernatants at 4°C for 30 min, washed once with medium, and stained with a combination of fluorescein-conjugated goat anti-mouse IgM and goat anti-mouse IgG (Meloy Laboratories, Springfield, Va.) for 30 min at 4°C. After a final wash step, fluorescent antibody-coated cells were enumerated on a Cytofluorograf FC200/480A (Ortho Instruments, Westwood, Mass.). Hybridoma cultures containing antibodies reactive with large adherent cells but negative for T lymphocytes and human B-cell lymphoblastoid cell lines were selected and cloned by the limiting dilution method in the presence of feeder cells. Selected clones were subsequently maintained by i.p. injection of 10^6 cells into BALB/c mice primed with pristane. Antibody-containing ascites were used in all subsequent experiments.

Isolation of Human Peripheral Blood Elements

Peripheral Blood Mononuclear Cells

PBM were obtained by venipuncture of volunteer donors or from the leukocyte-rich residues of blood bank platelet bags (plateletpheresis). Venipuncture blood specimens were either heparinized or defibrinated by constant shaking over glass beads for 15 min. PBM
were then isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradient centrifugation.18

Adherent PBM

Monocyte-enriched adherent cells (AC) were obtained by incubating PBM in Eagle's Minimal Essential Medium (Microbiological Associates, Walkersville, Md.) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 1 mM sodium pyruvate, 1% solution of nonessential amino acids (0.1 mM each), 4 mM L-glutamine, and 10% fetal calf serum (FCS) (in selected experiments pooled human AB serum or autologous serum was substituted for FCS) on plastic culture dishes (Falcon Plastics, Oxnard, Calif). In selected experiments, dishes were precoated with either FCS or autologous serum (37°C, 15 min). After adherence for 60 min at 37°C, the nonadherent lymphoid cells were removed and the dishes washed with 4 changes of culture medium. The remaining adherent cells were routinely 60%-80% monocytes as defined by alpha-naphthyl butyrate (nonspecific) esterase (NSE). On some occasions, AC were removed immediately by scraping with a plastic policeman after a 30-min incubation in cold medium containing 5 mM EDTA. Alternatively, the AC were cultured for varying lengths of time in the presence of culture medium at either 37° or 4°C. The culture vessel consisted of either the original plastic culture dish or a 50-mL polypropylene tube (Falcon Plastics). For culture times exceeding 16 hr, fresh medium was added on alternate days. After prolonged culture, dead cells were sedimented by Ficoll-Hypaque density gradient centrifugation.

Monocyte-enriched PBM were also isolated by density gradient centrifugation of washed Ficoll-Hypaque interface cells on a single-step Percoll (Pharmacia Fine Chemicals) gradient (50% Percoll in PBS, 1.066 g/cc; 1100 g, 15 min, 25°C). The lower density cells resting at the Percoll interface were highly enriched for monocytes, being >75% positive for NSE.

Granulocytes

Granulocytes were isolated from the pellet formed during Ficoll-Hypaque density gradient centrifugation of peripheral blood. Contaminating erythrocytes were excluded by gravity (1 g) sedimentation in the presence of 0.4% dextran according to the technique of Levy et al.22

Platelets

Platelets were obtained by differential centrifugation of platelet-erythrocyte residues. Aliquots of platelets were either used fresh or fixed in paraformaldehyde 1% in PBS.

T Cells

Nonadherent PBM were enriched for T lymphocytes by rosetting with sheep erythrocytes.1 Populations of activated T lymphoblasts were obtained by culturing nonadherent PBM for varying lengths of time (3-8 days) either in the presence of stimulatory concentrations of phytohemagglutinin (PHA) or with equal numbers of allogeneic nonadherent PBM [two-way mixed lymphocyte reaction (MLR)]. T lymphoblasts were examined selectively by focusing on the large cell peak on the cytofluorograf scatter pattern.

B Cells

Nonadherent PBM were enriched for surface immunoglobulin positive (slg+) cells by Sephadex G-200 anti-IgG, chromatography as previously described. In selected experiments, contaminating null cells and monocytes were depleted by complement-mediated lysis using anti-Mol.

Null Cells

E-rosette-negative nonadherent PBM that failed to bind to the anti-F(ab')2, column (E. slg-) were operationally defined as null cells. In selected experiments, contaminating T and B cells were excluded by complement-mediated lysis using anti-T3 and I-2 (la) monoclonal antibodies.

Purity of these populations was ascertained by the percentage of cells binding monoclonal antibodies specific for T (T3), B (Bl26 and I-2), monocytes (Mo2), null cells (Mo1), and granulocytes (Mo1).

Human Cell Lines

Epstein-Barr virus transformed B lymphoblastoid lines (Laz 156 and 388), T-cell lymphoblastoid lines (MOLT-4, CCRF-CEM, and HPB-ALL), CALLA-positive lymphoblastoid lines (NALM-1 and Laz 221), histiocytic lymphoma line U937, promyelocytic line HL-60, and erythrophagocytic line K562 were kindly provided by Dr. Herb Lazarus, Sidney Farber Cancer Institute. Myeloblastic leukemia cell line KG-1 was provided by Dr. David Golde, UCLA School of Medicine.

Human Leukemia Cells

After Human Subjects Committee approval, tumor cells were obtained from peripheral blood or bone marrow of 42 patients with acute leukemia. In all cases, the tumor populations used for antibody binding contained >75% blasts/tumor cells by Wright-Giemsa morphology. Binding of monoclonal antibody J-S27 served as a marker for acute lymphocytic leukemia (ALL) cells. Cells from patients with acute myelocytic leukemia were subclassified into acute myeloblastic (AML), acute monocytic (AMoL), and myelomonocytic (AMML) variants on the basis of morphology and histochemical staining.

Peritoneal Macrophages

Peritoneal macrophages were obtained from dialysis fluid of patients undergoing chronic peritoneal dialysis (kindly provided by Dr. Michael Lazarus, Brigham and Women's Hospital, Boston, Mass.); the interface cells isolated after Ficoll-Hypaque density gradient centrifugation were >90% positive for NSE.

Immunofluorescence Studies

Analysis of monoclonal antibody binding with normal and malignant cells was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (7S) and IgM as described above. Wash medium contained deoxyribonuclease (Millipore Corp., Freehold, N.J.) 150 U/mg and human gammaglobulin (Fraction II, Miles Laboratories, Elkhart, Ind.) 1 mg/ml to prevent cell clumping from dead cells and to block nonspecific Fc receptor binding, respectively. Antigen expression is represented by the percentage of 10,000 cells demonstrating fluorescence (weak or strong) above that of cells exposed to a control antibody nonreactive with normal PB elements.

Quantitative Absorptions

To compare antigen densities on monocytes before or after overnight culture, 100 μl aliquots of antibody-containing ascites (at an appropriate limiting dilution) were absorbed with an equal volume containing graded numbers of Percoll-gradient isolated monocytes (0, 3, 1, 6.3, 12.5, 25.0, or 50.0 x 106 cells). After 2 hr at 4°C, the absorbed antibodies were collected after centrifugation and stored at -80°C until use. To assay the relative amounts of residual antibodies, 150 μl of each absorbed ascites was incubated with test
monocytes known to express the antigens in question. After indirect immunofluorescence labeling, the extent of absorption was calculated using the following formula:

\[
\text{Percent absorption} = 100\% \times \left(1 - \frac{\text{Percent of test cells positive with absorbed antibody}}{\text{Percent of test cells positive with unabsorbed antibody}}\right)
\]

Enzymatic Treatment of Monocytes

A quantity of 60-100 x 10^6 adherent cells were suspended in varying concentrations of trypsin (lyophilized trypsin 217 U/mg, Millipore Corp.), papain (25 U/mg, Millipore Corp.), or neuraminidase (Vibrio cholerae, dase) at a final substrate concentration of 7.5-10.0 x 10^6 cells/ml and incubated for 30 min at 37°C. All cell suspensions contained deoxyribonuclease (Millipore Corp.) 100 μg/ml to prevent clumping by released DNA from dead cells. Papain was activated by the addition of l-cysteine to a final concentration of 5 mM. At the end of enzyme treatment, the proteolytic reactions were stopped by the addition of ice-cold FCS (plus a twofold excess of ovomucoid trypsin inhibitor (Millipore Corp.) in reactions containing trypsin). Treated cells (always >95% viable) were washed 3-4 times in serum containing medium prior to analysis or overnight culture. Sham-treated cells were subjected to identical conditions with the exclusion of enzyme.

Culture of Monocytes in Puromycin

In selected experiments, monocytes were cultured for 16 hr in medium containing puromycin dihydrochloride (crystalline, Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 2.5-5 μg/ml. At this concentration range, cell viability was preserved, but incorporation of ^35S-methionine into TCA-preipitable proteins was inhibited (>80%).

HL-60 Differentiation Induced by Conditioned Medium or Phorbol Diester

HL-60 promyelocytic cells were cultured at 37°C for 96 hr in the presence of 10% PHA-leukocyte-conditioned medium (prepared according to the method of Ave38 and kindly supplied by Dr. James Griffin, Sidney Farber Cancer Institute) or 1-2 x 10^-5 M 12-0-tetradecanoyl phorbol-13-acetate (TPA)(Consolidated Midland Corp., Brewster, N.Y.) (diluted in acetone at 1 mM). At the end of this incubation, adherent cells were resuspended by scraping with a plastic policeman in a medium containing 5 mM EDTA; dead cells were sedimented by Ficoll-Hypaque density gradient centrifugation. Under these conditions a majority of the promyelocytes transform into cells bearing the morphological and histochemical features of macrophages.39,40

Determination of Subclass of Monoclonal Antibodies

Immunoglobulin subclass was determined by Ouchterlony immunodiffusion in 1% Agar. Antibody-containing ascites diluted 1/100 in PBS was placed in a center well that was surrounded by wells containing undiluted rabbit anti-mouse gamma-A, G1, G2a, G2b, and M (Litton Bionetics, Inc., Kensington, Md.). After overnight incubation at 37°C, the agar dishes were examined for precipitin lines. Ascites, produced by a nonsecreting hybridoma served as control.

RESULTS

Development and Initial Characterization of Monoclonal Antibodies Directed Against Monocyte Surface Antigens

As described in our previous report,1 a BALB/c mouse was immunized with human peripheral blood (PB) adherent cells (overnight adherence), and its splenocytes fused with NSI myeloma cells to form antibody-producing hybridomas. In addition to clones 94 (anti-Mo1) and 116 (anti-Mo2), 3 other hybridomas made antibodies that bound to PB adherent cells but failed to show significant reactivity for sheep erythrocyte rosette-positive (E+) cells (T-cell-enriched) and B-cell lymphoblastoid cell lines. Passage of clones 17, 22, and 69 in BALB/c mice resulted in ascites containing high titers of IgM antibodies (as characterized by Ouchterlony immunodiffusion using specific rabbit anti-mouse immunoglobulin reagents), which were used in all subsequent experiments. The antibodies produced by these clones were designated anti-Mo3 (clone 17), anti-Mo4 (clone 22), and anti-Pit-1 (clone 69).

Expression of Monocyte Antigens on Normal Peripheral Blood Elements and Human Cultured cell Lines

Table 1 summarizes the reactivity (by indirect immunofluorescence) of the 3 monoclonal reagents for human PB elements. All 3 demonstrated a variable degree of binding to PB adherent cells (large cell scatter) but showed little or no significant reactivity for populations enriched for T, B, and null cells, and erythrocytes. Anti-Mo4 and anti-Pit-1 occasionally showed weak activity on granulocytes, but this was not a consistent finding. T3-positive lymphoblasts that had undergone in vitro activation in response to either PHA and MLC stimuli were uniformly negative when tested with these antibodies. To our surprise, platelets strongly and uniformly expressed the antigens defined by anti-Mo4 and anti-Pit-1.

Except for moderate reactivity for histiocytic lymphoma cell line U937 by anti-Mo3, cultured lines of B, T, CALLA+ lymphoblastic, erythrocytic, and myeloid origin were largely negative (data not shown).

Variability of Antigen Expression on Adherent Cells and Macrophages

A source of considerable frustration and confusion was the wide range of antigen expression on adherent cells from different individuals. This was most apparent for anti-Mo3 whose reactivity ranged from <10%...
reactivity was weak or discovered that for anti-Mo3, or autologous serum) after a 60-mm incubation. It was collected from dishes (either plain or coated with FCS culture dishes; more recently, adherent cells have depended on how the adherent cells were isolated. antigen expression within our donor population, it depended on how the adherent cells were isolated.

However, rather than a polymorphism of antigen expression within our donor population, it depended on how the adherent cells were isolated.

When these studies were initiated, PB adherent cells were isolated after overnight incubation on plastic dishes after 60 min were subsequently cultured in "nonadherable" polypropylene tubes. Similarly, the same phenomenon was observed if monocytes were isolated by Percoll density sedimentation and then cultured either at 4°C or 37°C in polypropylene tubes (Fig. 1). In Fig. 1, this is seen as a dramatic shift of the fluorescence curves from weak or background staining to more intense fluorescence. Quantitative absorptions of anti-Mo3 using graded numbers of either virgin monocytes or cells cultured for 16 hr at 37°C confirm the relative differences in antigen density occurring during culture as compared to the minimal change in the density of Mo2 (Table 3). When the protein synthesis inhibitor puromycin was added during culture at 37°C (at concentrations known to block the incorporation of 35S-methionine into TCA-precipitable proteins), expression of Mo3 was partially blocked (Fig. 2 and Table 4). These observations suggest that to >80%. However, rather than a polymorphism of antigen expression within our donor population, it became apparent that the degree of antigen expression depended on how the adherent cells were isolated.

When these studies were initiated, PB adherent cells were isolated after overnight incubation on plastic culture dishes; more recently, adherent cells have been collected from dishes (either plain or coated with FCS or autologous serum) after a 60-min incubation. It was discovered that for anti-Mo3, reactivity was weak or absent for cells harvested after 60 min [Table 2, AC(t,)].

Likewise, adherent cells kept under similar culture conditions but at 4°C demonstrated weak antigen expression relative to cells incubated at 37°C [Table 2, AC (37, t,)]. This culture-dependent augmentation of antigen density also occurred if adherent cells were cultured in up to 50% autologous serum, or if adherent cells, harvested from plastic dishes after 60 min were subsequently cultured in "nonadherable" polypropylene tubes. Similarly, the same phenomenon was observed if monocytes were isolated by Percoll density sedimentation and then cultured either at 4°C or 37°C in polypropylene tubes (Fig. 1). In Fig. 1, this is seen as a dramatic shift of the fluorescence curves from weak or background staining to more intense fluorescence. Quantitative absorptions of anti-Mo3 using graded numbers of either virgin monocytes or cells cultured for 16 hr at 37°C confirm the relative differences in antigen density occurring during culture as compared to the minimal change in the density of Mo2 (Table 3). When the protein synthesis inhibitor puromycin was added during culture at 37°C (at concentrations known to block the incorporation of 35S-methionine into TCA-precipitable proteins), expression of Mo3 was partially blocked (Fig. 2 and Table 4). These observations suggest that

Table 1. Antigen Expression on Peripheral Blood Elements by Indirect Immunofluorescence

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AC(t,)</th>
<th>E(\times)</th>
<th>E(\times)</th>
<th>E(\times)</th>
<th>E(\times)</th>
<th>PMN(\times)</th>
<th>PLT(\times)</th>
<th>RBC(\times)</th>
<th>T(\times)</th>
</tr>
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<tbody>
<tr>
<td>Mo3</td>
<td>50 ± 36(\times)</td>
<td>1 ± 1</td>
<td>3 ± 3</td>
<td>2 ± 1</td>
<td>6 ± 8</td>
<td>1 ± 1</td>
<td>0</td>
<td>6 ± 5</td>
<td></td>
</tr>
<tr>
<td>Mo4</td>
<td>70 ± 14</td>
<td>2 ± 1</td>
<td>10 ± 3</td>
<td>8 ± 4</td>
<td>16 ± 15</td>
<td>92 ± 1</td>
<td>0</td>
<td>5 ± 1</td>
<td></td>
</tr>
<tr>
<td>Pht-1</td>
<td>38 ± 15</td>
<td>2 ± 1</td>
<td>6 ± 5</td>
<td>8 ± 3</td>
<td>13 ± 14</td>
<td>93 ± 2</td>
<td>0</td>
<td>3 ± 3</td>
<td></td>
</tr>
<tr>
<td>Mo1</td>
<td>79 ± 9</td>
<td>8 ± 9</td>
<td>11 ± 10</td>
<td>48 ± 23</td>
<td>84 ± 14</td>
<td>1 ± 2</td>
<td>0</td>
<td>8 ± 1</td>
<td></td>
</tr>
<tr>
<td>Mo2</td>
<td>69 ± 10</td>
<td>1 ± 1</td>
<td>5 ± 4</td>
<td>1 ± 1</td>
<td>3 ± 2</td>
<td>2 ± 2</td>
<td>0</td>
<td>1 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Monocyte-enriched adherent cells (large cell fraction); 60%–80% NSE positive; 14 determinations.
‡T-cell-enriched, E-rosette + cells; 3 determinations.
§B-cell-enriched, E-rosette + surface Ig + cells; 3 determinations.
¶Null-cell-enriched, E rosette − cells; 3 determinations.
‖Polymorphonuclear leukocytes (granulocytes); 5 determinations.
*Platelets (fixed in 1% PFA); 4 determinations.
††Erythrocytes.
§§Mean percent ± SD. Cell fractions demonstrating significant reproducible positivity are underlined for emphasis.

Table 2. Antigen Expression on Virgin or Cultured Monocytes and Peritoneal Macrophages

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AC(4, t,)</th>
<th>AC(37, t,)</th>
<th>AC(4, t,)</th>
<th>M0(4, t,)</th>
<th>Peritoneal M0†</th>
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<tbody>
<tr>
<td>Mo3</td>
<td>17 ± 15*</td>
<td>78 ± 9</td>
<td>9 ± 8</td>
<td>77 ± 11</td>
<td>4 ± 5</td>
</tr>
<tr>
<td>Mo4</td>
<td>75 ± 9</td>
<td>59 ± 20</td>
<td>56 ± 19</td>
<td>77 ± 10</td>
<td>44 ± 8</td>
</tr>
<tr>
<td>Pht-1</td>
<td>41 ± 13</td>
<td>35 ± 19</td>
<td>14 ± 16</td>
<td>18 ± 6</td>
<td>3</td>
</tr>
<tr>
<td>Mo1</td>
<td>78 ± 12</td>
<td>76 ± 10</td>
<td>77 ± 15</td>
<td>33 ± 8</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>Mo2</td>
<td>68 ± 12</td>
<td>70 ± 11</td>
<td>69 ± 15</td>
<td>80 ± 5</td>
<td>65 ± 8</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Large adherent cells examined after 60 min adherence; 7 determinations.
‡Large adherent cells examined after culture overnight at 37°C; 23 determinations.
§Large adherent cells examined after culture overnight at 4°C; 5 determinations.
¶Monocyte-enriched cells isolated by adherence or Percoll-gradient centrifugation and then cultured for 6–8 days at 37°C; 5 determinations.
†Peritoneal macrophages isolated from dialysis fluid; 2 determinations.
**Mean percent ± SD.
the apparent increase in antigen density is (1) dependent on metabolic activity by the cultured monocytes (inhibited by incubation at 4°C or with puromycin); (2) is probably not due to an uncovering of sequestered antigenic sites blocked by autologous serum proteins; and (3) occurs independently of monocyte adherence.

After prolonged culture of 6–8 days, during which time monocytes develop the morphological and biochemical characteristics of macrophages, the density of Mo3 remains high (Table 2; Fig. 3, D–F). It will be noted (Table 2) that Mo1 and Mo2 are well expressed by both virgin and overnight cultured monocytes; by 6–8 days of culture, Mo1 but not Mo2 is diminished.

The expression of antigen Plt-1 is relatively weak with considerable variability among adherent cells isolated and maintained under identical conditions (Table 2). After prolonged culture, relatively insignificant expression is a uniform observation (Table 2, \( \text{MO} (t_{6.8}) \)). Antigen Mo4 also shows some variability in expression (Table 2) but without significant change after culture either overnight or for up to 8 days.

These results with virgin and cultured macrophages were then compared with the degree of antigen expression evident on peritoneal macrophages isolated from the dialysis fluid of patients with end-stage renal disease undergoing peritoneal dialysis. These cells expressed only antigens Mo1, Mo2, Mo4, and I-2 (Ia)(Table 2; Fig. 3, A–C) (peritoneal cells cultured overnight did not express Mo3). Identical results were seen when peritoneal macrophages from patients undergoing intraperitoneal therapy for ovarian carcinoma were examined (Bast and Todd, unpublished).
Fluorescence Intensity

**Fig. 2. Effect of puromycin on expression of Mo3 by overnight cultured adherent cells.** Aliquots containing $36 \times 10^6$ adherent cells ($2 \times 10^6$ cells/ml in culture medium) were incubated overnight at $4^\circ$C (A and D), $37^\circ$C (B and E), or $37^\circ$C in medium containing puromycin $2.5 \mu $g/ml (C and F). The cultured cells were then assayed for antigen expression by indirect immunofluorescence (dashed line represents the background fluorescence produced by cells exposed to control antibody nonreactive for normal PB elements).

**Anti-platelet Reactivity of Anti-Mo4 and Anti-Plt-1**

We were surprised to find that in addition to a variable degree of binding to adherent cells, anti-Mo4 and anti-Plt-1 were strongly reactive for human platelets. It has been the observation of other investigators (Perussia et al., personal communication) that platelets may adhere to monocytes isolated from heparinized human peripheral blood (the method employed to isolate adherent cells used for immunization). This “contamination” can be minimized by defibrinating the blood prior to Ficoll-Hypaque sedimentation (Perussia, personal communication). To determine if the variable degree of binding by anti-Mo4 and anti-Plt-1 to monocytes was due to reactivity for absorbed platelets, a direct comparison was made between antibody binding to adherent cells isolated from heparinized or defibrinated blood. Table 5 shows that anti-Plt-1 displays insignificant reactivity toward defibrinated monocytes relative to heparinized monocytes from the same individual; reactivity of anti-Mo4, on the other hand, demonstrated binding irrespective of the method of isolation. These observations suggest that anti-Plt-1 is in fact platelet-specific, whereas Mo4 is expressed by both monocytes and platelets. The possibility that anti-Mo4 contains two antibodies, one specific for monocytes and the other for platelets, was excluded by

**Table 4. Inhibition of Mo3 Expression by Culture in Puromycin**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Percent Positive Cells*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AC (37,t,)†</td>
</tr>
<tr>
<td>Mo3</td>
<td>$78 \pm 8$‖</td>
</tr>
<tr>
<td>Mo1</td>
<td>$79 \pm 11$</td>
</tr>
<tr>
<td>Mo2</td>
<td>$76 \pm 4$</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Large adherent cells cultured overnight at $37^\circ$C.
‡Large adherent cells cultured overnight at $4^\circ$C.
§Large adherent cells cultured overnight at $37^\circ$C in medium containing puromycin 2.5–5.0 $\mu $g/ml.
‖Mean percent ± SD (3 separate experiments).
the fact that absorption of anti-Mo4 with platelets removed all activity for monocytes (data not shown).

Regeneration of Monocyte Antigens After Proteolytic Cleavage

Since our results suggested that PIt-1 is not a true monocyte antigen, it was important to demonstrate that Mo3 and 4 are in fact structures intrinsic to the monocyte. We had previously observed that the expression of Mo2 is lost after treatment of monocytes with trypsin but is regenerated after overnight culture, indicating that Mo2 is synthesized by the monocyte.*

With this rationale in mind, we determined the effect of enzymatic treatment of monocytes on the expression

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Percent Positive Cells*</th>
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<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td></td>
<td>HEP§</td>
</tr>
<tr>
<td>PIt-1</td>
<td>45</td>
</tr>
<tr>
<td>Mo4</td>
<td>78</td>
</tr>
<tr>
<td>Mo2</td>
<td>84</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Large adherent cells examined immediately after 60 min adherence.
‡Large adherent cells examined after overnight culture at 37°C.
§Cells isolated from heparin-anticoagulated blood.
¶Cells isolated from defibrinated blood.
of Mo3 and 4. Table 6 illustrates the result of treatment of overnight cultured monocytes with proteolytic enzymes trypsin and papain. It can be seen that the expression of all antigens disappeared after proteolysis with the exception of Mol and framework Ia. This sensitivity was evident using enzyme concentrations down to 10 μg/ml (data not shown). Treatment with neuraminidase had no effect on the degree of antigen expression.

To determine whether the expression of these protease-sensitive antigens would recover after culture in enzyme-free medium, trypsin (or sham) treated cells were incubated for 16 hr at 4° or 37°C and then reassayed for antigen expression. Table 7 indicates the results of 3 such experiments (one of which illustrated in Fig. 4) which do in fact demonstrate full antigen recovery after overnight culture. That minimal reexpression occurs when cells were cultured in the presence of puromycin suggests that recovery depends on protein synthesis (as opposed to reexpression of preformed antigen).

Expression of Monocyte Antigens on Tumor Cells From Patients With Acute Leukemia

In our previous report1 we demonstrated that the degree of expression of Mo1 and Mo2 by tumor cells of patients with acute myeloid leukemia was associated with the coexistence of monocytic characteristics (e.g., the presence of alpha-naphthyl butyrate esterase). In Table 8 a comparison is made between the expression of Mol and Mo2 and that of Mo3 and 4 on blast forms from patients with acute myeloblastic, monocytic, and lymphoblastic leukemia. It will be noted that Mo3 and 4 also tend to be evident on cells with monocytic maturation, but that the frequency of expression is less than that shown by Mo1 and Mo2. None of the antigens is borne by lymphoblastic cells indicating their specificity for the myeloid lineage.

Expression of Monocyte Antigens on HL-60 Promyelocytic Cells Induced Toward Macrophage Differentiation

Earlier studies have shown that HL-60 promyelocytic leukemia cells, which acquire macrophage characteristics as a result of in vitro differentiation stimulated by leukocyte-conditioned medium or by phorbol diester, also acquire the expression of Mol and Mo2.22 We have therefore examined these differentiated cells for the acquisition of the other monocyte markers. Table 9 demonstrates the augmented expression of antigen Mo3 by both PHA-LCM and TPA-induced cells. Some degree of binding by anti-Mo4 is also apparent when HL-60 is induced by PHA-LCM but not by TPA (as is the case with Mo2). As expected, P1t-1 is not expressed by differentiated HL-60 cells.

### Table 6. Antigen Sensitivity to Treatment With Trypsin or Papain

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Control</th>
<th>Trypsin</th>
<th>Papain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3</td>
<td>75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mo4</td>
<td>33</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>P1t-1</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mol</td>
<td>72</td>
<td>82</td>
<td>76</td>
</tr>
<tr>
<td>Mo2</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-2</td>
<td>67</td>
<td>61</td>
<td>77</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive with PB elements.
†Sham-treated cells.
‡Trypsin 1000 μg/5 × 10⁶ cells/ml, 37°C, 30 min.
§Papain 1000 μg/5 × 10⁶ cells/ml, 37°C, 30 min.

### Table 7. Recovery of Antigen Expression After Trypsin Treatment: Inhibition by Puromycin

<table>
<thead>
<tr>
<th>Antigen</th>
<th>C 37†</th>
<th>T 4‡</th>
<th>T 37§</th>
<th>T 37 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3</td>
<td>76 ± 5</td>
<td>0 ± 0</td>
<td>71 ± 13</td>
<td>13 ± 11</td>
</tr>
<tr>
<td>Mo4</td>
<td>49 ± 12</td>
<td>0 ± 2</td>
<td>31 ± 11</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>P1t-1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Mol</td>
<td>50 ± 3</td>
<td>79 ± 5</td>
<td>61 ± 14</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Mo2</td>
<td>70 ± 4</td>
<td>1 ± 1</td>
<td>66 ± 8</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>1-2</td>
<td>54 ± 20</td>
<td>74 ± 2</td>
<td>57 ± 23</td>
<td>81 ± 6</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Sham-treated large adherent cells cultured overnight at 37°C.
‡Trypsinized (1000 μg/5 × 10⁶ cells/ml; 37°C; 30 min) large adherent cells cultured overnight at 4°C.
§Trypsinized large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 37°C in medium containing puromycin 2.5–4 μg/ml.
†Mean percent ± SD of 3 experiments.

### Table 8. Expression of Monocyte Antigens on HL-60 Promyelocytic Cells Induced Toward Macrophage Differentiation

<table>
<thead>
<tr>
<th>Antigen</th>
<th>C 37†</th>
<th>T 4‡</th>
<th>T 37§</th>
<th>T 37 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3</td>
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<td>71 ± 13</td>
<td>13 ± 11</td>
</tr>
<tr>
<td>Mo4</td>
<td>49 ± 12</td>
<td>0 ± 2</td>
<td>31 ± 11</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>P1t-1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
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<td>50 ± 3</td>
<td>79 ± 5</td>
<td>61 ± 14</td>
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<tr>
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</tr>
<tr>
<td>1-2</td>
<td>54 ± 20</td>
<td>74 ± 2</td>
<td>57 ± 23</td>
<td>81 ± 6</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive with PB elements.
†Sham-treated large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 4°C.
§Trypsinized large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 37°C in medium containing puromycin 2.5–4 μg/ml.
†Mean percent ± SD of 3 experiments.

### Table 9. Expression of Monocyte Antigens on HL-60 Promyelocytic Cells Induced Toward Macrophage Differentiation

<table>
<thead>
<tr>
<th>Antigen</th>
<th>C 37†</th>
<th>T 4‡</th>
<th>T 37§</th>
<th>T 37 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0 ± 0</td>
<td>71 ± 13</td>
<td>13 ± 11</td>
</tr>
<tr>
<td>Mo4</td>
<td>49 ± 12</td>
<td>0 ± 2</td>
<td>31 ± 11</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>P1t-1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Mol</td>
<td>50 ± 3</td>
<td>79 ± 5</td>
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</tr>
<tr>
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</tr>
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<td>57 ± 23</td>
<td>81 ± 6</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive with PB elements.
†Sham-treated large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 4°C.
§Trypsinized large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 37°C in medium containing puromycin 2.5–4 μg/ml.
†Mean percent ± SD of 3 experiments.

### Table 10. Expression of Monocyte Antigens on HL-60 Promyelocytic Cells Induced Toward Macrophage Differentiation

<table>
<thead>
<tr>
<th>Antigen</th>
<th>C 37†</th>
<th>T 4‡</th>
<th>T 37§</th>
<th>T 37 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3</td>
<td>76 ± 5</td>
<td>0 ± 0</td>
<td>71 ± 13</td>
<td>13 ± 11</td>
</tr>
<tr>
<td>Mo4</td>
<td>49 ± 12</td>
<td>0 ± 2</td>
<td>31 ± 11</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>P1t-1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Mol</td>
<td>50 ± 3</td>
<td>79 ± 5</td>
<td>61 ± 14</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Mo2</td>
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<td>1 ± 1</td>
<td>66 ± 8</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>1-2</td>
<td>54 ± 20</td>
<td>74 ± 2</td>
<td>57 ± 23</td>
<td>81 ± 6</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive with PB elements.
†Sham-treated large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 4°C.
§Trypsinized large adherent cells cultured overnight at 37°C.
‡Trypsinized large adherent cells cultured overnight at 37°C in medium containing puromycin 2.5–4 μg/ml.
†Mean percent ± SD of 3 experiments.

### Discussion

In an earlier report, we described the identification of two antigens, Mo1 and Mo2, which are expressed on the surface of human monocytes.1 As part of an ongoing study of the surface characteristics of human monocytes and macrophages as these features relate to differentiation and function, we have detected an additional three antigens that are distinct from Mo1 and Mo2 and which display certain unique characteristics (see Table 10 for summary).

One IgM monoclonal antibody (anti-Mo3) defines an antigen that is weakly expressed by virgin PB monocytes but becomes strongly detectable if the cells are cultured for 16 hr at 37°C. This change in antigen expression is temperature dependent, failing to occur
Fig. 4. Loss and recovery of antigen expression after trypsin treatment. Aliquots containing 50 × 10⁶ adherent cells were either sham-treated (A and E) or exposed to trypsin (1000 µg trypsin/7.5 × 10⁶ cells/ml; 37°C; 30 min), washed in ovomucoid trypsin-inhibitor, and cultured overnight at 4°C (B and F) or 37°C (C and G). One trypsin-treated aliquot was cultured overnight at 37°C in medium containing puromycin 4 µg/ml (D and H). Following culture, the cells were assayed for antigen expression by indirect immunofluorescence (dashed line represents the background fluorescence produced by cells exposed to control antibody nonreactive for normal PB elements).

Table 8. Expression of Antigens on Myeloid and Lymphoid Leukemia Cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML (n = 17)</td>
</tr>
<tr>
<td>Mo3</td>
<td>4(25)</td>
</tr>
<tr>
<td>Mo4</td>
<td>15(95)</td>
</tr>
<tr>
<td>Mo1</td>
<td>3(18)</td>
</tr>
<tr>
<td>Mo2</td>
<td>11(69)</td>
</tr>
</tbody>
</table>

*Number of patients whose leukemic cells are positive (>20% of leukemic cells expressing antigens by indirect immunofluorescence). Number in parentheses is percent patients positive.

Table 9. Expression of Antigens on HL-60 Cells Induced Toward Macrophage Differentiation by PHA-LCM and Phorbol Diester

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Control†</th>
<th>PHA-LCM‡</th>
<th>TPA§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3</td>
<td>5 ± 2</td>
<td>67 ± 4</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>Mo4</td>
<td>3 ± 4</td>
<td>8 ± 11</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Mo1</td>
<td>14 ± 6</td>
<td>40 ± 12</td>
<td>18 ± 21</td>
</tr>
<tr>
<td>Mo2</td>
<td>11 ± 4</td>
<td>48 ± 22</td>
<td>28 ± 12</td>
</tr>
</tbody>
</table>

*Percentage of 10,000 cells demonstrating fluorescence above that of cells exposed to control antibody nonreactive for PB elements.
†Sham-treated cells; 4 determinations.
‡HL-60 cultured in 10% PHA-LCM for 96 hr; 2 determinations.
§HL-60 cultured in 1-2 × 10⁸ M TPA for 96 hr; 2 determinations. Mean percent ± SD.

Table 10. Summary of Mol-4 and Pit-1 Expression

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Virgin Monocyte</th>
<th>Cultured† Monocyte</th>
<th>Peritoneal Macrophage</th>
<th>PMN</th>
<th>Null Cell</th>
<th>Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo1</td>
<td>++ + + + + + +</td>
<td>++ + + + + + + +</td>
<td>++ + + + + + + + +</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Mo2</td>
<td>++ + + + + +</td>
<td>++ + + + + + + +</td>
<td>++ + + + + + + + +</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Mo3</td>
<td>++ + + + + +</td>
<td>++ + + + + + + +</td>
<td>++ + + + + + + + +</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Mo4</td>
<td>++ + + + + + +</td>
<td>++ + + + + + + +</td>
<td>++ + + + + + + + +</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>Pit-1</td>
<td>++ + + + + +</td>
<td>++ + + + + + + +</td>
<td>++ + + + + + + + +</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
</tbody>
</table>

*Cells cultured from 1 to 8 days.
†Degree of antigen expression ranging from (−−−−) few or no cells expressing antigen to (++++) most cells expressing antigen.
‡Assuming monocytes derived from defibrinated blood.
at 4°C, and is partially blocked at 37°C in medium containing puromycin. These observations suggest that the full expression of Mo3 requires metabolic activity by the monocyte. Substrate adherence per se does not contribute to the change in antigen expression (unlike an antigen characterized by Hogg et al.4) since monocytes isolated by Percoll density gradient centrifugation and then cultured on “nonadherable” polystyrene tubes undergo the same phenomenon. While it is technically difficult to completely exclude the possibility that this change in expression is the result of an elution of extrinsic material adsorbed to the monocyte surface (e.g., serum proteins or platelets), culture in the presence of high concentrations of autologous serum does not prevent the increase in Mo3, and uncultured monocytes isolated from defibrinated blood show no enhanced expression of this antigen. It is intriguing to speculate that the full expression of Mo3 may represent an early event in the maturational process during which monocytes transform into macrophages.31

Mo4 is a unique antigen whose major distinguishing feature is its expression by both monocytes and platelets. Absorptions with platelets removes monocyte reactivity, excluding the possibility of two distinct antibodies detecting separate antigens. The expression of Mo4 on PB monocytes from different individuals is more variable than Mo1 and Mo2 (ranging from 40% to 80% of cells positive), but, unlike Mo3, this variation is unrelated to the technique of isolation or culture conditions.

One additional IgM antibody binds to an antigen, Plt-1, which is probably restricted to platelets alone. The unexpected discovery of anti-platelet antibodies arising from an immunization with adherent cells is apparently the result of platelets adsorbed to monocyte surfaces as has been previously noted (Perussia, personal communication). The adsorption of platelets is reduced when blood specimens are defibrinated prior to cell fractionation and monocytes isolated from defibrinated blood failed to bind significant amounts of anti-Plt-1. Long-term cultured monocytes, peritoneal macrophages, and macrophages derived from in vitro HL-60 differentiation likewise do not express Plt-1, further indicating that this antigen is not an intrinsic macrophage marker.

That Mo3 and 4 are in fact true monocyte membrane antigens is suggested by the regeneration of these structures following protease treatment of intact monocytes. As in the case of Mo2, exposure to even small concentrations of trypsin and papain completely removed the antigenic activity of Mo3 and Mo4 (neuraminidase had no effect on antigen expression). More importantly, monocytes stripped of Mo2–Mo4 could resynthesize these markers following overnight incubation at 37°C. Culture of enzyme-treated cells either in the cold or at 37°C in medium containing puromycin retarded this reexpression. These experiments provide several insights into the nature of the Mo antigens: (1) these protease-sensitive antigens are in fact synthesized by the monocyte and, unlike Plt-1, are not contaminating extrinsic proteins; (2) trypsin or papain-sensitive cleavage suggests the presence of exposed polypeptide structures; (3) the kinetics of membrane turnover of these antigens falls within a 16-hr period; (4) new protein synthesis may be required for antigen reexpression; and (5) Mo1 (and framework Ia) has unique characteristics (either intrinsic structural properties or related to protection by the local government) that make it relatively resistant to protease treatment.

Given the fact that the PB monocyte represents a maturational subset along the path of differentiation toward macrophages,31 it was of interest to evaluate the expression of Mo1–4 on both immature myeloid forms as well as fully differentiated macrophages. Mo3 and 4 are rarely expressed on early myeloid cells as represented by leukemic myeloblasts; they are however found on monocytic forms from a fraction (one-fourth and one-half, respectively) of patients with monocytic leukemia (a frequency of expression less than Mo1 and Mo2). Leukemic promyelocytes (as represented by HL-60) induced to differentiate in vitro into cells bearing the morphological and histochemical characteristics of macrophages29,30 also acquire Mo1–4 with some variation in expression depending on the mode of induction. Likewise, monocytes that have transformed into macrophages during in vitro culture retain Mo1–4 as surface markers. Interestingly, however, the expression of Mo3 is lost on macrophages derived from the peritoneal cavity, suggesting that this antigen is a transient feature during the maturational process. Taken together, these observations may indicate that the Mo antigens represent differentiation markers within the monocyte-macrophage lineage. Mo2, for example, being expressed on PB monocytes and certain noncirculating macrophages appears to be a lineage-specific antigen, while the appearance of Mo3 may be characteristic of a maturational subset within this lineage. Coexpression of antigens between monocytes and granulocytes (Mo1) or platelets (Mo4) may suggest either a differentiative link among the monocyte, granulocyte, and platelet-megakaryocyte lineages or may signify common membrane features unrelated to differentiation (e.g., Ia antigens shared between the monocyte and the B lymphocyte). Studies to determine
the function of the Mo antigens may shed light on this issue.

In addition to Mo1−4, several other antigens whose expression is restricted to human monocytes or shared among cells of the myeloid lineage have been recently described. We have previously compared Mo1 and Mo2 with Mac-120 (expressed on 38% of PB monocytes), OKM1 (expressed by monocytes, granulocytes, and null cells), and UC-45 (expressed by PB monocytes only after substrate adherence). In addition, 63D3 is a 200,000-dalton antigen strongly expressed by PB monocytes and weakly expressed on granulocytes. Whereas Mo2 is distinct from these other markers, recent biochemical observations suggest that anti-Mo1 and OKM1 are directed against distinct antigenic sites on the same parent glycoproteins (consisting of two subunits of 94,000 and 155,000 daltons).* Similar to Mo2 with respect to monocyte specificity is a determinant reported by Anderson and colleagues, which unlike Mo2, is trypsin resistant with a molecular weight of 90,000 and 100,000 daltons (two subunits). Likewise, a series of monoclonal reagents with apparent specificity for human monocyte-macrophages at various stages of maturation has been developed by Dimitriu-Bona et al. and Linker-Israeli et al. The possible relationships of these determinants and the Mo series awaits further published information from these investigators.

Griffin et al. have raised monoclonal antibodies whose specificity is directed against antigens common to the myeloid series. MY3 and MY4 are antigens strongly expressed by normal monocytes and weakly expressed by granulocytes and normal bone marrow cells. In contrast, MY7 is strongly evident on granulocytes but weakly evident on monocytes. MY8, which is distinct from Mo1/OKM1, is borne by normal monocytes, granulocytes and all peroxidase-positive bone marrow cells. Similarly, Perussia et al. have developed monoclonal reagents recognizing antigens on granulocytes and monocytes as well as immature myeloid forms. It would appear, therefore, that there is a family of differentiation antigens shared by members of the myeloid series that are expressed to a variable degree by cells committed to the monocyte-macrophage path of differentiation.

TA-1* and 4F2* are antibodies that define markers shared by PB monocytes and T lymphocytes or T-cell lymphoblastoid cell lines, respectively. These distinctive antigens are in a class of their own and may prove to be of functional significance with regard to monocyte–T-cell collaboration.

In this article, we have shown that Mo3 and Mo4 are unique from Mo1 and Mo2, and based on either distinct patterns or frequencies of cellular distribution, have features dissimilar to Mac-120, 63D3, and the antigens reported by Hogg, Anderson, and their colleagues. Interestingly, however, Waldrep et al., using conventional heteroantisera, have detected human monocyte antigens whose expression is induced only after exposure to lymphokines, are inhibited by protein synthesis inhibitors, and are protease sensitive. Whereas the expression of Mo3 does not appear to require specific activation, the similarity between these two observations is striking and at least provides precedent for the rise in Mo3 density. A similar augmentation of antigen expression has been reported for human monocyte Ia (also suppressed by cold temperature and puromycin), although quantitatively, this change appears less significant than that observed for Mo3.

Regarding the functional nature of the Mo antigens, none appear to bind selectively to Fc receptors, nor to markers that are functionally required in monocyte presentation of soluble antigen to helper T cells. Moreover, analysis to date demonstrates no specificity for a functionally distinct subset of monocyte such as has been asserted for Mac-120. Studies are in progress to determine whether platelet binding by antibodies directed against Plt-1 or Mo4 has functional significance, such as inhibition of platelet aggregation.

Ultimately, it is expected that an expanded library of myeloid-macrophage antigens will be defined by monoclonal reagents. With appropriate “cross-referencing” among the many investigators now working in this field, these reagents may prove useful in identifying discrete morphological and functional stages along the path of differentiation from the CFU-C to the mature macrophage. As in the T-lymphocyte system, specific membrane markers may delineate cells within the macrophage lineage that perform distinct functions that modulate the immune system.

ACKNOWLEDGMENT

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Analysis of antigenic determinants on human monocytes and macrophages

RF 3d Todd and SF Schlossman