Antigenic Heterogeneity of Human Mononuclear Phagocytes: Immunohistologic Analysis Using Monoclonal Antibodies

By Wayne W. Hancock, Heddy Zola, and Robert C. Atkins

Eight monoclonal antibodies to cell surface antigens of human monocytes were evaluated as immunologic markers for recognition of macrophages in sections of normal and diseased tissues, using immunoperoxidase and enzyme histochemical techniques. Monoclonal antibodies assessed were PHM2, PHM3, FMC17, FMC32, FMC33, FMC34, OKM1, and 63D3. Sites studied were human bone marrow, blood, lymph node, spleen, thymus, liver, kidney, lung, and peritoneal lavages, rejecting renal allografts containing inflammatory macrophages, and granulomatous showing epithelioid and multinucleate giant cell formation. All antibodies bound to at least some tissue macrophages and, except for FMC32 and FMC33 antibodies, which were identically distributed, each antibody had a distinctive tissue distribution. Some antigens were shared by other bone-marrow-derived cells (megakaryocytes and cortical thymocytes), endothelium, epithelium, and dendritic cells. Antigenic differences were also detected between mononuclear phagocytes present at different sites, different stages of differentiation, and likely different stages of activation. These studies provide evidence of major antigenic differences between various populations of human mononuclear phagocytes. They therefore indicate the need for careful evaluation of experiments involving the recognition of macrophages in tissue sections and smears based solely on the use of antimonocyte monoclonal antibodies.

CELLS OF THE MONONUCLEAR phagocyte system arise from a common bone marrow stem cell, enter the circulation while still in an undifferentiated state, and eventually lodge in various target tissues where, as macrophages, they undergo final maturation.1,2 Macrophages are involved in the disposal of cell debris, defense against microorganisms, and interaction with lymphoid cells during the immune response.3 However, most studies of macrophage maturation and function have been performed in vitro, where macrophages can be readily identified and purified using their properties of phagocytosis and adherence to glass.4 Little is known of macrophage maturation and function in vivo, because variations in macrophage morphology, enzyme content, and surface receptors hinder their recognition and distinction from other cell types.5,6 Moreover, this problem of macrophage identification has not been overcome using serologic techniques, due, in part, to the difficulties involved in producing suitably macrophage-specific polyclonal antisera.5,9

Monoclonal antibodies provide the potential for detailed analysis of cell lineage and differentiation, as has been shown for human T cells.10,11 Such studies are of significance for the present investigation because of the finding of T-cell differentiation antigens specific to intrathymic and other stages of development, plus antigens shared between T cells and unrelated tissue components. The production of monoclonal antibodies to monocyte antigens has been recently reviewed.12 However, although a number of antibodies displaying relative specificity for human monocytes have now been produced,14,16 the reactivities of these antibodies with macrophages or other cell types in tissues have not yet been determined. The use of antimonocyte monoclonal antibodies as markers for some or all of the stages of macrophage maturation should allow the detailed in situ analysis of human macrophage differentiation and activation, which has not been possible using previously available techniques.12,15

This article describes an approach to the study of human mononuclear phagocytes through the in situ analysis of macrophage surface antigens at different stages of cell maturation and stimulation, and under normal and pathologic conditions. First, the extent of binding of eight antimonocyte monoclonal antibodies to macrophages in many tissues was examined to determine whether there were antigens specific to the cell surface of all mononuclear phagocytes. Next, to determine whether cell maturation from dividing precursor cells in the bone marrow to more stable tissue forms was associated with the expression of differentiation antigens, the cell membrane antigens of bone marrow and blood monocytes were compared with those of tissue macrophages. Finally, to assess whether there were antigenic differences between resident and activated or inflammatory macrophages, the membrane antigens of macrophages participating in acute and chronic inflammatory reactions were analyzed, including after epithelioid and multinucleate giant cell transformation.

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MATERIALS AND METHODS

Cells and Tissues

The reactivity of each antibody was initially assessed using histologically normal human surgical specimens to contain cells of the mononuclear phagocyte system. These included bone marrow aspirates (x4); peripheral blood mononuclear cells (x4) and platelets (x3) (greater than 99% pure by morphology) obtained by flotation of venous blood on Ficoll-Isoopaque;2,5,7,8 plus specimens of normal abdominal lymph node (x6), spleen (x6), thymus (x4), liver (x5), alveolar (x6) and peritoneal (x3) lavages, and kidney (x6). Peritoneal macrophages (greater than 90% pure by latex ingestion) were isolated from peritoneal lavages by adherence to plastic Petri dishes, as previously described.1,7 The reactivity of each antibody with inflammatory macrophages was then determined using renal biopsies (x24) displaying acute cellular allograft rejection. Antigen expression on epithelioid and Langerhans-type multinucleate giant cells was also determined, using lymph nodes containing sarcoid (x4), cryptococcal (x1), and tuberculous (x1) granuloma. Small tissue blocks or cytospin cell smears (Shandon, England) were fixed in periodate-lysine-parafomaldehyde (PLP)29 at pH 7.2, washed in 0.05 M phosphate buffer solution (PBS), pH 7.2, with 7% sucrose, quick frozen in liquid nitrogen, and stored at −70°C. PLP-fixed tissues were used since preliminary experiments with several fixatives showed PLP-fixation alone provided superior morphological detail compared to unfixed tissues or tissues fixed after sectioning, without significantly reducing the antigenicity of tissues for this group of antibodies. This comparison, based on cell binding assays and immunohistochemistry, was performed using methods recently described in detail.30

Monoclonal Antibodies

A series of 8 monoclonal antibodies reactive with human peripheral blood monocytes was used as undiluted tissue culture supernatants, except for monoclonal antibody 63D3, which was obtained as ascites fluid (BRL, Maryland), and was diluted 1:500 in PBS. The production and specificity testing of 7 of these antibodies has been previously described in detail and is summarized in Table 1. The eighth marker, FMC34, was produced following repeated immunization of a BALB/c mouse with 107 human peripheral blood mononuclear cells, fusion with the nonsecretory P3-x63-Ag8.653 line, and screening of wells for binding to mononuclear cells by indirect immunofluorescence, as described.32 The immunoglobulin subclass of FMC34 was IgM, as determined by Ouchterlony diffusion using subclass-specific antisera (Bionetics, Maryland). A monoclonal antibody to a monomorphic determinant of human Ia-like antigen, 7.2,33 and a rabbit antiserum to human muramidase (lysozyme) (Dako, Denmark), whose specificity has been previously characterized,34 were also employed.

Table 1. Previously Reported Specificity of Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Reference</th>
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<tr>
<td>OKM1</td>
<td>Monocytes, granulocytes, and some NK and null cells</td>
<td>14</td>
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<tr>
<td>63D3</td>
<td>Monocytes and granulocytes</td>
<td>15</td>
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<tr>
<td>PHM2</td>
<td>Monocytes plus 5%-10% T cells</td>
<td>17</td>
</tr>
<tr>
<td>PHM3</td>
<td>60%-70% Monocytes</td>
<td>17</td>
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<tr>
<td>FMC17</td>
<td>Monocytes</td>
<td>23</td>
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<td>FMC32</td>
<td>Monocytes</td>
<td>23</td>
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<tr>
<td>FMC33</td>
<td>Monocytes</td>
<td>23</td>
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<tr>
<td>FMC34</td>
<td>20%-30% Monocytes, plus granulocytes</td>
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*Unpublished.

Immunoperoxidase Technique

A sensitive 4-layer peroxidase-antiperoxidase (PAP) technique was used for tissue localization of monoclonal antibodies.30 Briefly, serial sections (4 μ) of PLP-fixed tissues and cytospin smears were incubated sequentially with 10% normal swine serum (NSS), monoclonal antibody, 1:400 rabbit anti-mouse immunoglobulin serum,1,7 1:400 swine anti-rabbit immunoglobulin serum (Dako), and 1:100 rabbit peroxidase-antiperoxidase complexes (Dako) for 15 min at room temperature. Dilutions were performed in 1% NSS, 1% normal human serum, and 1% fetal calf serum (FCS) (Commonwealth Serum Laboratories, Melbourne, Australia). Localization of the antimuramidase antisera was performed by omission of the monoclonal antibody step and dilution of the primary antibody in 1% FCS alone. Each incubation was followed by 3 x 5 min washes in PBS with 0.2% gelatin added. Peroxidase activity was detected using the substrate diaminobenzidine (DAB) (Sigma, Melbourne, Australia), 0.6mg DAB/ml in 0.05 M Tris-HCl, pH 7.6, with 0.03% H2O2 for 3–5 min. Slides were then counterstained with hematoxylin and mounted. Endogenous peroxidase was blocked by the addition of sodium azide (0.3%) to the DAB solution.35 Specificity controls consisted of substitution of test antibodies by rabbit-specific monoclonal antibodies (6.36 and 6.19; W. Hancock, unpublished results), by the preincubation of test antibodies with blood monocytes purified by overnight adherence to glass,32 and by preincubation of sections and cytospin smears with heat-aggregated Cohn fraction II human gamma globulin to block Fc receptor binding of antibodies.33 FMC34 and antimuramidase antibodies were also absorbed (24 hr, 4°C) with serial dilutions of normal human serum prior to their immunohistochemical application to renal sections.

Enzyme Histochemistry

Histochemical reactions for alpha-naphthyl esterase (ANAE), naphthol AS-D chloracetate esterase, adenosine triphosphatase (ATPase), and acid phosphatase were performed on tissue sections and cytospin cell smears according to Van Heerde et al.34

Criteria for Identification of Cells as Mononuclear Phagocytes

Mononuclear phagocytes in tissue sections were identified using a combination of standard morphological, serologic, and enzyme histochemical criteria. Morphological features were those described by Van Furth.2 Serologic markers consisted of labeling by anti-Ia1 and antimuramidase35 antibodies. Histochemical markers were an appropriate diffuse reaction for ANAE, and in some cases, chloroacetate esterase and acid phosphatase.2 Blood, peritoneal, and alveolar mononuclear phagocytes were also identified using these criteria, plus the in vitro properties of adherence to glass and phagocytosis of latex beads.37 Mononuclear phagocytes defined by this combination of markers were then assessed for reactivity with each monoclonal antibody, using serial sections.

RESULTS

Control sections incubated with rabbit-specific monoclonal antibodies, or antimonocyte monoclonal antibodies previously absorbed with purified human monocytes, were unstained (see below). In contrast, distinctive staining patterns were obtained for 7 of the 8 antimonocyte monoclonal antibodies when applied to sections of tissues known to contain normal and inflammatory cells of the mononuclear phagocyte system. FMC32 and FMC33 antibodies were found to
Table 2. Varying Patterns of Antigen Expression on Tissue Mononuclear Phagocytes as Determined by Immunoperoxidase Labeling With Antimonocyte Monoclonal Antibodies*

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Bone Marrow</th>
<th>Blood</th>
<th>Lymph Node and Spleen</th>
<th>Peritoneal Cavity</th>
<th>Inflammation</th>
<th>Exudate</th>
<th>Granuloma</th>
<th>Dendritic† Cells</th>
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<tr>
<td>PHM2</td>
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<td>PHM3</td>
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<td>FMC32</td>
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<td>OKM1</td>
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* Determined using a 4-layer immunoperoxidase technique applied to PLP-fixed cryostat sections.
† Types of lymphoid and interstitial dendritic cells examined for reactivity are described in detail in Results.
‡ Continuous line denotes reactivity with some or all of the mononuclear phagocytes at the specified site. Broken line denotes trace reactivity with only very infrequent cells being labeled. Absence of any line indicates no reactivity with mononuclear phagocytes at that site.

have an identical distribution, though differing in staining intensity, and probably bind to different epitopes of the same antigen. The possibility that all immunostaining results were due to Fc binding of antibodies to mononuclear phagocytes was excluded by the absence of reactivity with control anti-rabbit monoclonal antibodies of the same immunoglobulin subclasses, by the use of fixed tissue sections, which is known to abolish Fc receptor binding,36 and by the finding that saturation of Fc receptors by preincubation with aggregated gamma globulins had no effect on tissue staining.

A summary of the overall results of tissue labeling is presented in Table 2. Details of labeling of individual tissues and organs are described below.

**Bone Marrow**

Labeled cells were expressed as a proportion of total marrow cells, unless nuclear morphology, cell size, and histochemical criteria allowed further classification. PHM2 antibody stained 1.6% ± 0.7% (mean ± SD) of total cells; these labeled cells included very large multilobed megakaryocytes, scattered smaller cells (15–25 µm) with oval nuclei and abundant cytoplasm suggestive of promonocytes, and more frequent cells (10–15 µm) with lesser cytoplasm and indented or reniform nuclei, similar to blood monocytes. Both the latter mononuclear cell types were found, in adjacent smears of the same marrow specimen, to possess strong diffuse ANAE and antimuramidase activity, but only weak acid phosphatase and no ATPase activity, indicating their monocyte lineage. By contrast, PHM3 antibody did not stain any bone marrow cells. FMC17 (4.5% ± 0.2%), FMC32 (3.8% ± 0.5%), and FMC33 (3.5% ± 0.6%) antibodies all stained very similar proportions of cells; moreover, these cells possessed the same morphological and histochemical characteristics as those labeled by PHM2 antibody (excluding megakaryocytes). FMC34 (74.6% ± 2.6%) and OKM1 (60.9% ± 4.0%) antibodies differed by staining mainly multilobed myeloid cells, although small numbers of monocyte-like cells were also included. 63D3 antibody (9.6% ± 0.6%) stained a mixture of monocytes and some myeloid cells.

**Peripheral Blood**

Immunoperoxidase staining of peripheral blood smears resulted in similar specificities to those previously described (summarized in Table 1). However, the labeling of marrow megakaryocytes by PHM2, as shown in the present study, led to an examination of platelet smears using all 8 antibodies; PHM2 alone was found to also stain platelets. The previously unreported antibody, FMC34, stained only some 26.2% ± 3.1% (mean ± SD) of blood monocytes and greater than 95% of granulocytes.

**Lymph Node**

The majority of subcapsular sinus, medullary cord, and medullary sinus histiocytes displayed only weak antimuramidase reactivity and diffuse ANAE activity. These cells, plus lymphoid follicles and branched cells in paracortical and interfollicular areas, were also la-positive. Few histiocytes were identified outside of these areas. Histiocytes were only weakly and irregularly labeled by PHM2, which also stained endothelial and some fibroblast cells. PHM3 and FMC17 generally failed to stain any cells of normal lymph nodes. In contrast FMC32 and FMC33 antibodies labeled histiocytes (Fig. 1), like those seen with PHM2, but also cells present within lymph node germinal centers and paracortical areas. Within germinal centers, FMC32 and FMC33 antibodies diffusely stained large oval cells in addition to some extracellular matrix labeling. In paracortical and interfollicular areas, as previously noted,23 these antibodies strongly labeled large cells with pale nuclei and branching cytoplasm; these branched cells possessed long cell processes that often
Fig. 1. Immunoperoxidase labeling of human mononuclear phagocytes with antimonocyte monoclonal antibody FMC32. Section of lymph node showing staining of medullary sinus macrophages (M), but not adjacent polymorphonuclear granulocytes (G) or lymphocytes (L) (x1,000).

Fig. 2. Immunoperoxidase labeling with FMC32. Section of liver showing staining of stellate Kupffer cells (K) lining hepatic sinusoids, but not of parenchymal cells (x250).

Fig. 5. Immunoperoxidase labeling with FMC32. Cytocentrifuge smear of alveolar lavage cells showing staining of alveolar macrophages (M), but not granulocytes (G) or alveolar epithelial cells (E) (x160).

Fig. 6. Immunoperoxidase labeling with FMC32. Section of a pulmonary lymph node from a patient with sarcoidosis showing staining of granulomata (Gran), which consist of epitheloid and multinucleate giant cells, plus adjacent interstitial macrophages (M) (x160).
Fig. 3. Immunoperoxidase labeling of peritoneal cells, purified by adherence to glass, using the antimonocyte monoclonal antibody FMC32. Field of control slide selected to show some of the cell types obtained by this procedure, including macrophages (M), lymphocytes (L), and granulocytes (G). Note the absence of either membrane or cytoplasmic staining on this slide, which was stained with FMC32 antibody that had been previously absorbed with blood monocytes (hematoxylin counterstain, x 100).

extended into direct contact with adjacent lymphocytes. Branched cells were also stained with the anti-Ia antibody, 7.2, but did not stain with the antimuramidase serum. In addition, they possessed considerable ATPase activity, but did not show any reaction for ANAE, chloracetate esterase, or acid phosphatase. The remaining three antibodies, FMC34, OKM1, and 63D3, showed variable labeling of macrophages and granulocytes. FMC34 labeled only granulocytes within lymph nodes, and OKM1 stained histiocytes and granulocytes. 63D3 antibody stained histiocytes, an occasional granulocyte, and also weakly labeled arteriolar smooth muscle and endothelial cells.

Spleen

Analogous results to lymph node tissues were found using sections of spleen, except that macrophages (identified morphologically and using antimuramidase, anti-Ia, and ANAE markers) were mainly present in the splenic red pulp. These cells and endothelial cells were weakly stained by PHM2. As found using lymph node sections, PHM3 and FMC17 antibodies were demonstrated at most on only a few macrophages in each section of spleen. However, pulp macrophages and Ia-positive, ATPase-positive, muramidase-negative, ANAE-negative large branched dendritic cells in periarteriolar areas were again stained by FMC32 and FMC33 antibodies. Pulp macrophages and granulocytes were stained by OKM1 and 63D3 antibodies, but FMC34 stained granulocytes alone. No labeling of lymphoid cells was detected.

Thymus

Scattered large oval cells with abundant cytoplasm were stained using the antimuramidase and anti-Ia antibodies. These thymic macrophages, mainly confined to the thymic medulla, also possessed diffuse ANAE but little ATPase activity. Adjacent medullary and septal dendritic cells (interdigitating reticulum cells) expressed Ia antigen and ATPase, but not muramidase or ANAE reactivities. Thymic macrophages were stained by PHM2, which also labeled capillary endothelial cells, and by FMC32, FMC33, OKM1, and 63D3 antibodies. Interdigitating reticulum cells were stained by FMC17, FMC32, and FMC33 antibodies. Cortical thymocytes alone were strongly stained by PHM3 antibody. Thymic epithelial cells, including Hassall’s corpuscles, were labeled
using FMC34 antibody. Except for PHM3, no staining of lymphoid cells was detected.

Liver

Stellate cells lining hepatic sinusoids displayed considerable antimuramidase and diffuse ANAE activity and dense Ia-antigen expression. These Kupffer cells were probably weakly labeled by PHM2, but the labeling of endothelial and connective tissue cells by this antibody made the distinction of Kupffer cells from sinusoidal endothelial cells difficult. However, this distinction was not difficult with the other antibodies, since they produced a more discrete, discontinuous staining of cells lining sinusoids. PHM3 stained only weakly scattered Kupffer cells, and FMC17 was usually negative. Kupffer cells were also stained by FMC32 (Fig. 2), FMC33, and 63D3 antibodies, with FMC34 producing a particularly dense reaction. OKM1 antibody did not stain liver sections, apart from an infrequent granulocyte. With the exception of PHM2 antibody, hepatic parenchymal and biliary tract cells were unstained.

Peritoneal Macrophages

Peritoneal macrophages displaying characteristic morphology, latex phagocytosis, ANAE activity, and antimuramidase and anti-Ia reactivity comprised over 90% of the cultured peritoneal cells examined; the remaining cells included a mixture of granulocytes, lymphocytes, and fibroblasts. Peritoneal cells were not stained by monoclonal antibodies that had been previously absorbed with blood monocytes (Fig. 3). PHM2 stained both macrophages and fibroblasts. Only occasional macrophages (less than 10% of cells) were stained by PHM3, FMC17, and FMC34 antibodies. FMC32 and FMC33 antibodies stained peritoneal macrophages alone (Fig. 4), while OKM1 and 63D3 antibodies stained all macrophages and granulocytes but not lymphocytes or fibroblasts.

Alveolar Macrophages

Alveolar macrophages displayed ANAE, anti-Ia, and antimuramidase reactivity. Additional main morphologically distinct cell types present in smears of alveolar lavages included alveolar and occasionally bronchial or squamous epithelia, lymphocytes, and granulocytes. Alveolar macrophages were stained by PHM2, FMC17, FMC32 (Fig. 5), FMC33, and 63D3 antibodies, variably by OKM1 antibody, but not consistently by PHM3 or FMC34 antibodies. However, both FMC34 and OKM1 antibodies stained granulocytes present within lavage smears, and FMC34 also stained occasional upper airway, but not alveolar, epithelial cells.

Kidney

The reactivity of normal kidney tissues, particularly interstitial cells, was found to vary between antimono-ocyte antibodies. Infrequent interstitial mononuclear cells expressed anti-Ia, antimuramidase, and ANAE activities. However, these cells varied considerably from large oval cells with considerable cytoplasm, to elongated branched cells that were difficult to distinguish from adjacent Ia-positive and partially compressed, intertubular capillaries. Such macrophages were only rarely detected in cross-sections of normal glomeruli. They were stained using PHM2, FMC32, FMC33, OKM1, and 63D3 antibodies but generally not with PHM3, FMC17, or FMC34 antibodies. At least an equal number of interstitial dendritic cells, lacking ANAE or lysozyme, but strongly Ia-positive, were also labeled using FMC32 and FMC33 antibodies. Occasional granulocytes were labeled by FMC34 and OKM1 antibodies. In addition, endothelial cells throughout the kidney were stained with PHM2; glomerular basement membrane (GBM) showed weak punctate labeling using 63D3 antibody; and proximal tubules were stained with both antimuramidase and FMC34 antibodies. However, FMC34 antibody is probably not directed against muramidase (lysozyme), since its staining, unlike the antimuramidase serum, was not abolished following absorption with human serum. Finally, no staining of mesangial cells was found using any of the antibodies tested.

Exudate Macrophages

Sections of renal biopsies from patients undergoing acute cellular allograft rejection included a high proportion of infiltrating large ruffled macrophages within the interstitium, in addition to smaller intravascular and perivascular monocytes, T lymphocytes, and granulocytes, as recently described. These exudate, apparently activated macrophages, were stained by PHM2, FMC32, FMC33, OKM1, and 63D3 antibodies; PHM2 produced only weak labeling, which was also present on endothelium and connective tissues, and OKM1 labeled overall somewhat lesser numbers of macrophages than the other antibodies. Exudate macrophages were not stained by PHM3, FMC17, or FMC34 antibodies, though perivascular monocytes were labeled. In addition, granulocytes were stained by FMC34 and OKM1, and variably by 63D3 antibody.

Granulomatous Macrophages

Selected lymph nodes displaying sarcoid, tuberculous, and cryptococcal granulomata showed typical epithelioid and Langerhans-type multinucleate giant
cells, in addition to inflammatory macrophages between and around granulomata. Foreign-body-type granulomata were not examined. The intensity of staining of granulomata varied considerably, but both giant cells and epithelioid cells stained with PHM2, FMC32 (Fig. 6), FMC33, and 63D3 antibodies, though generally not with PHM3, and FMC17, OKM1, or FMC34 antibodies. Single interstitial OKMI, giant cells and epithelioid cells stained with PHM2, staining of granulomata varied considerably, but both granulomata were not examined. The intensity of HUMAN MACROPHAGE ANTIGENIC HETEROGENEITY 1277 described for normal lymph node tissues.

sections maintained the same pattern of reactivity as erogeneity has not been previously determined, since exudate macrophages. Whether human mononuclear macrophages stained only with PHM2, FMC32, FMC33, OKM1, and 63D3; no labeling was seen with PHM3, FMC17, or FMC34 antibodies. Surviving lymphoid tissues present within adjacent areas of these sections maintained the same pattern of reactivity as described for normal lymph node tissues.

DISCUSSION

This study presents a fresh immunologic approach to the problem of recognition of human mononuclear phagocytes in situ, in both normal and disease states. Previous studies in laboratory animals have shown that variable antigen expression can occur between macrophages present at the same site, between macrophages present at different sites, and between resident and exudate macrophages. Whether human mononuclear phagocytes exhibit a similar degree of antigenic heterogeneity has not been previously determined, since research into human mononuclear phagocytes has been only fragmentary. Using a sensitive immunoperoxidase technique, we initially established that monoclonal antibodies to human monocyte surface antigens could be used to identify normal resident mononuclear phagocytes at different stages of maturation and in several different tissues. These antibodies were then used to detect exudate macrophages participating in both acute and chronic inflammatory responses. This approach, in conjunction with accepted serologic and histochemical markers for macrophages, therefore allowed documentation of the changing antigenic profile present during human monocyte/macrophage differentiation.

Four main conclusions were apparent from this study. First, surface antigen expression may correlate with, and in some instances be limited to, particular stages of mononuclear phagocyte differentiation. Thus, PHM3 antibody labeled a differentiation antigen that was expressed on monocytes only after their entry into the circulation. Indeed, apart from very low levels of antigen on peritoneal macrophages and Kupffer cells, PHM3 binding appears to be restricted to circulating monocytes. Similarly, FMC17 antibody labeled bone marrow precursors and circulating monocytes, but was not detected on tissue macrophages, except, as discussed below, in the lung. FMC17 there-fore appears to define a second and different differentiation antigen to PHM3.

Second, certain mononuclear phagocyte antigens, such as those defined by FMC17 and FMC34 antibodies, have a restricted organ distribution. Outside the circulation, FMC34 antigen was expressed only on Kupffer cells. Since FMC34 labeled some 20%-30% of circulating monocytes, and in the mouse, an estimated 56% of circulating monocytes leave the blood to lodge in the liver as Kupffer cells, we can speculate that FMC34 antigen expression on monocytes could be of functional significance in determining which cells are destined to become Kupffer cells. This remains to be investigated. Alternatively, FMC34 antigen detection on only a subpopulation of blood monocytes may reflect technical limitations, since in some experiments, all monocytes cultured in vitro have been shown to bind FMC34 antibody (H. Zola, unpublished observations); clearly extrapolation of in vitro results to tissues requires some caution. Like FMC34, FMC17 labeling also appears to be organ selective, being confined to alveolar macrophages. However, since FMC17 binds to almost all circulating monocytes, any relationship of FMC17 antigen expression specifically to alveolar macrophage ontogeny is difficult to assess. A related antigen defined by a monoclonal antibody VEP6,40 with specificity for alveolar macrophages, has recently been described in the rat. However, the VEP6 antigen is also expressed on platelets but not peripheral blood monocytes, and hence, is almost certainly not an analogous antigen to that bound by either FMC17 or PHM2 antibodies, which both bind blood monocytes.

Third, antigenic differences were found between circulating monocytes and macrophages present within inflammatory foci. This occurred in both the acute and chronic inflammatory models we studied. Previous studies have shown that mononuclear phagocytes, in varying stages of differentiation, contribute significantly to the cellular infiltrate present within transplanted kidneys undergoing acute interstitial rejection. In this study involving 25 different rejecting renal allografts, exudate macrophages were stained by PHM2, FMC32, FMC33, and 63D3 antibodies. Varying proportions of macrophages were also stained by OKM1, and OKM1 and FMC34 antibodies labeled all infiltrating granulocytes. However, except for infrequent perivascular monocytes, these macrophages were not labeled by PHM3, FMC17, or FMC34. Similarly, epithelioid or multinucleate giant cells within granulomata stained with PHM2, FMC32, FMC33, and 63D3 antibodies but usually not with OKM1, despite the presence of OKM1-positive macrophages adjacent to these granulomata. Neither granulomata nor adjacent macrophages labeled with PHM3,
FMC17, or FMC34 antibodies. Therefore, monoclonal antibodies are available that recognize antigens present on circulating monocytes but which are lost or markedly decreased in quantity on entry into inflammatory sites. This loss of antigen from the macrophage cell surface may conceivably be related to susceptibility to local extrinsic factors, such as a high concentration of proteolytic enzymes, rather than an intrinsic change in surface antigens related to cell differentiation. Conversely, some monocyte antigens, such as those defined by PHM2, FMC32, FMC33, and 6D3 antibodies, persist through all stages of mononuclear phagocyte differentiation, including end-stage granuloma formation, and hence are apparently unaffected by in vivo macrophage stimulation or activation. These four antibodies, directed against cell lineage antigens, are therefore valuable immunohistologic markers for mononuclear phagocytes throughout maturation.

Fourth, these studies illustrate the sharing of antigens between mononuclear phagocytes and other hemopoietic cells, and also with unrelated cell types. Such sharing may suggest underlying relationships, including related cell ontogeny. Thus, the labeling of macrophages and interdigitating reticulum cells by FMC17, FMC32, and FMC33 antibodies is in agreement with previous studies based on enzyme histochemistry, which have suggested that dendritic cells belong to the mononuclear phagocyte system.41-43 However, sharing of monocyte antigens with endothelium and fibroblasts (PHM2), epithelium (FMC34), or cortical thymocytes (PHM3) does not appear to be of functional or developmental significance and is perhaps analogous to the sharing of Thy-1 antigen between thymus and neurones. Whatever the mechanism, this antigen sharing between cell types would be of importance if attempts are made to discriminate macrophages from other cells in tissue sections or in cell suspensions from various organs, using these antibody markers.

Other monoclonal antibodies reactive with human monocytes have been described. 16, 18, 22, 24-26 However, the precise tissue distribution of each of these markers has not been determined beyond blood and, in some cases, bone marrow, though UC45 antigen has been detected on neurones.21 A preliminary analysis of the tissue reactivity of several of these monocyte markers has shown that at least some also bind to tissue macrophages.46 In addition, further evidence was detected of the sharing of monocyte antigens with cells of disparate lineage, such as epithelial and endothelial cells of various organs.

The availability of antimonocyte monoclonal antibodies with demonstrable reactivities with either particular subpopulations or all mononuclear phagocytes provides an important tool for future studies of macrophage differentiation and function. Moreover, such antibodies may have clinical application through their use as markers for classification of histiocytic tumors and for other diseases involving mononuclear phagocytes. However, the choice of appropriate markers for use in studies involving a particular organ or inflammatory process appears critical due to the antigenic heterogeneity of human mononuclear phagocytes demonstrated in this study.

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HUMAN MACROPHAGE ANTIGENIC HETEROGENEITY


Antigenic heterogeneity of human mononuclear phagocytes: immunohistologic analysis using monoclonal antibodies

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