# *Dendritic Cell and Macrophage Staining by Monoclonal Antibodies in Tissue Sections and Epidermal Sheets*

THOMAS J. FLOTTE, MD,
TIMOTHY A. SPRINGER, PhD,
and G. JEANETTE THORBECKE, MD, PhD

*From the Department of Pathology, New York University School of Medicine, New York, New York, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

**Mouse tissue sections were stained by monoclonal antibodies to macrophage antigens (Mac-1 (M1/70), Mac-2 (M3/38), Mac-3 (M3/84) with the use of immunoperoxidase. Mac-1 was located diffusely in the cytoplasm of round cells in a high percentage of alveolar macrophages, resident peritoneal and bone marrow cells, in splenic red pulp, and in rare perivascular cells in the thymus. Mac-1 was absent in epithelial cells and Langerhans cells. Mac-2 was strongly positive in many dendritic cells in the thymic medulla, more than the cortex, in paracortex and medulla of lymph nodes, sparing the follicles, and in the marginal zone of spleen. There were a few positive cells in germinal centers. Mac-2 was located in a low percentage of bone marrow and a high percentage of resident peritoneal cells. When positive in sections Mac-3 always showed granular cytoplasmic staining. Bone marrow showed a high percentage of cytoplasmic staining (>50%), as compared with low surface staining (<1%). It was found in hematopoietic cells, and in all endothelium, including postcapillary venules and lining of sinuses. It was probable that the resulting dendritic staining pattern for Mac-3 in paracortex of lymph node, white and red pulp, thymic cortex, and medulla included dendritic cells other than endothelial cells. Alveolar macrophages and Kupffer cells were positive for Mac-2 and Mac-3. Mac-3 also stained bile canaliculi. Clearly different staining patterns were found in epithelial cells for Mac-2 and Mac-3 in kidney tubules, intestinal mucosal lining, bronchi, choroid plexus, and epidermis. (Am J Pathol 1983, 111:112–124)**

MACROPHAGES AND MONOCYTES are a heterogenous group of cells distinctive for their abilities to phagocytize and to carry out various functions in the immune response. Recently it became evident that several groups of morphologically dendritic cells, including Langerhans cells,[1-7] interdigitating dendritic cells in the lymph node,[8-11] follicular dendritic cells,[12-15] lymphoid dendritic cells[16-19] of lymphoid cell suspensions, and the thymic mesenchymal cells,[20-24] should be regarded as distinct from ordinary macrophages. Although some of these cells share features in common with macrophages, such as cell membrane adenosine triphosphatase (ATPase), Ia antigens, and Fc and C3b receptors, they differ in their dendritic shape, location, and the presence of certain surface antigens (recently reviewed by Thorbecke et al[25] and Tew et al[26]). OKT6 has been shown to be present on Langerhans cells and on some dendritic cells in the paracortex as well as 70% of thymocytes, but not on monocytes.[8,9,27] S100 was previously thought to be limited to nerve tissue and is absent from monocytes but has been recently shown to be present on Langerhans cells and interdigitating dendritic cells.[28,29]

In the present study we were interested in looking at the tissue distribution of certain recently described murine macrophage antigens to determine whether distinctive subsets could be found and in particular, whether these antigens would also be present on some

Supported by NIH Grants AI-03076, AM-26091 and CA-31799, Council for Tobacco Research Grant 1307, Training Grant GM-07552 from the NIGMS, NIH, and the American Cancer Society Junior Faculty Fellowship.

Accepted for publication November 12, 1982.

Address reprint requests to G. Jeanette Thorbecke, MD, Department of Pathology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

0002-9440/83/0407-0112$01.45 

of the dendritic cells. A set of mouse macrophage cell membrane antigens (Mac-1, Mac-2, Mac-3) are identified by rat anti-mouse monoclonal antibodies M1/70, M3/38, M3/84, respectively. The presence of the antigens on hematopoietic cell surfaces has been previously determined by immunofluorescence. Mac-1[30-32] is an antigen containing 95,000 and 170,000 $M_r$ subunits and is present on resident peritoneal macrophages, splenic macrophages, granulocytes, monocytes, and 50% of bone marrow cells. It is also present on peritoneal exudate cells elicited by thioglycollate, protease peptone, lipopolysaccharide (LPS), concanavalin A (Con A), and *Listeria monocytogenes*. Thymocytes and peripheral lymph node cells lack Mac-1. Mac-3,[33,34] a 110,000 $M_r$ polypeptide, is present on unelicited peritoneal macrophages and on peritoneal exudate macrophages elicited by a wide variety of agents. Thymocytes, peripheral lymph node cells, and greater than 95% of bone marrow cells are negative. Mac-2,[34,35] a 32,000 $M_r$ polypeptide, is not on resident peritoneal macrophages but is present on thioglycollate- and on 20% of protease peptone-induced peritoneal exudate cells. It is not present on peritoneal exudate cells induced by LPS, Con A, or *L monocytogenes*, as shown by immunofluorescence, but 10- to 30-fold lower quantities than on thioglycollate-elicited cells can be detected by $^{35}$S-methionine labeling and immunoprecipitation. Mac-2 is negative by immunofluorescence and $^{35}$S-methionine incorporation in lymph node lymphocytes, and is also negative by immunofluorescence on splenic macrophages, granulocytes, thymocytes, and >95% of bone marrow cells.

## Materials and Methods

### Animals

BALB/c mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

### Monoclonal Antibodies

The derivations of subcloned lines M1/70,[30] M3/38,[34,35] M3/84[33,34] M5/114,[36] M1/89,[34] M17/5,[37] and 2.4G2[38] have been described previously. In brief, spleen cells from rats immunized to mouse spleen cells (M1/70, M5/114, M1/89) or thioglycollate-elicited peritoneal exudate cell glycoproteins depleted of previously recognized antigens with monoclonal immunoabsorbents (M3/38, M3/84, M17/5) or J774 cell line (2.4G2) were fused with the mouse myeloma NSI or P3U1. The cells were grown in Dulbecco's Modified Eagle's medium supplemented with 10% horse serum or 5% fetal calf serum. The culture supernatants were harvested and used as the source of monoclonal antibodies.

### Tissue Sections

BALB/c mice were sacrificed by cervical dislocation and portions of lymph nodes, thymus, spleen, bone marrow, kidney, liver, intestine, lung, brain, and skin were taken for processing in paraffin and in polyester wax (BDH Chemicals Ltd., Poole, England) and for frozen sections. Resident peritoneal exudate cells were obtained by washing the peritoneal cavity with heparinized RPMI 1630 (GIBCO, Grand Island, New York). The cells were pelleted by centrifugation and processed for polyester wax and frozen sections.

Tissue for paraffin was fixed in Carnoy's fixative for 45 minutes, transferred to 60% ethanol, processed in an 8-hour cycle in an Auto-Technicon Ultra from alcohol to paraffin (Paraplast, Brunswick Company, St. Louis, Mo) and finally embedded in paraffin. Tissue for polyester wax was fixed in cold ethanol for 2 hours and subsequently processed through alcohol, alcohol/polyester wax, and polyester wax at 37 C. Four-micron sections were cut from the paraffin- and polyester-wax–embedded tissues. Tissue for frozen sections was placed in OCT compound (Miles Laboratories, Naperville, Ill), frozen, and cut to approximately 6 $\mu$ in a cryostat (Ames Cryostat II, Miles Laboratories). The sections were stored in a freezer at −20 C until stained.

### Epidermal Sheets

The mice were killed by cervical dislocation, shaved, depilated, rinsed with saline, and skinned. The pelts were cleaned of underlying tissue, cut into 1-sq cm pieces, and floated dermal-side-down on 1 M sodium bromide for 30 minutes at 37 C. The epidermis was peeled from the dermis with fine forceps. The sheets were rinsed in phosphate-buffered saline.

### Immunoperoxidase

Sections were deparaffinized in xylene and alcohol or dewaxed in alcohol. Frozen sections were thawed and dried, and the OCT compound was then removed in water. The sections were incubated in 0.3% hydrogen peroxide in methanol for 30 minutes to eliminate endogenous peroxidase activity. The sections were incubated with the primary antibody, followed by the bridge antibody and then rat peroxidase–antiperoxidase complexes. The primary anti-

body was either M1/70, M3/38, M3/84, M1/89, M17/5, 2.4G2 (diluted to 1–10 μg/ml), normal rat serum (diluted 1:10), or purified rat IgG (diluted to 10 μg/ml, Pel-Freez Biologicals, Rogers, Ariz). The bridge antibody was rabbit anti-rat γ1, γ2a, or γ2b (diluted 1:20, Pel-Freez Biologicals). The rat peroxidase–antiperoxidase complexes (Sternberger-Meyer Immunocytochemicals Inc., Jarretsville, Md) were diluted 1:100. The incubations in antiserums were separated by washing in two changes of Tris-buffered saline (0.05 M, pH 7.6). The sections were stained with 0.075% diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo) and 0.003% hydrogen peroxide in Tris-buffered saline for 7 minutes. The reaction was terminated by washing with water. The sections were counterstained with methyl green.

Epidermal sheets were stained with the use of similar protocols except that the epidermal sheets were floated on 50 μl of the appropriate reagents. The reagents, antibodies, and washing solutions contained 0.1% sodium azide. Incubations and washes were performed at 4 C for 1 hour and 20 minutes, respectively. The sections were fixed in 5% neutral buffered formalin. No counterstain was used.

## Results

### Effects of Processing

BALB/c lymph nodes were processed for paraffin, polyester wax, and frozen sections as described in Materials and Methods. Sections of lymph nodes, thymus, spleen, bone marrow, skin, and kidney were stained by the immunoperoxidase method for Mac-1, Mac-2, Mac-3, and Ia antigens. The frozen sections showed staining for all four antigens, but the morphologic characteristics were not as well preserved as with the other methods. The polyester wax sections showed staining for Mac-2, Mac-3, and Ia antigens, but not for Mac-1. The morphologic characteristics were better than those of frozen sections, although not as good as those of paraffin sections. The paraffin sections only showed staining for Mac-2. Therefore, in this study, frozen sections were used for the study of the distribution of Mac-1, paraffin for Mac-2, and polyester wax for Mac-3 and Ia antigens. It should be emphasized that the staining characteristics, ie, granular or diffuse, and pattern were not altered by the method of processing.

### Antigen Distribution

The staining patterns seen in lymphoid and nonlymphoid organs are described in Tables 1 and 2 and illustrated in Figures 1–4. The staining of the cytoplasm of the cells was diffuse and homogeneous for Mac-1 and Mac-2, whereas it was discrete and granular for Mac-3.

The previously described immunofluorescent surface staining and $^{35}$S-methionine incorporation studies[30–32,35] and the immunoperoxidase staining of sections in the present study corresponded well for both Mac-1 and Mac-2 antigens. The weakly positive staining for Mac-2 in resident peritoneal macrophages in sections could represent a true difference between surface and cytoplasmic localization. It could also represent the greater sensitivity of the immunoperoxidase technique than of the immunofluorescence technique. This interpretation is in agreement with the finding that resident peritoneal cells are negative by immunofluorescence but weakly positive by immunoprecipitation of $^{35}$S-labeled antigen.[35] The intracellular distribution for Mac-3 differed from that previously obtained by surface immunofluorescence. Bone marrow cells showed <5%

Table 1—Primary and Secondary Lymphoid Organs

| Organ | Mac-1 | Mac-2 | Mac-3* |
|---|---|---|---|
| Bone marrow | 40% positive cells | 5% positive cells | 50% positive cells |
| Thymus | Rare round positive perivascular cells | Dendritic cells, more in medulla than in cortex | Dendritic pattern in cortex and medulla |
| Spleen | Round cells in marginal sinus, red pulp | DC in white pulp, round cells in marginal zone, red pulp, few in germinal centers | Dendritic pattern in white pulp, many cells in red pulp including hematopoietic cells |
| Lymph node | Round cells in marginal sinus, medullary cord | Numerous DC paracortex, medullary cords, round cells in marginal sinus | Dendritic pattern in paracortex, endothelial cells in sinuses, medullary cords and postcapillary venules, 50% of round cells in marginal sinus |
| Gut-associated lymphoid tissue | Few positive round cells | Dendritic cells in T cell areas and scattered round cells | Dendritic pattern |
| Resident peritoneal cells | 80% positive | 5% strongly positive, remainder weakly positive | 80% positive |

* Mac-3 antigen was found in lymphatic and vascular endothelial cells in every organ. It was not possible to be certain whether the dendritic pattern represented dendritic cells and endothelium or endothelium alone.

Table 2—Nonlymphoid Organs

| Organ | Mac-1 | Mac-2 | Mac-3* |
|---|---|---|---|
| Liver | Negative | Cells lining the sinusoids | Cells lining the sinusoids, bile canaliculi |
| Lung | Alveolar macrophages | Alveolar macrophages, bronchial epithelium | Alveolar macrophages and lining cells, bronchial epithelium |
| Intestinal villi | Round cells in lamina propria | Round cells in lamina propria, cytoplasm of epithelial cells increasing from bases to tips of villi | Round cells in lamina propria, luminal border of epithelial cells evenly distributed |
| Kidney | Negative | Only some tubules stained, all levels of the tubular epithelium and urothelium were positive | Luminal borders of all renal tubular epithelia were positive but much stronger in cortex than medulla, glomeruli positive |
| Skin† | Negative | Keratinocytes, hair follicles, sweat ducts positive | Keratinocytes, hair follicles, sweat ducts positive |
| Brain‡ | Negative | Choroid plexus and ependyma positive | Choroid plexus and ependyma positive |

* Mac-3 antigen was found in lymphatic and vascular endothelium in every organ.
† Langerhans cells received special attention. They were unstained with Mac-1, but they could not be definitively evaluated for Mac-2 and Mac-3 because of the keratinocyte staining.
‡ In view of the paucity of microglia in normal brain, a definitive statement regarding microglial staining is not possible.

surface staining[33] and 50% cytoplasmic staining in sections.

The staining of lymphoid organs was different for each antibody. Lymphocytes were not stained by any of the antibodies in any organ. Round, free-lying macrophages, such as peritoneal, alveolar, red pulp, marginal sinus, and medullary cord macrophages, stained with all three monoclonal antibodies. Mac-1 stained only these cells and did not stain any dendritic cells (Figure 3).

Dendritic cells were stained both by Mac-2 and Mac-3, but in two different patterns (Figure 1). Mac-2 stained more cells in the thymic medulla than in the cortex, and in peripheral lymphoid tissue more cells in the paracortex and white pulp, sparing the follicles, than in the medulla and the red pulp. The few positive cells in the germinal centers were large, had the typical appearance of tingible body macrophages, and were not in the location of the follicular dendritic cells (corona) described by Nossal and co-workers.[15] There were many more positive cells in the lymph nodes than in the spleens. Mac-2 stained a subpopulation of 5% resident peritoneal cells strongly and the remainder weakly. This appears to correlate with the finding that peritoneal macrophages elicited by different techniques vary 30-fold in the quantity of Mac-2 expressed.[35]

Mac-3 stained dendritic cells that were more evenly distributed between the thymic medulla and the cortex and that were also present throughout the lymph nodes and splenic white pulp, sparing only the follicles. The staining of lymphatic and vascular endothelium made the interpretation of the Mac-3 pattern difficult, since it was not certain whether it included dendritic cells and endothelium or endothelium alone (Figures 1 and 2). Our interpretation was, however, that the staining was too extensive to be limited to endothelium and, indeed, represented both endothelium and dendritic cells. The endothelium of the postcapillary venules and the lining cells of the sinuses in the medulla were among the cells staining darkest for Mac-3 in the lymph node (Figure 2).

Staining for Ia antigens in the thymus showed a pattern of dendritic cells throughout the cortex and medulla that was far greater than that for either Mac-2 or Mac-3, which is consistent with previous reports that Ia antigens are expressed on both epithelial and mesenchymal dendritic cells.[24]

In nonlymphoid organs, the Mac-1 antigen was not located in the nonlymphoid tissues such as epithelia (Figures 3 and 4). It was present on the classical macrophages in lymphoid infiltrates such as in the lamina propria of the gut and in alveolar macrophages but not in Kupffer cells.

Mac-2 was present in some cells of lymphoid infiltrates, alveolar macrophages (Figure 3), and cells lining the sinusoids (Figure 4), as well as in certain epithelial cells. Even within a single organ the epithelia did not stain uniformly (Figure 4). Intestinal epithelial cells at the base of villi stained weakly, whereas those at the tips of the villi stained stronger. This pattern for Mac-2 in the intestine is similar to the degree of cytoplasmic eosinophilia. The differential ability to absorb fat from the lumen follows a similar distribution.[39] Some renal tubules stained, whereas others did not. The pattern was apparently unrelated to the level of the tubule involved. Glomeruli were negative.

Mac-3 was present in many more cells than Mac-2. In the lung, staining was seen in alveolar macrophages, bronchial epithelium, and alveolar lining cells (Figure 3). In the liver, bile canaliculi as well as the cells lining the sinusoids stained (Figure 4). In the intestinal epithelium, Mac-3 was evenly distributed

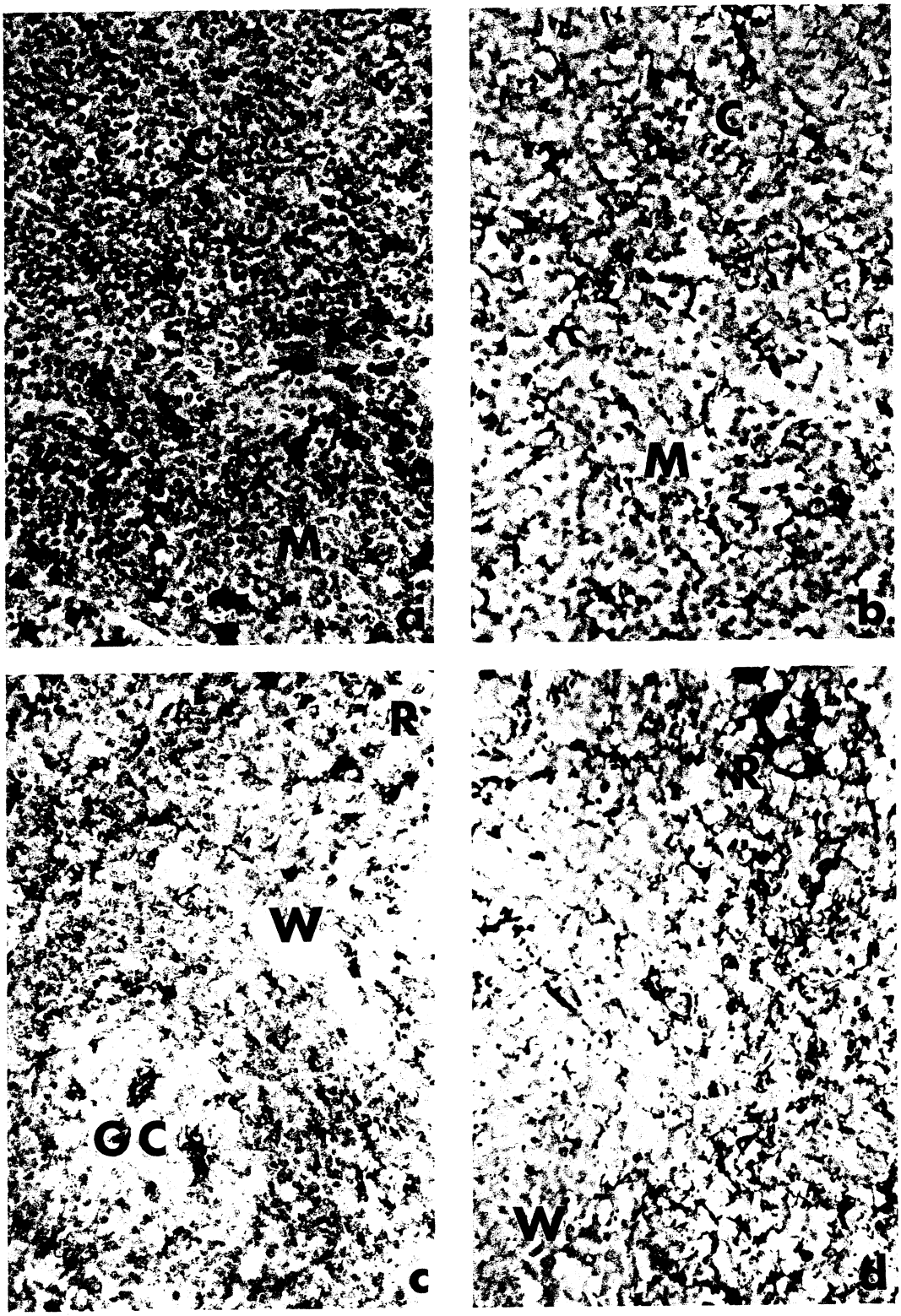


**Figure 1a** – Mac-2 staining of thymus. More positive dendritic cells are present in the medulla (*M*) than in the cortex (*C*). **b** – Mac-3 staining of thymus. There is a relatively even distribution of positive cells, but if anything, they are more frequent in the cortex. **c** – Mac-2 staining of spleen. Dendritic cells in the white pulp (*W*) and dendritic cells and macrophages in the red pulp (*R*). Notice the positive "tingible" body macrophages in the germinal center (*GC*). **d** – Mac-3 staining of spleen. Dendritic cells in the white pulp and dendritic and round cells in the red pulp. The round cells are hematopoietic cells, including megakaryocytes, and macrophages. (Immunoperoxidase, nuclear staining by methyl green, ×250)

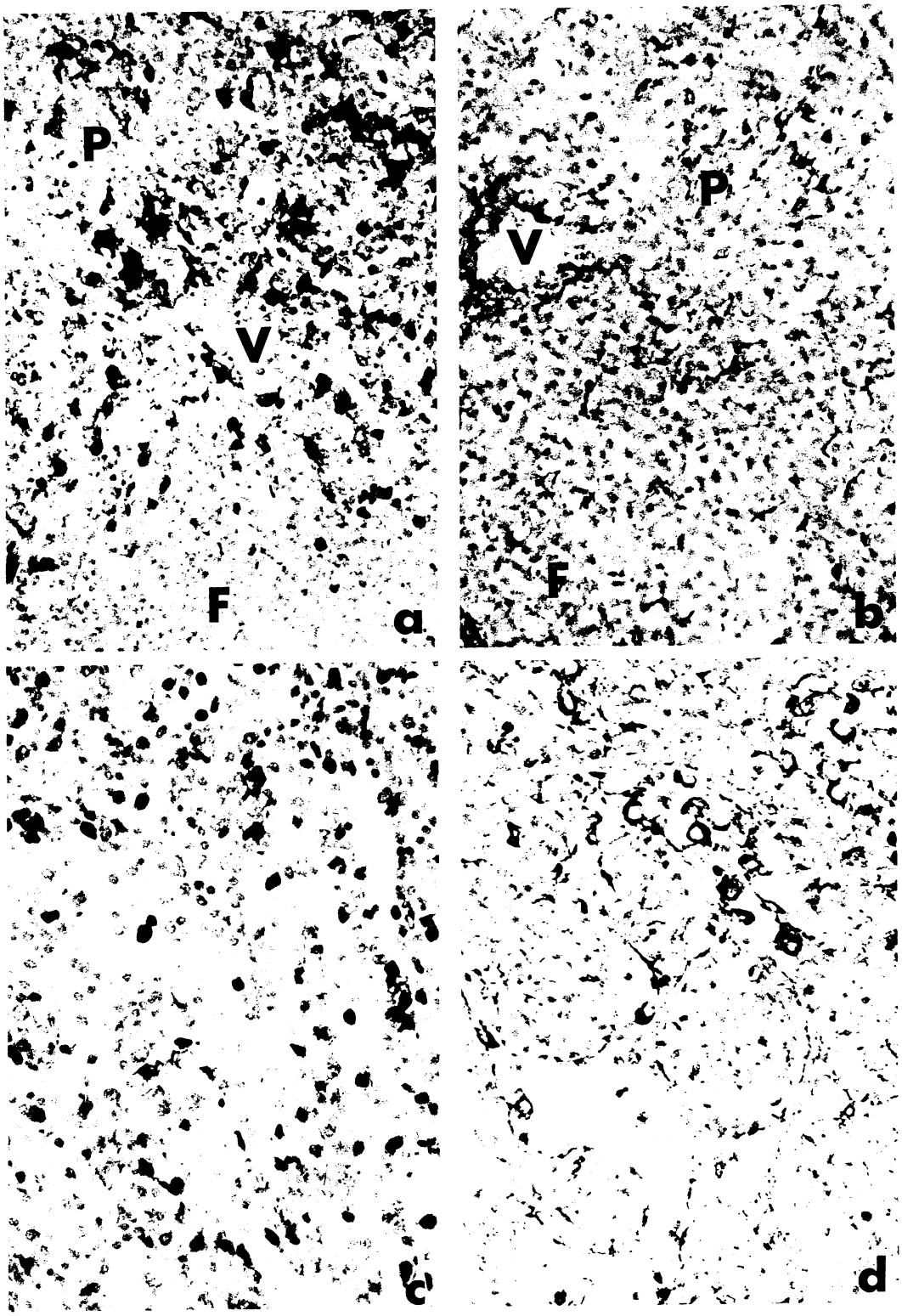


**Figure 2a** — Mac-2 staining of lymph node cortex. Many positive cells are present in the paracortex (*P*), while few are positive in the follicle (*F*). Notice that the postcapillary venule (*V*) is negative. **b** — Mac-3 staining of lymph node cortex. There are many positive dendritic cells in the paracortex, but also a few staining cells in the follicle. Notice the positive postcapillary venule. **c** — Mac-2 staining of lymph node medulla. Note many positive tissue macrophages in the medullary cords and some free lying cells in sinusoids. **d** — Mac-3 staining of lymph node medulla. Many positive cells are seen lining the sinuses and lying free in the sinusoids. The cytoplasmic processes of the cells lining the sinuses are clearly visible. (Immunoperoxidase, methyl green, ×250)

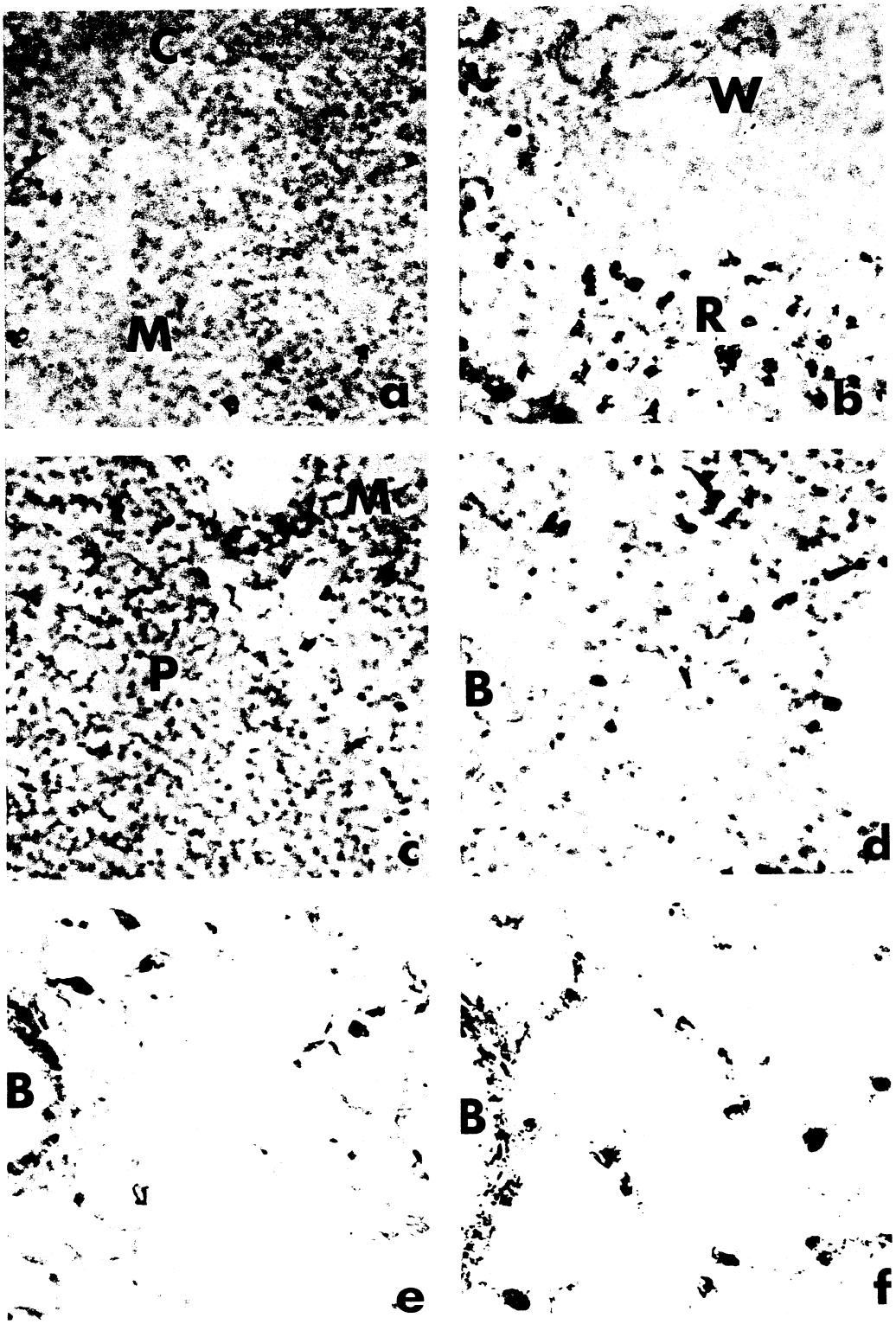


**Figure 3a** — Mac-1 staining of thymus. Scattered, round, positive cells are most frequent in cortico(*C*)-medullary (*M*) junction. **b** — Mac-1 staining of spleen. Round, positive cells are present in the red pulp (*R*) and absent from the white pulp (*W*). **c** — Mac-1 staining of lymph node. Round positive cells are seen in the medulla (*M*). The follicles and paracortex (*P*) are negative. **d** — Mac-1 staining of lung. Alveolar macrophages are positive. Bronchiolar epithelium (*B*) is negative. **e** — Mac-2 staining of lung. Alveolar macrophages and bronchiolar epithelium are positive. **f** — Mac-3 staining of lung. Alveolar macrophages, bronchiolar epithelium, and alveolar lining cells are positive. Although not well-illustrated here, free-lying macrophages are also positive. (Immunoperoxidase, methyl green, ×250)

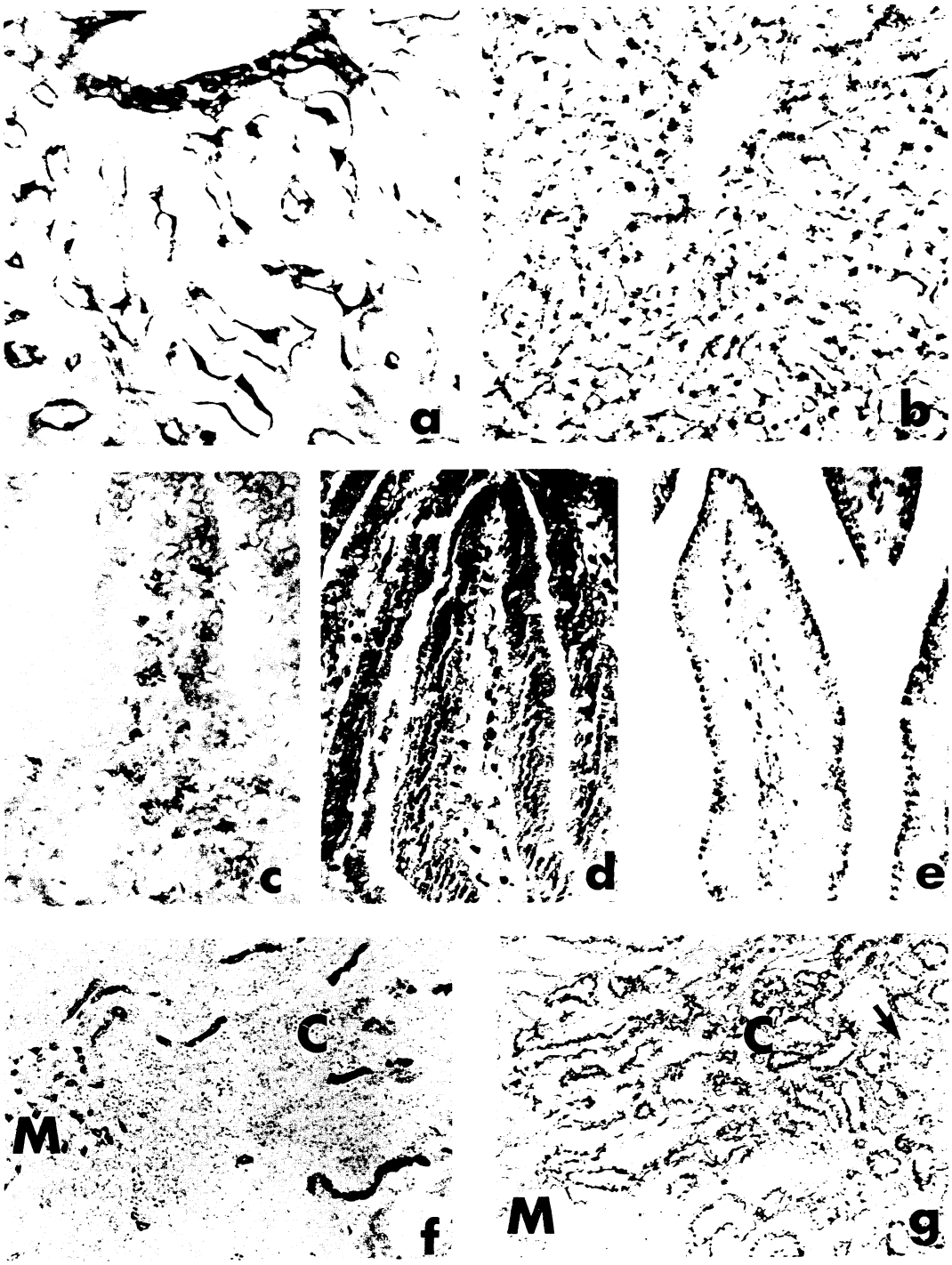


**Figure 4a** – Mac-2 staining of liver. The cells lining the sinusoids, including Kupffer cells, are positive. **b** – Mac-3 staining of liver at the same magnification as **a**. The cells lining the sinusoids, including Kupffer cells and the bile canaliculi are positive. **c** – Mac-1 staining of small intestine. There are positive, round cells in the lamina propria. The epithelium is negative. **d** – Mac-2 staining of small intestine. Note positive, round cells in the lamina propria. The cytoplasm of epithelial cells shows increasing staining from the base to the tips of villi. **e** – Mac-3 staining of small intestine. There are positive cells in the lamina propria. There is staining of the luminal borders of epithelial cells evenly distributed from the base to the tips of villi. **f** – Mac-2 staining of kidney. Some tubules are stained in both the cortex (*C*) and the medulla (*M*). **g** – Mac-3 staining of kidney. Positive staining is seen in the glomeruli (*arrow*) and tubules in the cortex. Medulla is predominantly negative. (Immunoperoxidase, methyl green, **a**–**e**, ×250; **f** and **g**, ×150)

from the base of the villi to the tips. The cortical renal tubules were positive, but the medulla was not. Glomeruli were positive.

Epidermis was studied with the use of both sections and epidermal sheets. No staining of epidermis, either of Langerhans cells or of keratinocytes, was seen in frozen sections or epidermal sheets for Mac-1. Sections and epidermal sheets stained for Mac-2 and Mac-3 showed diffuse staining of the keratinocytes. It could not be determined by light-microscopic examination of these slides whether Langerhans cells were also positive. Epidermis stained by monoclonal antibodies for the common leukocyte antigen (Figure 5), Fc receptors, and Ia antigens (Figure 5) all show suprabasalar dendritic cells in sections and nonoverlapping dendritic pattern with increased density about hair follicles, characteristic of Langerhans cells, in epidermal sheets.

## Discussion

Our results highlight two technical points of interest. First, the method of processing the tissue has marked effects on the results produced. Paraffin and frozen sections are the most common methods of processing tissues. However, polyester wax can provide better preservation of morphologic characteristics than frozen sections. It also removes or destroys fewer antigens than does paraffin embedding because of its lower melting point (37 C), and because it is miscible with alcohol, it thus requires no clearing agents such as xylene. Second, there are an ever increasing number of antibodies being produced to surface antigens. These antibodies are generally only screened for surface staining in cell suspension or for absorption by cells or insoluble membranes. This study demonstrates the dramatic differences that can be seen when the antibodies are also screened on tissue sections, where they may demonstrate a predominantly cytoplasmic localization not realized previously. Expression of antigens on the surface and intracellularly is not unexpected. Recyclable receptors are an interesting class of proteins found both on cell surface and in intracellular compartments. For example, the phosphomannose receptor, with transfers extracellular phosphomannosyl enzymes to lysosomes, is found 10% in plasma membranes and 90% in endoplasmic reticulum, Golgi bodies, and lysosomes.[40]

Immunoperoxidase staining of lymphoid and nonlymphoid tissues for macrophage antigens with rat anti-mouse monoclonal antibodies showed interesting patterns of distribution. While none of the antibodies stained lymphocytes, they did define at least two populations of cells. The first group shows staining for all three macrophage antigens tested, Mac-1, Mac-2, Mac-3. Morphologically, these cells are round and usually have abundant cytoplasm. They also tend to have limited contact with the surrounding tissues, such as is the case for alveolar, peritoneal, and sinus macrophages. This group is composed of those cells that are generally thought of as "classic" macrophages. An exact definition may be those cells that express both Mac-1 and Mac-2 antigens. Mac-1 alone would almost be the best definition, except that it is present on granulocytes and 50% of bone marrow cells. Mac-2 is present on a variety of cells but not on granulocytes or on 95–99% of bone marrow cells. Therefore, the expression of both Mac-1 and Mac-2 appears to be limited to those cells that have been thought of as "classic" macrophages. The second population consists of dendritic cells located in primary and secondary lymphoid organs and liver. These cells do not contain Mac-1 but do contain Mac-2. Mac-3 is most likely present in these cells, but that cannot be stated with certainty, because in most organs the abundance of endothelial cells that definitely stain with Mac-3 precludes a clear evaluation of the staining of other dendritic cells, such as the ones stained by Mac-2.

Langerhans cells were shown to express the common leukocyte antigen and Fc receptors but not Mac-1. The common leukocyte antigen is equivalent to Ly-5 (T. A. Springer, unpublished observations) and is expressed by all leukocytes and hematopoietic stem cells but not by nonhematopoietic tissues.[41,42] This represents further evidence of the hematopoietic origin of these cells. Other experiments[43] using epidermal cell suspensions have shown that Langerhans cells express Mac-2 and Mac-3 antigens.

The relation of the dendritic cells stained by these antibodies to the previously described dendritic cells is not clear. The thymus contains two dendritic cell populations,[20–24] the mesenchymal and the epithelial, both of which are thought to express Ia antigens. The reported staining pattern for Ia antigens,[24] as well as that seen in our laboratory, is much more extensive than that seen for either Mac-2 or Mac-3. Therefore, these antibodies must be recognizing only one of the Ia+ populations or a subset of one. In the lymph node and spleen one can find at least three types of dendritic cells, the follicular,[12–15] interdigitating,[8–11] and reticular cells.[44] None of the antibodies stained the follicular dendritic cells. Mac-2 and probably Mac-3 antigens were found in the interdigitating dendritic cells in the T-cell zones as well as in additional cells located in red pulp and medullary cords, which may or may not include the reticular cells. The loca-

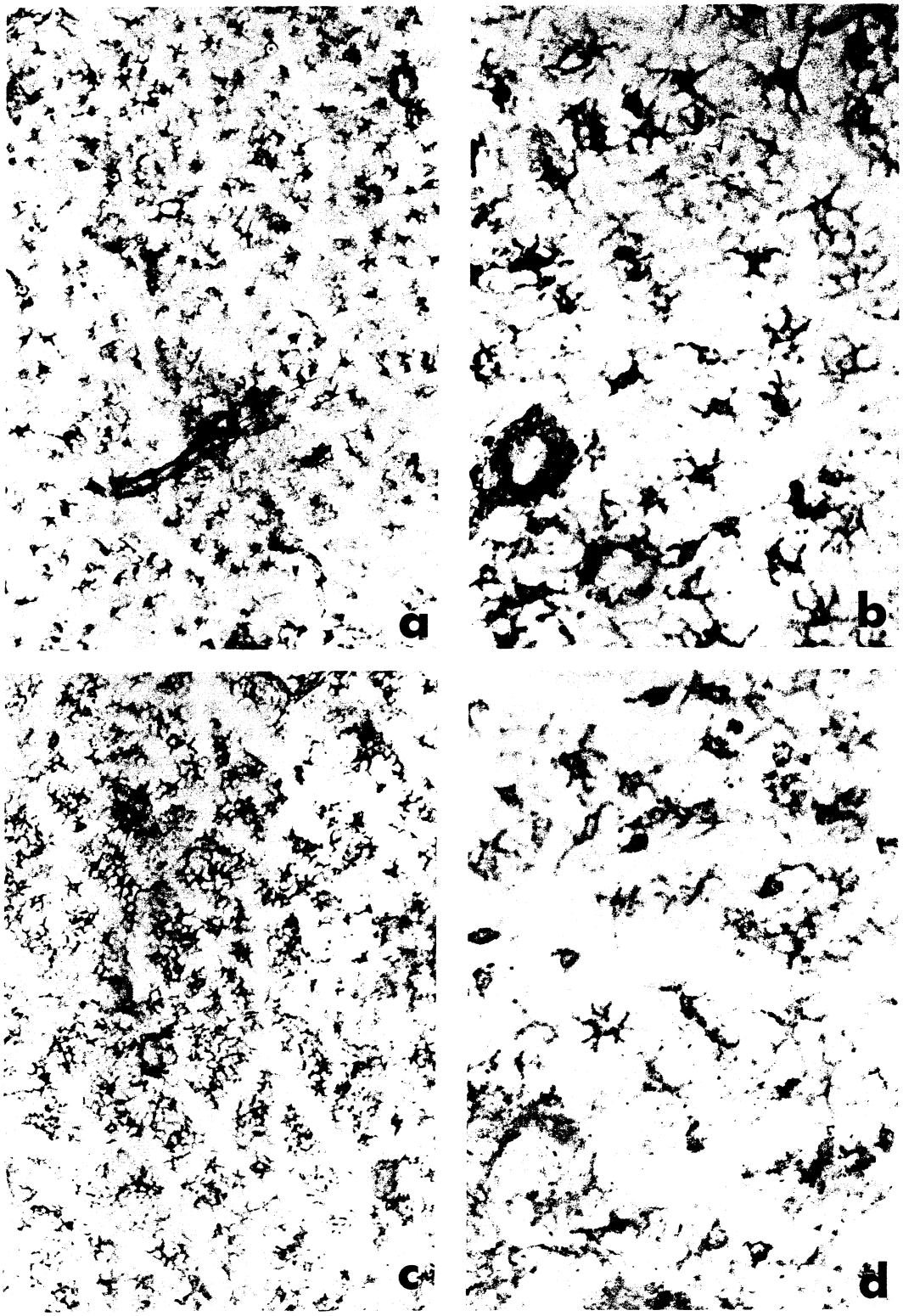


**Figure 5a** and **b** – I-A staining of skin sheets from BALB/c mice showing positive Langerhans cells. **c** and **d** – CLA staining of skin sheets showing positive Langerhans cells. (Immunoperoxidase without counterstain, **a** and **c**, ×100; **b** and **d**, ×250)

tion of Mac-3 in the spleen was also difficult to evaluate because of the staining of hematopoietic cells.

The lymphoid dendritic cell characterized by Steinman, Cohn, and co-workers,[16-19] has not been localized in tissue sections and therefore cannot be included in the present study. However, it is interesting to note that Steinman et al[45] find more of these cells in the spleen than in the lymph node suspensions, whereas Mac-2 is demonstrated in a greater number of cells in the lymph node than in the spleen sections in the present study. Steinman et al[45] have previously reported these cells to be negative for Mac-1.

The patterns of lymph node and splenic staining for Mac-2 showed striking similarities to the staining for S100 in human tissues. S100 was previously thought to be limited to nerve tissue; however, it has recently been shown to be present in other tissue. Staining for S100 has been shown in dendritic cells in the paracortex of the lymph node, in the thymic medulla, and in fewer cells in the spleen.[29] It stains Langerhans cells (LC) in the skin but not keratinocytes.[28] Mac-2 is present on both keratinocytes and LC.[43] Mac-2 does not stain astrocytes. Thus, although there are similarities between Mac-2 in the mouse and S100 in the human, important differences in their distributions also exist.

It is interesting to compare the wide yet specific distributions of Mac-1, Mac-2, and Mac-3 with three other antigens defined by monoclonal antibodies. Their distributions overlap, but no two are the same. Unfortunately, each of the three other monoclonal antibodies that have been described to stain dendritic cells of the lymphoid system is directed against a different species. OKT6, a mouse anti-human reagent, stains Langerhans cells, some of the dendritic cells in the paracortex, as well as 70% of thymocytes.[8,9,27] MRC OX2, a mouse anti-rat antibody, reacts with follicular dendritic cells, marginal sinus cells, endothelium of postcapillary venules, B cells (weakly), smooth muscle, peripheral nerves, brain, spinal cord, and thymocytes.[46] It precipitates a glycoprotein of 60,000 molecular weight. 33D1, a rat anti-mouse reagent, reacts with lymphoid dendritic cells as studied in cell suspensions of spleen and lymph node.[45] It is negative on cell suspensions of thymus, peritoneal exudate cells, and blood monocytes. Unfortunately, no information is available regarding the distribution of 33D1-positive cells in tissue sections.

The staining patterns of epithelial structures observed with Mac-2 and Mac-3 were entirely different. Staining of lymphoid and nonlymphoid tissue by the same antibodies raises the question of whether the monoclonal antibodies are recognizing the same molecule in all tissues. It has been recently reported that a monoclonal anti-mouse Thy-1 cross-reacts with an intermediate filament, probably vimentin.[47] Although these two molecules have very different molecular structures, they apparently share a common antigenic determinant to which the antibody is directed. While a definitive statement in this regard cannot be made about either Mac-2 or Mac-3, it seems likely that M3/84 (anti-Mac-3) is recognizing the same antigen in the lymphoid and nonlymphoid tissues, since the distinctive granular staining is very similar in both. We know of no specific organelle that would account for the distribution in all tissues. It seems likely that it is at least present in secretory granules and lysosomes.

Table 3 presents the data from this and previous studies, comparing the dendritic cells of the lymphoid system. The Mac-1, 2, 3 phenotypes divide the cells into three categories that have other correlates in addition to these antigens. The Mac-1$^{\pm}$, 2$^{+}$, 3$^{+}$ phenotype is limited to macrophages. The Mac-1$^{-}$, 2$^{+}$, 3$^{+}$

Table 3—Comparison of Properties of Various Dendritic Cells and Macrophages

| Species | Property/antigen | Macrophage | Langerhans cell | Interdigitating dendritic cell | Follicular dendritic cell | Lymphoid dendritic cell | Kupffer cell |
|---|---|---|---|---|---|---|---|
| Rodent | Ia | +/−[48] | +[49] | +[49] | −[50] | +[19] | +[51] |
| | Mac-1 | +[30] | − | − | − | −[52] | − |
| | Mac-2 | +[35] | +[43] | + | − | ND | + |
| | Mac-3 | +[33] | +[43] | +* | − | ND | + |
| | Ly-5 | +[42] | + | ND | ND | +[52] | ND |
| | MRCOX2 | −[46] | ND | −[46] | +[46] | ND | ND |
| | FcR | +[53] | +† | ND | +[50] | −[18] | +[55] |
| | C3R | +[56] | +[57] | ND | +[50] | −[18] | +[55] |
| | ATPase | +[58] | +[59] | ND | +[58] | −[17] | ND |
| Human | Ia | +/−[60] | +[5] | +[9] | ND | +[61] | +[62] |
| | OKT6 | −[9] | +[27] | +/−[9] | −[9] | −[61] | ND |
| | S100 | −[29] | +[28] | +[29] | − | ND | ND |

* The staining pattern observed is most consistent with the interpretation that these cells are positive.

† Demonstrated both by binding of immune complexes[54] and by staining with the 2.4G2 monoclonal anti-Fc-receptor (this study).

phenotype includes Langerhans cells, interdigitating dendritic cells, and thymic dendritic cells. These cells have all been shown to contain Birbeck granules, S100, Ia, are only minimally phagocytic and have interactions with T cells. Kupffer cells are also Mac-1$^-$, 2$^+$, 3$^+$, but can be very phagocytic; they thus appear to have properties intermediate between macrophages and the other dendritic cells with this phenotype. The third phenotype, Mac-1$^-$, 2$^-$, 3$^-$, is found on follicular dendritic cells. These cells differ from the other dendritic cells, in expressing MRC OX2, by electron microscopy, and in their location in B cell areas. Unfortunately, the Mac-1, 2, 3 phenotype for lymphoid dendritic cells is not yet known, but other surface properties such as FcR$^-$, C3R$^-$, and ATPase$^-$ make them much different from other dendritic cells. The manner in which these cells are obtained in cell suspensions may select for less mature cells than are seen in tissue sections.

## References


1. Birbeck MS, Breathnach AS, Everall, JD: An electron microscopic study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. J Invest Dermatol 1961, 37:51–64
2. Katz SI, Tamaki KI, Sachs DH: Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 1979, 282:324–326
3. Klareskog L, Malmnas-Tjernlund U, Fossum U, Peterson PA: Epidermal Langerhans cells express Ia antigens. Nature 1977, 268:248–250
4. Langerhans P: Über die Nerven der menschlichen Haut. Virchows Arch (Cell Pathol) 1868, 44:325–337
5. Rowden G, Lewis MG, Sullivan AK: Ia antigen expression on human epidermal Langerhans cells. Nature 1977, 268:247–248
6. Silberberg I, Baer RL, Rosenthal SA, Thorbecke GJ, Berezowsky V: Dermal and intravascular Langerhans cells at sites of passively induced allergic contact sensitivity. Cell Immunol 1975, 18:435–453
7. Stingl G, Katz SI, Clement L, Green I, Shevach EM: Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 1978, 121:2005–2013
8. Bhan AK, Reinherz EL, Poppema S, McCluskey RT, Schlossman SF: Location of T-cell and major histocompatibility complex antigens in the human thymus. J Exp Med 1980, 152:771–782
9. Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T-cell subsets in human lymph nodes. J Exp Med 1981, 153:30–41
10. Veldman JE: Histophysiology and electron microscopy of the immune response (Academic thesis). State University of Groningen, The Netherlands, N.V. Boekdrukkerij Kijkstra Niemeyer, 1970
11. Veerman AJP, van Ewijk W: White pulp compartments in the spleen of rats and mice: A light and EM study of lymphoid and non-lymphoid cells in T- and B-areas. Cell Tissue Res 1974, 156:427–441
12. Chen LL, Adams JC, Steinman RM: Anatomy of germinal centers in mouse spleen, with special reference to "follicular dendritic cells." J Cell Biol 1978, 77:148–164
13. Klaus GGB, Humphrey JH, Kunkl A, Dongworth DW: The follicular dendritic cell: Its role in antigen presentation in the generation of immunological memory. Immunol Rev 1980, 53:3–28
14. Mandel TE, Phipps RP, Abbot A, Tew JG: The follicular dendritic cell: Long term antigen retention during immunity. Immunol Rev 1980, 53:29–59
15. Nossal GJV, Abbot A, Mitchel J, Lummis Z: Antigens in immunity: XV. Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. J Exp Med 1968, 127:277–290
16. Nussenzweig MC, Steinman RM: Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. J Exp Med 1980, 151:1196–1212
17. Steinman RM, Cohn ZA: Identification of a novel cell type in peripheral lymphoid organs of mice: I. Morphology, quantification, tissue distribution. J Exp Med 1973, 137:1142–1162
18. Steinman RM, Cohn ZA: Identification of a novel cell type in peripheral lymphoid organs of mice: II. Functional properties *in vitro*. J Exp Med 1974, 139:380–397
19. Steinman RM, Nussenzweig MC: Dendritic cells: Features and functions. Immunol Rev 1980, 53:125–147
20. Barclay AN, Mayrhofer G: Bone marrow origin of Ia-positive cells in the medulla of rat thymus. J Exp Med 1981, 153:1666–1670
21. Janossy G, Thomas JA, Bollum FJ, Granger S, Pizzolo G, Brandstock KF, Wong L, McMitchel A, Ganeshaguru K, Hoffbrand AV: The human thymic microenvironment: An immunohistologic study. J Immunol 1980, 125:202–212
22. Kaiserling E, Stein H, Muller-Hermelink HK: Interdigitating reticulum cells in the human thymus. Cell Tissue Res 1979, 155:47–55
23. Olah I, Dunay C, Rohlich P, Toro I: A special type of cell in the medulla of the rat thymus. Acta Biol Acad Sci Hung 1968, 19:97–113
24. Rouse RV, van Ewijk W, Jones PP, Weissman IL: Expression of MHC antigens by mouse thymic dendritic cells. J Immunol 1979, 122:2508–2515
25. Thorbecke GJ, Silberberg-Sinakin I, Flotte TJ: Langerhans cells as macrophages in skin and lymphoid organs. J Invest Dermatol 1980, 75:32–43
26. Tew JG, Thorbecke GJ, Steinman RM: Dendritic cells in the immune response: Characteristics and recommended nomenclature. J Reticuloendothel Soc 1982, 31:371–380
27. Fithian E, Kung P, Goldstein G, Rubenfeld M, Fenoglio C, Edelson R: Reactivity of Langerhans cells with hybridoma antibody. Proc Natl Acad Sci USA 1981, 78:2541–2544
28. Cocchia D, Michetti F, Donato R: Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin. Nature 1981, 294:85–87
29. Takashi K, Yamaguchi H, Ishizeki J, Nakajima T, Nakazato Y: Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating reticulum cells of the human lymph node. Virchows Arch [Cell Pathol] 1981, 37:125–135
30. Springer T, Galfre D, Secher S, Milstein C: Mac-1: A macrophage differentiation antigen identified by monoclonal antibody. Eur J Immunol 1979, 9:301–306
31. Holmberg LA, Springer RA, Ault KA: Natural killer activity in the peritoneal exudates of mice infected with Listeria monocytogenes: Characterization of the natural killer cells by using a monoclonal rat anti-murine macrophage antibody (M1/70). J Immunol 1981, 127: 1792–1799
32. Ho MK, Springer TA: Mac-1 antigen: Quantitative expression in macrophage populations and tissues, and immunofluorescent localization in the spleen. J Immunol 1982, 128:2281–2286

33. Ho MK, Springer TA: Mac-3, a macrophage surface glycoprotein with heterogeneous molecular weight forms in different macrophage populations. Manuscript in preparation.
34. Springer TA: Monoclonal antibody analysis of complex biological systems: Combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. J Biol Chem 1981, 256:3833–3839
35. Ho MK, Springer TA: Mac-2, a novel 32,000 Mr macrophage subpopulation-specific antigen defined by monoclonal antibody. J Immunol 1982, 128:1221–1228
36. Bhattacharya A, Dorf ME, Springer TA: A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: Evidence for I region gene duplication. J Immunol 1981, 127:2488–2495
37. Kurzinger K, Reynolds T, Germain RN, Davignon D, Martz E, Springer TA: A novel lymphocyte function-associated antigen (LFA-1): Cellular distribution, quantitative expression, and structure. J Immunol 1981, 127:596–602
38. Unkeless JC: Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J Exp Med J 1979, 150:580–596
39. Ladman AJ, Padykula HA, Strauss EW: A morphological study of fat transport in the normal human jejunum. Am J Anat 1963, 112:389–394
40. Fischer HD, Gonzalez-Noriega A, Sly WS, Morre DJ: Phosphomannosyl-enzyme receptors in rat liver. J Biol Chem 1980, 255:9608–9615
41. Michaelson J, Scheid M, Boyse EA: Biochemical features of Ly-5 alloantigen. Immunogenetics 1979, 9: 193–197
42. Scheid MP, Triglia D: Further description of the Ly-5 system. Immunogenetics 1979, 9:423–433
43. Haines C, Flotte TJ, Springer TA, Gigli I, Thorbecke GJ: Staining of Langerhans cells with monoclonal antibodies to macrophages and lymphoid cells. Proc Natl Acad Sci USA (In press)
44. Clark SL: The reticulum of lymph nodes in mice studied with the electron microscope. Am J Anat 1962, 110:217–224
45. Nussenzweig MC, Steinman RM, Witmer MD, Gutchinov B: A monoclonal antibody specific for mouse dendritic cells. Proc Natl Acad Sci USA 1982, 79:161–165
46. Barclay AN: Different reticular elements in rat lymphoid tissue identified by localization of Ia, Thy-1, and MRC OX2 antigens. Immunology 1981, 44:727–736
47. Dulbecco R, Unger M, Bologna M, Battifora H, Syka P, Okada S: Crossreactivity between Thy-1 and a component of intermediate filaments demonstrated using a monoclonal antibody. Nature 1981, 292:772–774
48. Swartz R, Dickler HB, Sachs DH, Schwartz BD: Studies of Ia antigens on murine peritoneal macrophages. Scand J Immunol 1976, 5:731–743
49. Hoffman-Fezer G, Gotze D, Rodt H, Thierfelder S: Immunohistochemical localization of xenogeneic antibodies against Iak lymphocytes on B cells and reticular cells. Immunogenetics 1978, 6:367–375
50. Humphrey JH, Grennan D: Isolation and properties of follicular dendritic cells. Adv Exp Med Biol 1982, 149:823–827
51. Richman LK, Klingenstein JA, Richman JA, Strober W, Berzofsky J: The murine Kupffer cell: I. Characterization of the cell serving accessory function in antigen-specific T cell proliferation. J Immunol 1979, 123: 2602–2609
52. Nussenzweig MC, Steinman RM, Unkeless JC, Witmer MD, Gutchinov B, Cohn ZA: Studies of the cell surface of mouse dendritic cells and other leukocytes. J Exp Med 1981, 154:168–187
53. Berken A, Benacerraf B: Properties of antibodies cytophilic for macrophages. J Exp Med 1966, 123:119–144
54. Tamaki K, Stingl G, Gullino M, Sachs DH, Katz SI: Ia antigens in mouse skin are predominantly expressed on Langerhans cells. J Immunol 1979, 123:784–787
55. Steer CJ, Richman LK, Hague NE, Richman JA: Identification of receptors for immunoglobulins and complement on mouse Kupffer cells *in vitro*. Gastroenterology 1978, 75:988
56. Lay WH, Nussenzweig V: Receptors for complement on leukocytes. J Exp Med 1968, 128:991–1009
57. Burke K, Gigli I: Receptors for complement on Langerhans cells. J Invest Dermatol 1980, 75:46–51
58. Eikelenboom P: Dendritic cells in the rat spleen follicles: A combined immuno and enzyme histochemical study. Cell Tissue Res 1978, 190:79–87
59. Wolff K: Die Langerhans-Zelle Ergebnisse neuerer experimenteller Untersuchungen: I and II. Arch Klin Exp Dermatol 1967, 229:54–75
60. Raff HR, Picker LJ, Stobo JD: Macrophage heterogeneity in man. J Exp Med 1980, 152:581–593
61. Van Hoorhis WC, Hair LS, Steinman RM, Kaplan G: Human dendritic cells. J Exp Med 1982, 155:1172–1187
62. Lautenschlager I, Hayry P: Expression of the major histocompatibility complex antigens on different liver cellular components in rat and man. Scand J Immunol 1981, 14:421–426

## Acknowledgments

The authors would like to thank Dr. J. C. Unkeless for the gift of the monoclonal anti-Fc-receptor antibody 2.4G2, Mr. Francisco Aviles for processing and cutting numerous sections, and Anita Clark-Fanelli for typing the manuscript.