Human Macrophage Maturation and Heterogeneity: Analysis With a Newly Generated Set of Monoclonal Antibodies to Differentiation Antigens

By Reinhard Andreesen, Klaus J. Bross, Jurgen Osterholz, and Frank Emmrich

We have analyzed the expression of late differentiation antigens during terminal in vitro maturation of human macrophages (MØ) from blood monocytes (MO) in comparison to their distribution among mature MØ residing in various tissue sites. By immunizing mice with MØ derived from blood MO by culture on hydrophobic Teflon foils, monoclonal antibodies (mAbs) were developed (MAX.1, MAX.2, MAX.3, MAX.11) that reacted with lineage-restricted differentiation antigens. These antigens were expressed exclusively on MØ or were markedly increased after in vitro differentiation. The only overlap to another hemopoietic cell lineage was observed with MAX.3, which is shared by platelets and megakaryocytes. In the course of MØ maturation in vitro, the MAX.1 and MAX.3 antigens are detected within the cytoplasm two days before they appear on the cell surface. In contrast, the MAX.11 antigen is expressed simultaneously in the cytoplasm and at the cell surface, is found in varying degrees on a minor portion of blood MO and U937 cells, and is expressed rapidly at high density during early MØ differentiation in vitro. Among conventional mAbs that do not react with MO we found those against the transferrin (TF)-receptor, the BA-2, and the PCA1 antigen to label MØ. MØ matured in vivo and isolated from body fluids were positive with some but not all MAX mAbs. Distinctive patterns were observed with pulmonary MØ, exudate MØ from pleural and peritoneal effusions, synovial fluids, and early lactation milk. MØ from the alveolar space, for example, constantly expressed the MAX.2 antigen but not the MAX.3 antigen. Pleural effusion MØ, however, did not react with the MAX.1 mAb, but in most cases, it did react with the MAX.3 mAb. The detection of novel differentiation antigens, all expressed on monocyte-derived MØ but differently expressed on site-specific MØ in situ, underlines the remarkable heterogeneity among human MØ. The expression of these antigens is flexible because those MAX antigens that were not expressed in situ could be induced if cells from distinct tissue sites were cultured in vitro for several days. MAX mAbs may be of potential value to study both the sequential stages of maturation within the MØ lineage as well as differential developments induced by various culture conditions in parallel to environmental factors in vivo.

The mononuclear phagocyte system comprises cells of considerable heterogeneity in terms of localization, morphology, metabolism, and function. It seems likely that there are still more members of this cell family whose delineation comes from the common bone marrow stem cell committed to monocyte-macrophage differentiation. This can be proven or disproven if suitable markers become available. Whereas cumulative knowledge exists about the development of cells from bone marrow progenitors to monocytes (MO) entering the peripheral blood, data characterizing the terminal maturation beyond the blood monocyte stage of differentiation are still limited. The anatomical site may provide the environmental signal for the immigrating MO to develop into the individual cell type, eg, bone matrix may induce osteoclast formation and other local factors may be responsible for the development of hepatic Kupffer cells, pulmonary macrophages (MØ), microglia cells, fluid phase, and tissue or granulomatous MØ. On the other hand, because remarkable heterogeneity already exists within the circulating monocyte population, all of these mature cell types may derive from predestined monocyte subsets that are attracted to the specific anatomical site to which they are designated. The aim of this study was to detect and follow the expression of differentiation antigens on cells of the monocyte-macrophage lineage. We have used monocyte-derived MØ cultured on hydrophobic Teflon foils11,12 as immunizing cells for the development of monoclonal antibodies (mAbs) with specificity for surface molecules on terminal in vitro differentiation stages of human MØ. Here we report on the first five mAbs of the MAX series that could be studied at sequential stages of MØ differentiation in vitro. In addition, three nonlineage-restricted antigens (TF-receptor, BA-2, and PCA1) that are not present on blood MO could be found after in vitro differentiation. It was found that in vivo-matured MØ obtained from various body fluids were of considerable diversity in terms of expression of the differentiation antigens described.

Materials and Methods

Generation of MAX mAbs

Two-month-old BALB/c mice were injected five times intraperitoneally at weekly intervals with 5 x 10⁶ monocyte-derived MØ that were collected from hydrophobic Teflon bags after culture of blood-borne MO for at least ten days (for characterization of the cells see Table 1). Three and a half days after the last injection, immune spleen cells were fused with the HAT-sensitive line X63Ag8.653.14 Hybridoma supernatants were tested for binding to autologous B cells, T cells, MO, and monocyte-derived MØ by a cell-ELISA (enzyme-linked immunosorbent assay)15 and by the immunoperoxidase slide technique16 as described later. Finally, five hybridomas were selected because of their ability to discriminate between MO and MØ. They were subjected to recloning and further expansion. Cyttofluorometric analysis (ORTHO 50-11) was used to confirm the reactivity pattern as evaluated by cell-ELISA and the immunoperoxidase method.15

M Abs to Cell Surface Antigens Used in This Study

OKT3, OKT4, OKT8, OKT6, OKT9, OKM1, and OKIa1 were purchased from Ortho Pharmaceutical Corp, Raritan, NJ; antihuman...
monocytoma from Bethesda Research Lab Inc, Gaithersburg, MD; Coulter Clone B1; BA-1, BA-2, My4, and PCA1 from Coulter Immunology, Hialeah, Fla; Leu-2, Leu-3a, Leu-3b, Leu-7, and antihuman transferrin receptor from Becton Dickinson, Erembodegem, Belgium; antihuman IgG1, IgG2, IgG3, and IgG4 from Bio-Yeda Ltd, Rehovot, Israel; Ki-1, Ki-M1, and Ki-M5 from Behringwerke, Marburg, FRG; and anti-C3b from Dakopatts, Copenhagen.

MO S39 was a generous gift from Dr R. Winchester, Orthopedic Institute, Hospital for Joint Diseases, New York, and anti-Tac from Dr T. Waldmann, NIH, Bethesda, Md. A polyclonal rabbit antitransferrin antibody was purchased from Behringwerke AG.

### Cells

**Blood monocytes.** Buffy coat cells from normal blood donors or whole blood from patients with acute and chronic monocytic leukemias (classified by morphology, cytochemistry, and surface antigen analysis) were diluted 1:1 with phosphate-buffered saline (PBS) and centrifuged over Ficoll-Hypaque (F/H) to separate mononuclear cells (MNCs) from granulocytes and red cells. MO were separated from lymphocytes usually by adherence: 3 x 10^6 MNCs/mL RPMI 1640 (supplemented with 1-glutamine, antibiotics, 5 x 10^{-5} mol/L, 2-mercaptoethanol, and vitamins) plus 20% fetal calf serum (FCS) were incubated for 60 minutes in plastic Petri dishes. The nonadherent cells were removed by repeated washings with warm serum-free medium and the adherent cells (>90% MO as revealed by morphology and phenotype analysis) incubated overnight in RPMI 1640 (supplemented with MEM vitamers, penicillin 50 U/mL, streptomycin 50 μg/mL, 2-mercaptoethanol 5 x 10^{-5} mol/L, L-glutamine 2 mol/L) plus 10% human AB-group serum. MO could then be recovered from the dishes at 4 °C by vigorous pipetting. In some experiments, the MNCs were suspended in 0.1% EDTA containing PBS and loaded onto an elutriator centrifuge (J2-21, standard chamber, Beckman, Stockholm) with a flow rate of 10 mL/min. Lymphocytes were removed at 10 mL/min and 12.4 mL/min with 200 mL and 100 mL of PBS, respectively. The MO-enriched cell fraction was then collected at a flow rate of 14.0 mL/min with 150 mL PBS and contained >70% MO as judged by phenotype analysis.

Alternatively, MNCs were layered on top of a continuous Percoll (Pharmacia Fine Chemicals, Freiburg, FRG) gradient with densities ranging from 1.06 g/mL to 1.08 g/mL for 72 hours. At different times after the initiation of the culture, MO could be harvested from the bags and taken for FACS analysis.

**Granulocytes.** After F/H centrifugation of diluted buffy coat cells, the pelleted granulocytes and red cells were resuspended in equal volumes of PBS and hydroxyethylstarch. After sedimentation for 30 minutes at 1 g the granulocyte-rich supernatant was centrifuged and the remaining erythrocytes lysed by five-minute exposure to 0.16 mol/L ammonium chloride and 0.17 mol/L tris(hydroxymethyl)aminomethane (Tris, Merck, Darmstadt, FRG) at 37 °C.

**Megakaryocytes.** Megakaryocytes were identified by morphology in bone marrow MNC suspensions obtained from healthy volunteers by posterior iliac crest aspiration and subjected to F/H centrifugation.

**Thrombocytes.** Thrombocytes were collected from the plasma supernatants after F/H centrifugation and sedimented for 10 minutes at 800 g.

**T and B cells.** Cells were enriched by rosetting MNCs with AET-treated sheep erythrocytes and subsequent centrifugation over F/H. T cells were activated by incubation with concanavalin A (2 μg/mL) for 72 hours.

**Pulmonary MO.** Macrophages were collected from the broncho-alveolar lavage fluids of healthy volunteers and patients undergoing diagnostic bronchoscopy after giving informed consent.
**Pleural and peritoneal Mφ.** Macrophages were isolated from effusion fluids secondary to heart failure, portal hypertension, or malignancies.

**Synovial Mφ.** Macrophages were collected from samples obtained by diagnostic puncture of knee joints.

**Milk Mφ.** Macrophages were obtained from breast milk samples taken at day 3 to 9 postdelivery.

**Human tumor cell lines.** DHL-1 (diffuse histiocytic lymphoma, from Dr M. Scott, Stanford University, Calif.), U937 (histiocytic lymphoma, from Dr K. Nielsson, Wallenberg Lab, Uppsala, Sweden), HL-60 (promyelocytic leukemia, from Dr R. Vogler, Emory University, Atlanta), LBL6078 and LBL3283 (B lymphoblastoid cells), Mel-1 and Mel-2 (melanoma xenografts in nude mice, from Dr H. Fiebig, Medizinische Klinik, Freiburg, FRG), and Colo38 (melanoma cells, from Dr G. Moroni, University of Napoli, Italy).

**Immunoperoxidase Slide Technique.** For surface marker analysis, the cells were washed three times with cold PBS and transferred onto glass slides coated with 0.1% alcin blue in hydroxyethylstarch. After attachment of the cells to the negatively charged glass surface, they were fixed at 4°C with 0.05% glutaraldehyde in 0.1 mol/L PBS containing 10% glucose. They were then washed with a glycine containing medium and preincubated with a gelatine containing medium to prevent immunoglobulin binding to the glass surface. The staining procedure included the following steps: (1) 15-minute incubation with mAbs diluted in gelatine containing medium; (2) five-minute incubation with rabbit antimouse Ig (Dakopatts) diluted 1:1000, and absorbed with normal human serum and swine serum for five minutes; (3) another five-minute incubation with swine antirabbit Ig (Dakopatts) absorbed as described; (4) incubation with peroxidase-antiperoxidase complex from rabbits (Dakopatts) diluted 1:20; (5) incubation with diaminobenzidine-H₂O₂ for ten minutes, followed by postfixation with OsO₄. The slides were covered with a solution of 80% glycerine and 0.125% glutaraldehyde in 0.1 mol/L PBS. For detection of rabbit antibodies, the first two steps were omitted. The different sandwich sera served as negative controls. Unspecific Fc-receptor-mediated binding could be excluded by negative reactions with mouse IgG (MlsigG, Coulter Immunology) and several mAbs of different Ig class that did not react with the cells to be tested. Two hundred cells were evaluated for Mφ reactivity and were counted as positive when dark brown staining of the membrane was seen. In a few experiments the influence of the fixation reagent on the antigenic determinants was tested by adding mAbs to the cells before the fixation step. Data are usually expressed as percentage of positive macrophages. For intracellular staining, the cells were dried on alcin blue-coated glass slides in a medium containing 0.2% bovine serum albumin. After fixation with a solution of acetone, ethanol, and formaline (80:20:0.2) on ice for five minutes, the slides were transferred to 80% acetone and then dried quickly. This was followed by hydration in acetone of decreasing concentrations from 100% to 50% and, finally, in PBS. The slides were then incubated for 15 minutes at room temperature with a gelatine containing medium with 10% swine serum, 10% human serum, and 10% glycerine, which was also used for dilution of mAbs, rabbit antimouse Ig, swine antirabbit Ig, and the peroxidase-antiperoxidase complex. The staining procedure was performed as described but each incubation was extended to 15 minutes.

**RESULTS.**

To develop mAbs directed against lineage-restricted human Mφ differentiation antigens, we used monocye-derived Mφ cultured in hydrophobic Teflon foils. This method prevents the spreading of Mφ on the surface, and thus allows the recovery of cells without damage. Cells cultured in this way meet all the morphologic, cytochemical, antigenic, and functional criteria for Mφ ex vivo (see Table 1 for full characterization, and also refs 12, 20, 22). In this report we give detailed information on five mAbs of the MAX series that discriminate between Mφ and Mφ. The reactivity pattern of these antibodies is described in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Cellular Distribution of Surface Antigens Reactive With MAX. Monoclonal Antibodies</th>
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<tbody>
<tr>
<td><strong>Monoclonal Antibody Designation</strong></td>
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<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Monocyte-derived Mφ</td>
</tr>
<tr>
<td>Adherent monocytes (day 1)</td>
</tr>
<tr>
<td>Granulocytes</td>
</tr>
<tr>
<td>Platelets/megakaryocytes</td>
</tr>
<tr>
<td>B cells</td>
</tr>
<tr>
<td>T cells</td>
</tr>
<tr>
<td>Activated T cells</td>
</tr>
<tr>
<td>Permanent cell lines</td>
</tr>
<tr>
<td>L-428 (Hodgkin cells)</td>
</tr>
<tr>
<td>U-937 (histiocytic lymphoma)*</td>
</tr>
<tr>
<td>HL-60 (promyelocytic leukemia)</td>
</tr>
<tr>
<td>B lymphoblastoid cells†</td>
</tr>
<tr>
<td>Melanoma cells‡</td>
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</tbody>
</table>

Cell distribution was evaluated by immunoperoxidase staining; hybridoma supernatants were diluted 1:10. All cell lines were tested at least three times: Mφ, Mφ (10- to 15-day-old MO cultures). B cells (non-necrosing cell population containing 55% B1* cells, Mφ, and less than 2% OKT3* T cells) and cells (>90% purity) were obtained from ten donors, whereas activated T cells (about 40% la*-blast cells), platelets, and granulocytes were from three donors. +++, more than 90% of cells reactive; +, varying reactivity ranging from 15% to 90%; var, reactivity varied among experiments from negative to up to 20% positive cells; −, no reactivity.

**Varying portions of positive cells on repeated tests with the same line (15-80%).**

†L6078 and LBL3283.

‡Xenotransplanted tumors Mel-1 and Mel-2 (tested either freshly prepared from tissue specimen or after at least three serial passages in vitro) and permanent cell line Colo38.
During MØ maturation in vitro, the differentiation antigens defined by MAX. mAbs were expressed consecutively as shown in Fig 1A through D. Whereas nearly all mature MØ reacted with MAX.1 and MAX.3, only up to 70% of the cells were positive for the MAX.2 antigen. MAX.11 antigen appeared to be the first antigen to be fully expressed (after three days) and MAX.3 the latest antigen not being detectable in three-day-old MO cultures but was fully expressed by day 9.

MAX.1 and MAX.3 antigen appeared in the cytoplasm two to three days earlier than they were expressed on the cell surface (Fig 2), whereas cytoplasmic and surface labeling of MAX.11 antigen coincided (not shown). MAX.2 antigen seemed to be sensitive to the methanol treatment that was required for intracellular staining. This treatment completely abrogated the reaction of glutaraldehyde-fixed cells with this antibody. MAX.1 and MAX.3, but not MAX.2, reactivity was lost after fixation at room temperature.

In addition, a series of conventional mAbs that do not react with blood MO was tested routinely as control for binding to in vitro-matured MØ (OKT3, OKT4, OKT6, OKT9 directed to the TF-receptor, Leu-2, Leu-3a, Leu-3b, Leu-7, B1, BA-1, BA-2, PCA1, anti-Tac, Ki-1). Interestingly, mAbs directed to the TF-receptor,23 and the BA-2 antigen24 labeled mature MØ to varying degrees (Fig 3). Only two days later, during in vitro differentiation, TF molecules were detected on the cell membrane (Fig 3A). The PCA1 antigen25 also appeared to be expressed only on mature MØ with 17% to 72% of cells reacting (not shown). We observed in some experiments a weak reactivity of MØ with OKT4 and anti-Tac that, however, did not depend on the stage of maturation.

In order to investigate the expression after in vivo maturation of the differentiation antigens described, we isolated MØ from various body cavities (Table 3).

A considerable heterogeneity was found concerning membrane antigen expression in MØ from different sites and also between different individuals. Some generalizations seem to be possible: MAX.3 was absent from pulmonary and breast milk MØ but present on most pleural and some peritoneal MØ. MAX.1 could be detected only on minor portions of pulmonary and peritoneal MØ but never on pleural or breast milk MØ. However, if pulmonary and peritoneal MØ with distinct surface phenotypes were cultured on hydrophobic Teflon foils, the cells readily expressed these MAX antigens that were missing in situ (Table 4).

Up to now no significant difference could be detected in the MAX antigen expression on pulmonary MØ from smokers and nonsmokers, whereas with pleural and peritoneal

![Fig 1](image1.png)

**Fig 1.** Sequential expression of lineage-restricted differentiation antigens during maturation in vitro. MO (4 x 10⁷/mL) separated from other mononuclear cells by adherence were cultured on hydrophobic Teflon foils in supplemented RPMI 1640 plus 10% human AB group serum. At indicated days, the cells were recovered and subjected to immunoperoxidase staining with MAX.1 (A), MAX.2 (B), MAX.3 (C), and MAX.11 (D). Data are expressed as percentage of positive MØ (six experiments) that were identified by their reactivity with monocyte-specific mAbs.

![Fig 2](image2.png)

**Fig 2.** Intracytoplasmic detection of two MAX. differentiation antigens as compared with cell surface expression. (A) MAX.1 and (B) MAX.2. As described in Fig 1, MO were isolated and cultured on hydrophobic Teflon foils. Immunoperoxidase staining was performed on either glutaraldehyde-fixed cells (cell surface labeling) or cells that were dried on alcian blue-coated slides before fixation with acetone (intracellular staining). For experimental details, see Materials and Methods. Data are from one representative experiment.
antigens during macrophage maturation in vitro: the TF-receptor effusions, a problem that will be studied in more detail. No different diseases that had led to pleural or peritoneal details. see Fig 1.

and surface TF (A) and the BA-2 antigen (B). For experimental reaction of liver with collagenase do not express MAX.1, antigen. Preliminary experiments indicate that human Kupffer cells elutriated from cell suspensions obtained by perfusion of liver with collagenase do not express MAX.1, MAX.2, and MAX.3, but could be induced in culture to express MAX.2 (R. Andreesen and H. G. Leser, to be published).

Studies concerning in situ localization of MAX differentiation antigens using a sensitive four-layer immunoperoxidase technique are in progress.

DISCUSSION

We have analyzed the expression of antigens on human mononuclear phagocytes as they undergo terminal maturation. Differentiation from monocyte precursor cells as a more or less homogenous population in the peripheral blood leads to heterogeneous mature cells residing in various anatomical sites of the organism. Despite the changes in morphology, biochemistry, and function that occur while MO transform into mature cells, surprisingly little is known about the loss or acquisition of surface antigens beyond the blood monocyte stage of differentiation. With the new mAbs, we have identified two molecules (MAX.1 and MAX.2 antigens) on mature MO which are absent from MO and any other cell type tested so far, and a third molecule (MAX.3 antigen), which is shared only by platelets and megakaryocytes. Other antigens have been reported that are shared by the megakaryocyte and MO lineages, such as the MPA antigen, which differs, however, from MAX.3 as it is present on freshly harvested blood MO. As has been described for immunoglobulins during B cell differentiation, these maturation-related molecules are first detected in the cell cytoplasm before they appear on the outer cell surface.

All three antigens seem to be unique and different from any other reported so far: the PAM1 antigen detected by Biondi et al., like MAX.2, also binds to a 200-kd structure that, according to their studies, is not expressed on monocyte-derived MO and is restricted to pulmonary MO; the BMM1 mAb reported by the same authors to be specific for

![Fig 3. Expression of nonlineage-restricted differentiation antigens during macrophage maturation in vitro: the TF-receptor and surface TF (A) and the BA-2 antigen (B). For experimental details, see Fig 1.](image)

**Table 3. Heterogeneity of Human Macrophages as Defined by Surface Antigen Analysis**

<table>
<thead>
<tr>
<th>Source of Macrophages</th>
<th>Monoclonal Antibody Reactivity (%)</th>
<th>Monoclonal Antibody Reactivity (%)</th>
<th>Monoclonal Antibody Reactivity (%)</th>
<th>Monoclonal Antibody Reactivity (%)</th>
<th>Monoclonal Antibody Reactivity (%)</th>
</tr>
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<tbody>
<tr>
<td>Normal blood (n = 6)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>21 ± 17</td>
<td>None</td>
</tr>
<tr>
<td>Monocytic leukemia (n = 5)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>57 ± 28</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monocytic-derived (Teflon bag, n = 6)</td>
<td>&gt;90</td>
<td>61 ± 10</td>
<td>&gt;90</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion (n = 8)</td>
<td>&lt;1</td>
<td>23 ± 21</td>
<td>51 ± 28</td>
<td>41 ± 27</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Ascites fluid (n = 12)</td>
<td>17 ± 18</td>
<td>16 ± 18</td>
<td>60 ± 24</td>
<td>48 ± 28</td>
<td>22 ± 16</td>
</tr>
<tr>
<td>Alveolar lavage fluid (n = 14)</td>
<td>7 ± 6</td>
<td>84 ± 8</td>
<td>&lt;1</td>
<td>62 ± 30</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Breast milk (n = 4)</td>
<td>&lt;1</td>
<td>7 ± 7</td>
<td>&lt;1</td>
<td>39 ± 13</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Synovial fluid (n = 4)</td>
<td>7 ± 7</td>
<td>8 ± 4</td>
<td>23 ± 7</td>
<td>54 ± 19</td>
<td>11 ± 4</td>
</tr>
</tbody>
</table>

Monoclonal antibody reactivity was evaluated by immunoperoxidase staining on alcian blue-coated slides; MAX. mAbs were diluted 1:10 (hybridoma supernatant). OKT9 1:100 (ascites fluid). Values are given as mean percentage of positive monocyte-macrophages identified by morphology and in comparison by binding of monococyte-specific mAbs. The range within individual values is given in parentheses. MO were assayed for reactivity with mAbs in freshly prepared mononuclear cell suspensions where they represented 12% to 34% of total cells. All leukemic MO populations tested were of more than 90% purity. Except for alveolar lavage fluids, which always contained more than 80% of MO, the MO content in other body fluids varied from 18% to 68%. From www.bloodjournal.org by guest on September 23, 2017. For personal use only.
Table 4. Induction of MAX. Differentiation Antigens on Cultured Pulmonary and Peritoneal Macrophages

<table>
<thead>
<tr>
<th>MAX Monoclonal Antibodies</th>
<th>Pulmonary MØ (n = 5)</th>
<th>Peritoneal MØ (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAX.1</td>
<td>MAX.2</td>
</tr>
<tr>
<td>Freshly isolated</td>
<td>4 ± 3</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>Cultured on Teflon</td>
<td>85 ± 8</td>
<td>75 ± 7</td>
</tr>
</tbody>
</table>

MØ were obtained from alveolar lavage fluids from patients undergoing diagnostic bronchoscopy and from ascites secondary to portal hypertension or malignancies. Reactivity as evaluated by immunoperoxidase staining is expressed as the mean percentage of total M count. M were cultured at 3 x 10⁹/mL RPMI 1640 plus 10% AB serum for six days.

breast milk MΦ in fact reacted with minor subpopulation of a T cell line (HPB-ALL) and a minor portion of blood MØ; the 27F9 antigen reported by Zwadlo et al is present on melanoma cells, differs in the molecular weight, is expressed only up to 70% on monocyte-derived MΦ, and can be detected on Kupffer cells. MAX.11 reacts with a lineage-restricted molecule that, however, is found to variable degrees on a minor portion of blood MØ and U937 cells but is rapidly expressed on more than 95% of MΦ at day 3 in Teflon cultures of MØ. Somewhat similar seems to be the M3 antigen that is found, although at low density, on the majority of blood MØ and shows increased expression after overnight incubation.

Other groups have developed mAbs specific for the monocye-macrophage lineage but that are not able to distinguish between MØ and mature MØ: MΦ P15 and MØ S39 by Dimitriu-Bona et al.; Mo2 by Todd and Schlosmann; some of the FMC series by Hancock et al.; the PHM3 by Becker et al.; the 3C10 and 1D9 by Van Voorhis et al.; and the KiM1 by Radzun and Parwaresch.

The MΦ antigens described here do not seem to be the result of culture artifacts as (1) they all could be detected, although in a distinctive pattern, on in vivo-matured MØ by Radzun and Parwaresch; (2) the accessory cell function and cell lines were not induced to express the antigens under the same culture conditions (T cells, both activated and unactivated, B cells, melanoma xenografts, B lines); and (3) MAX.1- and MAX.3-negative Kupffer cells do not express these antigens after comparable culture periods. These antigens are obviously not differentiation antigens for the following reasons: (1) Cell activation by endotoxin or interferon-gamma usually is completed in less than 24 hours and is reversible, whereas cell differentiation to our belief is a continuous and irreversible process a cell is committed to undergo. (2) The kinetics of activation and differentiation are quite distinct, as MØ had to be cultured three days to see expression of differentiation antigens that, in the case of MAX.2, affects only a certain part of the cells (Fig 1). (3) In fact, when MØ were activated for tumor cytotoxicity by interferon gamma (up to 200 U/mL), MAX antigen expression was inhibited (to be published). (4) It has been reported that MΦ maturation is accompanied by an increase in some cell activities (see Table 1 and refs 12, 20, 21, 35; however, others, like the secretion of interleukin 1 and prostaglandin E₂, are even lost at a culture stage where MAX antigens are fully expressed (Table 1, to be published in detail).

Antigenic heterogeneity of mature MØ has been shown in the rat and, more recently, on human cells. Thus, end-stage maturation in vivo does not necessarily lead to the expression of MAX differentiation antigens; some may in fact be suppressed by environmental factors in the specific tissue site MØ have migrated to. Yet, the difference in the expression of antigens all present on monocyte-derived MØ among mature MØ from various anatomical sites is of considerable interest: it may indicate a functional correlation of specific membrane structures to the properties of cells residing in a given anatomical site. As these site-specific MØ can, however, be induced to express the missing antigens on in vitro culture, it supports the hypothesis that local factors in the microenvironment control the distinct phenotypes observed. This seems to be more likely than the existence of MØ subsets, which are committed to terminal differentiation into pulmonary, pleural, or peritoneal exudate MΦ, respectively.

The detection of antigens unique for late in vitro differentiation stages of MØ and at the same time differentially expressed on subsets of mature MØ of various anatomical sites might be of functional significance, as both maturation in vitro as well as generation of site-specific heterogeneity in vivo seems to be accompanied by quantitative changes in certain MØ functions: (a) the capacity to kill tumor cells in vitro, and (b) the bactericidal activity and hydrogen peroxide metabolism, (c) the accessory cell function and production of interleukin 1, and our own unpublished results).

MΦ differentiation in vitro is followed by an increase in ferritin synthesis, a change in the glycolipid pattern (M. Rössle and R. Andreesen, submitted for publication), and in cell adhesion behavior, the induction of cAMP-dependent protein kinase and calmodulin-binding protein, the expression of ecto-5' nucleotidase and creatine kinase. Thus, it has to be established whether the expression of the MAX. antigens is related to some of those features. This is subject to current investigations. Preliminary data seem to indicate that MAX. antigens are not involved in tumor cytotoxicity or in oxidative metabolism and Fe-receptor-mediated phagocytosis (to be published).

Besides lineage-restricted differentiation antigens, we found also nonlinear-restricted antigens that are newly expressed (TF-receptor, BA-2, TF-R, and PCA1). The TF-receptors may serve to deliver iron to intracellular stores. The detection of cell surface TF two days later than the expression of TF receptor in culture has to be explained. It may be due to incomplete expression of TF-receptors not yet capable of binding TF. Alternatively, membrane-bound TF by itself might be a MΦ product that is detected independent of the TF-receptor. Data from biosynthetic labeling experiments indicate that TF is synthesized by mature MΦ and probably processed to surface membrane sites distinct from the TF-receptor reactive with the OKT9 mAb (R. Andreesen and G.
HUMAN MACROPHAGE ANTIGENS

Bauer, manuscript in preparation). Both the TF-receptor and surface TF could function to facilitate tumor cell–MØ interaction, as has been shown for natural killer cell activity. Together with the MAX series, mAbs to the TF receptor, the BA-2, and the PCA-1 antigen seem to be useful for a phenotypic followup of MØ terminal maturation. The novel differentiation antigens described here may also serve to indicate distinct pathways of differentiation, although their functional significance has yet to be established.

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Human macrophage maturation and heterogeneity: analysis with a newly generated set of monoclonal antibodies to differentiation antigens

R Andreesen, KJ Bross, J Osterholz and F Emmrich