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Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution

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IDENTIFICATION OF A NOVEL CELL TYPE IN PERIPHERAL LYMPHOID ORGANS OF MICE

I. Morphology, Quantitation, Tissue Distribution*

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During the course of observations on the cells of mouse spleen that adhere to glass and plastic surfaces, it was clear that this population was quite heterogeneous. In addition to mononuclear phagocytes, granulocytes, and lymphocytes, we noticed a large stellate cell with distinct properties from the former cell types. In this paper, we describe the morphology, quantitation, and tissue distribution of this novel cell as identified in vitro. In following papers, we will further characterize it with respect to its functional properties in vitro, as well as its localization and properties *in situ*.

Materials and Methods

Mice.—Outbred NCS mice were maintained at The Rockefeller University. Inbred DBA/2J, C3H, BALB/cJ, C57BL, and C57BL × DBA/2J strains were obtained from the Jackson Laboratory, Bar Harbor, Maine. Mice, homo- and heterozygous at the nude locus, were kindly provided by Dr. P. Wernet of The Rockefeller University.

Preparation of Spleen Adherent Cells.—Single-cell suspensions of spleen were prepared in two general ways. The manual method involved teasing the spleen with fine forceps in Medium 199 at room temperature. The teased spleen was further disrupted by aspiration several times in a Pasteur pipette and then transferred to a plastic test tube (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). The remaining clumps of tissue were allowed to settle for 5 min. The supernatant was aspirated and centrifuged at 400 *g* for 10 min at room temperature. The cell pellet was resuspended in Medium 199–10% fetal calf serum ([FCS][1] heated for 30 min at 56°C; Medium 199 and FCS both were obtained from Grand Island Biological Company, Grand Island, N. Y.) and supplemented with 100 U/ml penicillin. Another method of harvesting spleen cells involved treating the teased spleen (fragments alone, or fragments and single cells) with clostridial collagenase (0.1% of Sigma Fx1 [Sigma Chemical Co., St. Louis, Mo.], 150 U/ml, or 0.05% of Worthington CLS [Worthington Biochemical Corp., Freehold, N. J.], 400 U/ml, both diluted in Medium 199) for 15 min in a 37°C water bath, without stirring. The small amount of residual undigested tissue was allowed to settle, and then the supernatant, single-cell suspension was aspirated, centrifuged, washed, and resuspended in culture medium as described above.

Aliquots of the cell suspension were cultivated on glass cover slips or in plastic Petri dishes (Nunc Plastics, Roskilde, Denmark; Falcon Plastics; Microbiological Associates, Inc., Be-

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[1] *Abbreviation used in this paper:* FCS, fetal calf serum.

thesda, Md.) for 1 h at 37°C. The nonadherent cells were removed with four to five washes of Medium 199 with swirling of the culture dishes between washes. A more vigorous procedure included the use of a single wash in which the jet of wash fluid was aimed with a syringe directly at the adherent cell monolayer, rather than to the side of the culture vessel. The adherent cells were then examined immediately or after further cultivation in Medium 199-10% FCS at 37°C.

Other Organs.—Adherent cells from the peritoneal cavity and bone marrow were harvested as previously described (24). Peritoneal exudate cells were obtained from mice injected 3 days previously with thioglycolate intraperitoneally. Thymus, lymph node, liver, Peyer's patches, and intestine were disrupted by the enzymatic procedure described for spleen.

Phase-Contrast Microscopy.—Phase-contrast microscope examination was performed on live cells cultivated in Sykes-Moore chambers (Bellco Glass, Inc., Vineland, N. J.), or on specimens fixed for 5 min at room temperature in 1.25–2.5% glutaraldehyde (Fisher Chemicals) buffered with 0.1 M sodium cacodylate, pH 7.4. Observations on living cells were recorded on Kodak Plus X negative film, no. 7231, using a series 500 cinephotomicrographic apparatus (Sage Instruments, Inc., White Plains, N. Y.).

Staining Procedures.—Vital staining for mitochondria and lysosomes was obtained by applying Janus green and neutral red, respectively, at concentrations of 1:10,000 (mg/ml Medium 199) for 1 min at room temperature. Cover slips fixed in absolute methanol were stained with Giemsa, while 4% formaldehyde solution in water was used for the periodic acid-Schiff and hematoxylin-eosin procedures. 0.1% toluidine blue in 0.1 M phosphate buffer, pH 6.2, was used to stain nucleic acids of glutaraldehyde-fixed cells after treatment with ribonuclease (RNase [Worthington Biochemical Corp.] 0.5 mg/ml) in 0.1 M phosphate, pH 7.0, for 2 h at 37°C. Several histochemical stains were applied to cells fixed 5 min in 1.25% glutaraldehyde including: acid phosphatase (16); magnesium-dependent adenosine triphosphatase (ATPase) (5); Perls' Prussian blue for ferric ion (17); and Graham and Karnovsky's technique for peroxidase (6). Kaplow's method for peroxidase (10) was also used.

Electron Microscopy.—Specimens for electron microscopy were prepared in two ways. Glutaraldehyde-fixed cells were scraped from cover slips and harvested as a pellet as previously described (8). The pellet was postfixed for 15 min in a cold glutaraldehyde-osmium tetroxide mixture (8) followed by en bloc staining for 15 min with 0.5% magnesium uranyl acetate (K & K Chemicals Inc., Plainview, N. Y.) in saline, pH 5.0. Alternatively cells were processed entirely on cover slips previously coated with vaporized carbon. The method was identical to that of Ross (20) except that 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, was the fixative.

RESULTS

Morphology of Mouse Spleen Adherent Cells.—

Phase-contrast observations: As has been noted by others (13, 19), some 10–50% of the nucleated cells in mouse spleen adhere to glass and plastic surfaces after 60 min of adherence at 37°C. Several types of nucleated cells, as well as dying cells, cell debris, red cells, and platelets, can be distinguished by phase-contrast microscopy of glutaraldehyde-fixed adherent cell preparations. Mature granulocytes, which in the mouse frequently have a doughnut-shaped nucleus, can be recognized as well by their content of small phase-dense granules (neutrophils) or larger refractile granules (eosinophils). The less mature myelocytes are large cells (10–15 μm), with an unruffled irregular cell outline, and they contain numerous phase-dense granules clustered about a clear Golgi zone and oval or kidney-shaped nucleus. Lymphoid cells also adhere to glass and generally

assume circular shapes with smooth cell surfaces. Small lymphocytes possess considerable phase-dense chromatin and a high nuclear to cytoplasmic ratio. Larger lymphocytes have a more refractile nucleus and more cytoplasm, in which one can discern many large, circular, phase-dense organelles. Plasma cells, especially in immunized animals, are also recognized by their characteristic eccentric "cartwheel" nuclei and clear Golgi zones. Mononuclear phagocytes containing typical oval or kidney-shaped nuclei are present in varying degrees of differentiation ranging from monocytes to large complex macrophages. The monocyte surface is ruffled, and cytoplasmic granules and vesicles are frequently in a perinuclear position. Macrophages (Figs. 4 and 5) also have a ruffled surface but exhibit larger numbers of cytoplasmic organelles: pinocytic vesicles, phase-dense lysosomes, long filamentous mitochondria, phagocytosed objects, and refractile inclusions. Frequently the macrophages are covered with lymphocytes, red cells, and dead cells forming clusters that can only be disrupted by vigorous washing.

In addition to granulocytes, lymphocytes, and mononuclear phagocytes, there is a fourth variety of adherent, nucleated cell whose morphological features are quite distinct (Fig. 1). The cytoplasm of this large cell is arranged in pseudopods of varying length, width, form, and number, resulting in a variety of cell shapes ranging from bipolar elongate cells to elaborate, stellate or dendritic ones. Most pseudopods are long, uniform in width, and have blunt terminations, but smaller spinous processes are also evident. The cytoplasm contains many large, circular, phase-dense granules, as well as infrequent refractile granules, probably lipid. There is no morphological evidence of active endocytosis, even if the cells are cultivated for several hours in high concentrations (40% vol/vol) of serum, conditions known to stimulate endocytosis in macrophages in vitro (4). Thus, the cell surface is not ruffled, and there are few if any phase-lucent vesicles or phagocytosed objects. The nucleus of this novel cell is very large, contorted in shape, and refractile. The nuclear membrane appears as a thick, uniform, phase-dense line. The nucleoplasm is lucent, except for several large, phase-dense chromatin masses, which frequently are opposed to the nuclear membrane.

Further morphological observations, both in vivo and in vitro (e.g. see microcinematography below), indicate that these novel cells can assume a variety of branching forms, and constantly extend and retract many fine cell processes. The term "dendritic" cell would thus be appropriate for this particular cell type.

Although all four classes of nucleated cells are represented in adherent populations of mouse spleen, it is clear that the absolute and relative numbers of each vary considerably depending on the details of the preparative procedure. Lymphocytes and granulocytes, in contrast to macrophages and dendritic cells, readily detach from the surface, e.g., with increased vigor of washing and/or duration of cultivation. Thus the percentage of dendritic cells in adherent spleen populations may range from 5 to 50% of the total. After manual disruption of

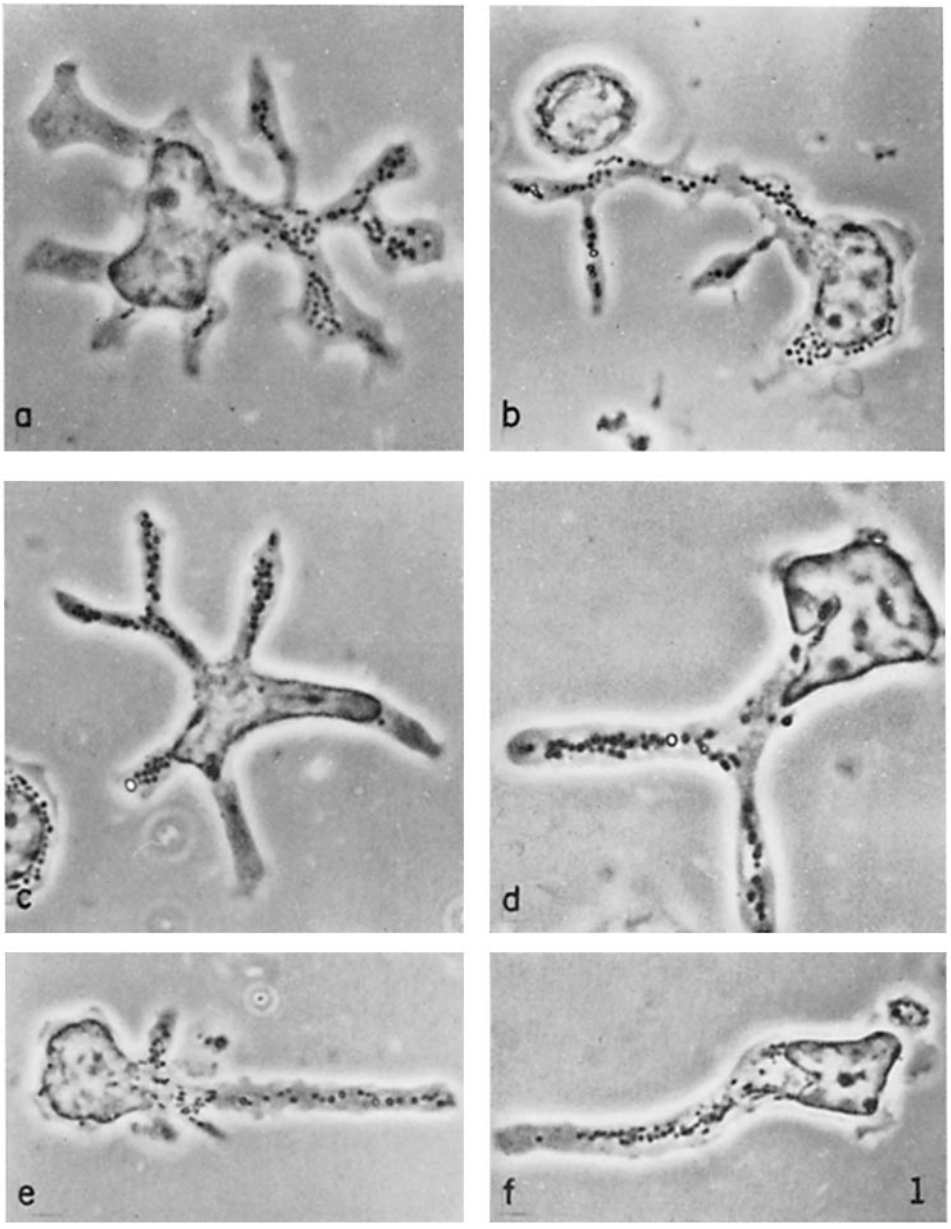


FIG. 1. Phase-contrast micrographs of dendritic cells isolated from peripheral lymphoid organs and fixed in glutaraldehyde. Figs. 1 *a–d* are from spleen, (*e*) from cervical lymph node, and (*f*) from Peyer's patch. The nucleus is large, irregular in shape, and has a refractile quality. The cytoplasm is arranged in processes of varying sizes and shapes, many of which contain spherical phase-dense mitochondria. Occasional refractile lipid granules are also present. A medium size lymphocyte in Fig. 1 *b* can be used as a size comparison. (*a*) × 4,500; (*b*) × 3,500; (*c*) × 3,200; (*d*) × 4,600; (*e*) × 3,200; (*f*) × 3,200.

the spleen, the number of macrophages is generally less (10–50%) than the number of dendritic cells. However, collagenase treatment of spleens strikingly increases the yield of large differentiated phagocytes without altering the total number of dendritic cells, such that the number of macrophages may exceed the number of dendritic cells by twofold. Collagenase treatment of the clumps of tissue that are normally discarded when spleens are disrupted manually consistently yields an additional 15–20% increase in nucleated cells, rich in macrophages.

Vital stains: The phase-dense spherical organelles filling the pseudopods of the dendritic cells stain vitally with Janus green and are thus mitochondria (Figs. 2 *a* and *b*). Vital staining of lysosomes with neutral red demonstrates that many dendritic cells contain a small perinuclear cluster of granules whereas some are not detectably stained. In contrast, granulocytes and macrophages contain many neutral red positive bodies of varying size.

Histological stains of fixed adherent cells: The nucleus and cytoplasm of the novel cell type in mouse spleen adherent cell populations stain weakly after the application of a number of basic dyes (toluidine blue, hematoxylin, Giemsa)

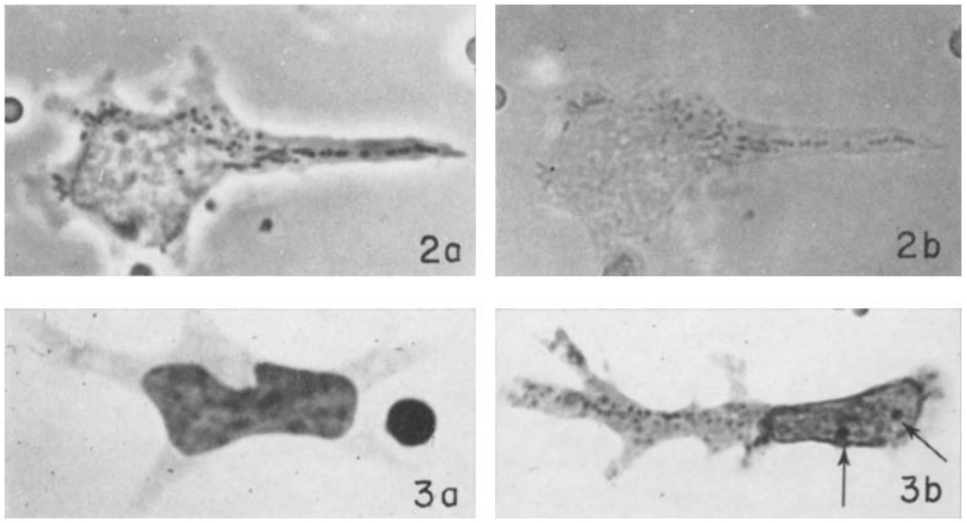


FIG. 2. Phase-contrast and bright-field micrographs of a dendritic cell stained vitally with Janus green. The spherical phase-dense cytoplasmic granules are thus identified as mitochondria. Control bright-field micrographs of dendritic cells in the absence of Janus green are available in Figs. 4 *b* and 5 *b*. × 4,600.

FIG. 3. Stained dendritic cells. (*a*) Hematoxylin and eosin stain after formaldehyde (4% in water) fixation. The scattered nuclear chromatin masses are stained with hematoxylin, but the cytoplasm stains weakly with this and other basic dyes. × 4,000. (*b*) Toluidine blue after glutaraldehyde fixation and DNase digestion. Two small discrete nucleoli (arrows) are seen. × 4,000.

after a variety of fixatives (glutaraldehyde, formaldehyde solution, methanol) (Fig. 3 *a*). Simultaneous identification of the four nucleated cell types by phase-contrast microscopy, as described above, was most readily made in glutaraldehyde-fixed preparations stained with toluidine blue. Macrophages stained similarly to dendritic cells with basic dyes, but the nucleus and cytoplasm of lymphoid cells were more strongly basophilic. The cytoplasm of dendritic cells did not stain with the periodic acid-Schiff reagent.

Toluidine blue can also be used to distinguish ribo- from deoxyribonucleic acid, by treating glutaraldehyde-fixed adherent cells with purified RNase or DNase. After RNase, toluidine blue stains scattered masses of presumptive nuclear chromatin, and the staining pattern is indistinguishable from nonenzyme-treated controls. However, after DNase, the dendritic cell nucleus exhibits only one to four (generally two), tiny, pink (metachromatic) nucleolar bodies (Fig. 3 *b*). Macrophage nuclei stain in a heterogeneous fashion after DNase treatment. Some nuclei contain little or no RNA in the form of nuclear masses, while others reveal one or more distinct nucleoli that can be two to four times the diameter of those seen in dendritic cells.

Histochemical stains: Dendritic cells differ strikingly from macrophages in three staining procedures. Macrophages contain an abundance of perinuclear acid phosphatase positive granules of varying size. Most dendritic cells contain a small cluster of positive tiny granules close to the nucleus, although some exhibit no histochemical reactivity (Figs. 4 *a* and *b*). The observations with acid phosphatase thus correspond to those seen with neutral red vital stains. All macrophages, identified by phase-contrast criteria, exhibit a divalent cation-dependent, membrane ATPase, while all dendritic cells lack this enzyme (Figs. 5 *a* and *b*). A third difference between the two cell types is that most macrophages contain Prussian blue positive granules, presumably hemosiderin, while dendritic cells do not.

Dendritic cells also do not stain for peroxidase by either the Kaplow or Graham-Karnovsky method. The observations on macrophages are more complex. The Kaplow technique reveals that rare, large macrophages have positive granules. A large number of small round cells have positive granules and are presumably granulocytes, rather than monocytes, because most disappear on prolonged (12 h) cultivation in vitro (monocytes would be expected to survive and differentiate into macrophages [2]). The results with the Graham-Karnovsky procedure are similar with two possible exceptions. First, macrophages contain hemosiderin granules whose endogenous color may be difficult to distinguish from the histochemical reaction product. Second, red cells are peroxidase positive by this procedure so that the phagocytic macrophages, especially from collagenase-treated spleen preparations, exhibit a good deal of histochemical reactivity, unless the red cells in the spleen suspension are lysed with ammonium chloride before cultivation.

Microcinematography of adherent cells: Phase-contrast cinematographic ob-

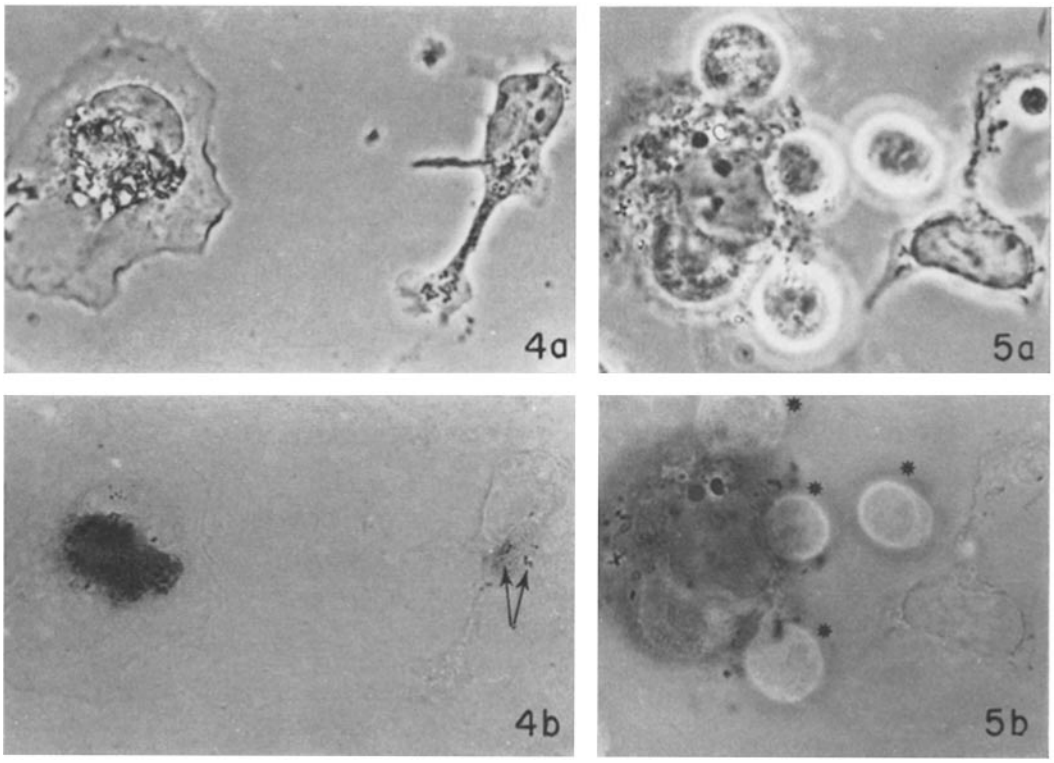
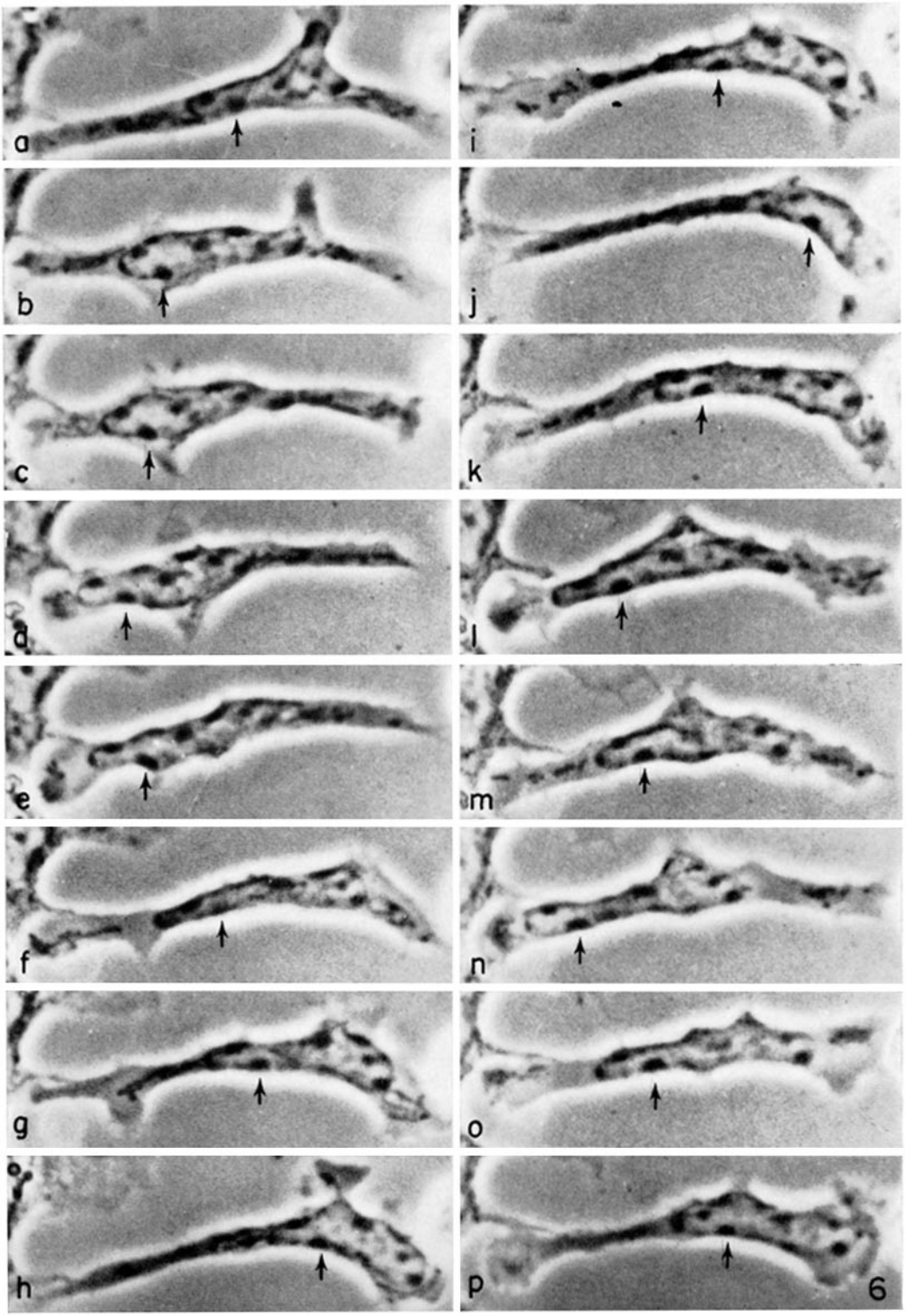


FIG. 4. Phase-contrast (*a*) and bright-field micrographs (*b*) after staining for acid phosphatase. The macrophage (note ruffles, vesicles, refractile inclusions) exhibits considerable reactivity in a perinuclear position. The dendritic cell has a few small reactive granules close to the nucleus (arrows). × 2,700.

FIG. 5. Phase-contrast (*a*) and bright-field micrographs (*b*) after staining for divalent cation-dependent ATPase. The predominant macrophage staining is consistent with a plasmalemma distribution of enzyme. The dendritic cell and lymphocytes (*) are negative. × 3,000.

servations (Fig. 6) of living, adherent populations reemphasize the unique morphological features of the dendritic cells, especially when compared with macrophages in the same preparation. The protoplasm of the dendritic cell, as exemplified by the nucleus, moves back and forth in a remarkable pulsatile fashion. The nucleus seems to fit the confines of the pseudopods and so assumes many unusual shapes. The cell constantly puts out and retracts small cytoplasmic dendrites and over larger periods, forms and/or reorients larger pseudo-

FIG. 6. Time-lapse, phase-contrast cinemicrographs of a dendritic cell at 1-min intervals. The nucleus moves to and fro within the confines of the cytoplasm. This movement is best followed by observing the movement of a marker mass of chromatin (arrows). Small cytoplasmic projections are rapidly formed and withdrawn. × 4,000.



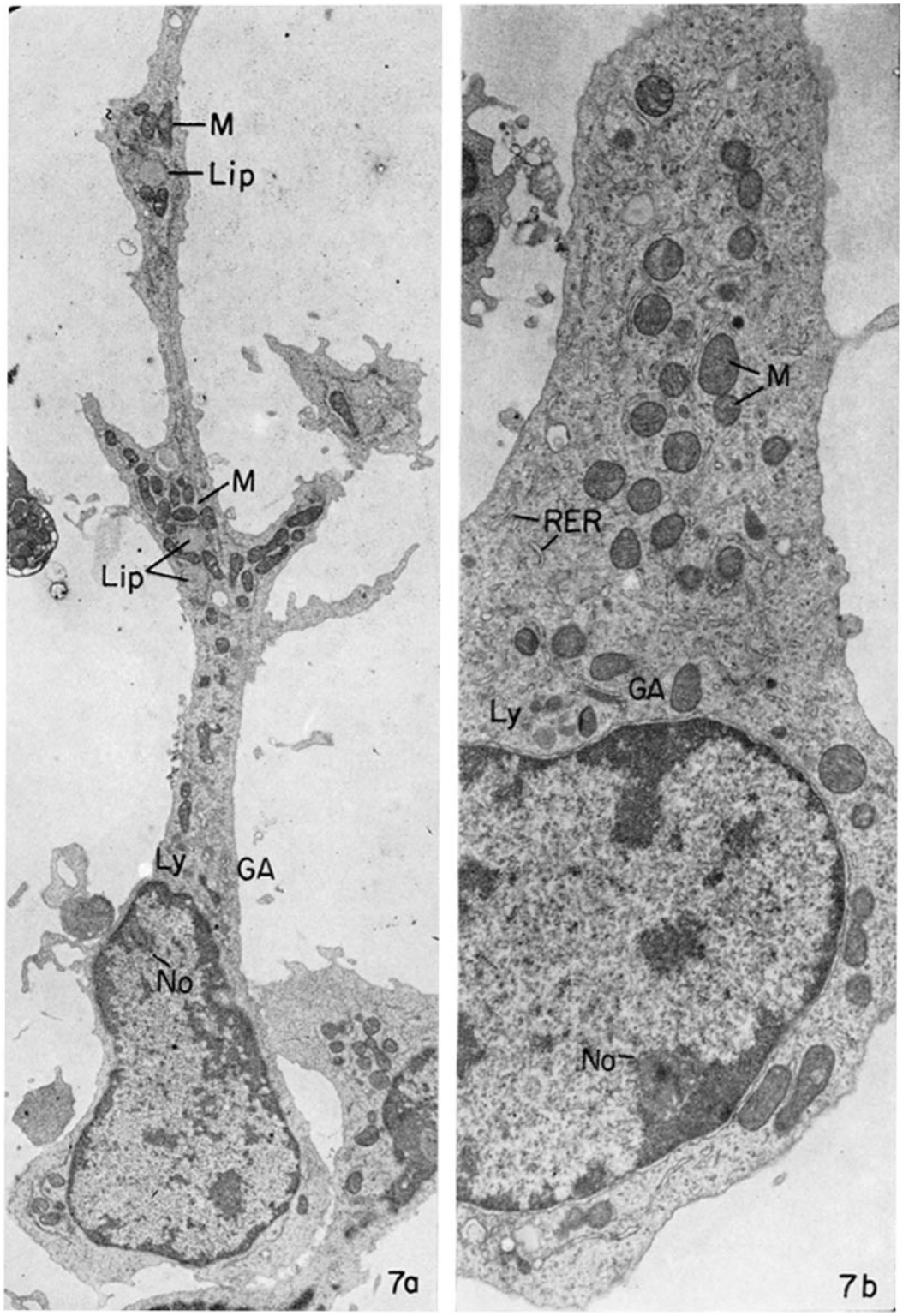
pods. Macrophages are much more sedentary during the period of these observations. There is little bulk reorientation of the cytoplasm or translocational movement of the cell. The nucleus is stationary or at most moves within a restricted region of the cytoplasm. The prominent activity consists of membrane ruffling, formation of pinocytic vesicles at the periphery, and the centripetal migration of these vesicles towards the perinuclear region, as has been described for macrophages in other locations.

Electron microscopy of adherent cells: The novel dendritic cell can be identified in thin sections on the basis of criteria established in the phase-contrast studies. We were especially concerned with distinguishing dendritic cells from mononuclear phagocytes. This distinction, on the basis of morphological criteria, has been corroborated by observations on cell populations enriched in dendritic cells and/or tested with suitable electron-dense markers to identify the highly endocytic macrophages (R. M. Steinman and Z. A. Cohn, manuscript in preparation).

Two types of large, well-spread cells are found in adherent cell preparations that have been removed as monolayers after cultivation, fixation, and embedding on carbon-coated cover slips. The predominant elongate cell corresponds to the dendritic cell seen in the light microscope (Figs. 7 *a* and *b*). It has a relatively smooth cell surface devoid of microprojections and surface lacunae. The cytoplasm contains large mitochondria, with well-developed cristae, and short slips of rough endoplasmic reticulum, generally in close association with the mitochondria. Membrane-bound lipid droplets are occasionally present in the pseudopods. The perinuclear region (Figs. 7, 9 *a*, and 10) is small and contains a Golgi apparatus, centriolar pair, rough and smooth surfaced vesicles, multivesicular bodies, and membrane-bound electron-dense granules (lysosomes). Long microtubules radiate from the perinuclear region along the axis of the pseudopods. A few 80-Å microfilaments can be distinguished, generally close to the plasmalemma, but large bundles of filaments are rarely observed. Ribosomes are relatively few in number and occur usually as scattered free polysomes or attached to the short cisternae of endoplasmic reticulum (Figs. 7 *b*, and 9 *a*).

The other large adherent cell in these preparations (Fig. 8 *a*) has features typical of tissue macrophages described previously in spleen and other locations. The cell surface may exhibit lacunae and/or microvilli as a result of the membrane ruffling seen at the light microscope level. Long cisternae of rough endo-

FIG. 7. Low-power electron micrographs of dendritic cells in preparations sectioned in the plane of the adherent cell monolayer. The elongate dendritic cells have smooth surfaces and large nuclei, in which the heterochromatin is deposited as a thin rim along the nuclear envelope. The nucleolus (*No*), when seen, is small. The cytoplasm contains large mitochondria with well-developed cristae (*M*), short slips of rough endoplasmic reticulum (*RER*), and occasional lipid droplets (*Lip*). The perinuclear area consists of a small Golgi apparatus (*GA*) and lysosomes (*Ly*). (*a*) × 6,300; (*b*) × 12,500.



plasmic reticulum are present and tend to be concentrated in the cell periphery. Mitochondria are numerous, but their diameters are small and cristae less well-developed when compared with lymphoid and dendritic cells. The macrophage perinuclear region (Figs. 8 *a* and 9 *b*) is distinctive, especially when compared with that of the dendritic cell (Fig. 9 *a*). The Golgi apparatus is hypertrophied, and is associated with many small vesicles and membrane-bound dense bodies (lysosomes) of varying content.

The nuclei of the two types of large adherent cells are indistinguishable in thin sections. In both macrophages and dendritic cells most of the heterochromatin is arranged along the nuclear envelope, and the nucleolus, when seen, contains typical fibrous and granular components.

Most splenic mononuclear phagocytes that adhere to glass after manual dissection are best classified as monocytes (Fig. 7 *b*). These smaller cells have a ruffled surface. The Golgi apparatus is well developed and is associated with membrane-bound granules. The cytoplasm contains abundant ribosomes and a dense matrix.

Distinguishing dendritic cells from other types of nucleated cells in thin sections is straightforward when large areas of the cells are available, as in preparations sectioned in the plane of the cell monolayer. The identification is more difficult when relatively small portions of the cells are included in the section, e.g., in sections of cells pelleted after scraping from cover slips. In these instances nondendritic cells can often be recognized positively by the presence of certain structures, e.g., endocytosed objects and surface ruffles in macrophages, abundant ribosomes or polysomes, and large nucleoli in lymphocytes. The most reliable identifying criterion of the dendritic cell is that its ground cytoplasm is relatively electron lucent, and this feature distinguishes it from other nucleated cells, especially lymphocytes (Fig. 10). The lucency of dendritic cell cytoplasm results from a paucity of defined small particles (ribosomes, glycogen, filaments) and the ill-defined matrix material.

Quantitation of Dendritic Cells.—We have examined several variables that could affect the yield of dendritic cells, i.e. the number of adherent cells per 100 nucleated cells plated, after cultivation of spleen cell suspensions in FCS-Medium 199. In each case, forty 800 × fields (each 86 μm in diameter) were counted on three to four cover slips of known diameter.

0.2 ml of spleen cell suspensions of varying concentrations were first plated for 1 h on 18-mm circular cover slips. The number of dendritic cells at first increases linearly with cell concentration (Fig. 11) but then plateaus and sometimes diminishes at concentrations greater than 2×10^7 cells/ml. If the nonadherent cells from the original platings are resuspended and replated at a level of 2×10^7/ml, additional dendritic cells are recovered, but only if the concentration of cells in the first plating was high (Fig. 11). A similar relation of plating efficiency with cell concentration is observed if smaller volumes (0.05–0.10 ml) of cells are adhered to smaller (12–13-mm) circular cover slips. These observations

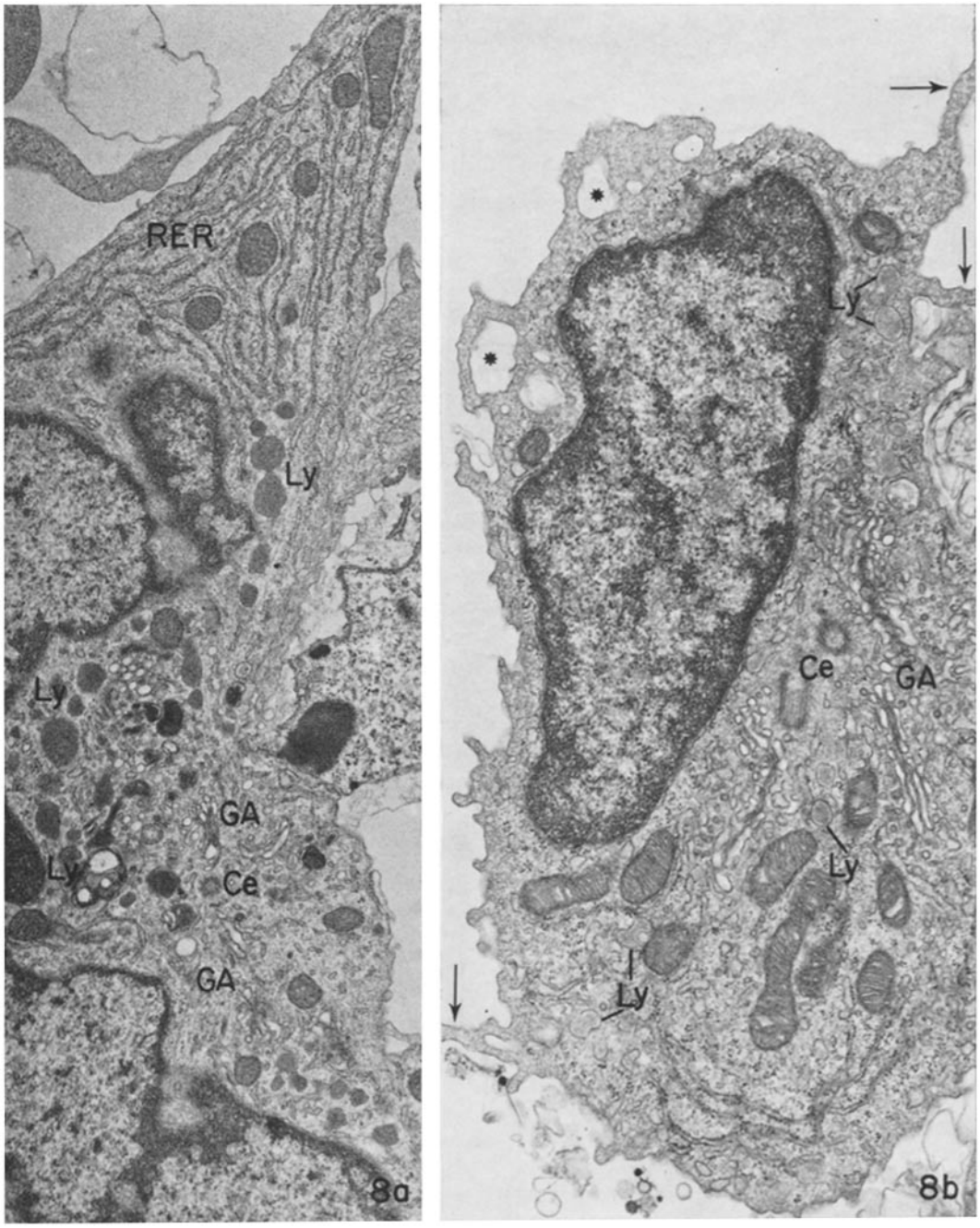


FIG. 8. Low-power electron micrographs of spleen mononuclear phagocytes. (*a*) A tissue macrophage with a typical complex perinuclear region containing Golgi apparatus (*GA*), centriole (*Ce*), and many membrane-bound lysosomes (*Ly*). The cisternae of rough endoplasmic reticulum (*RER*) are long and located peripherally in the cell. × 14,000. (*b*) A monocyte, the surface of which exhibits lacunae (✳) and microvilli (arrows) as a result of the membrane ruffling seen in living cells. The Golgi apparatus (*GA*) is well developed and is associated with a centriolar pair (*Ce*) and numerous, rather uniform, membrane-bound, electron-dense granules or lysosomes (*Ly*). The cytoplasm contains many ribosomes and relatively dense matrix material. × 17,600.

suggest that all spleen cells that are capable of exhibiting the morphological features of dendritic cells adhere to the glass surface after a single plating at cell concentrations of 0.5–2.0 × 10^7/ml.

Recovery of dendritic cells is similar when cells are plated in 5, 10, 20, or 40% fetal calf serum. In the absence of serum, many spleen lymphoid cells adhere nonspecifically to glass forming a tight monolayer in which dendritic cells cannot be easily identified. Most dendritic cells adhere within 30 min of culture at 37°C, but further cultivation (30–60 min) permits maximal recovery as well as an increase in the number and size of the cell processes. The yield of dendritic cells also diminishes if excessively large volumes of cells are plated, e.g., greater than 0.1 ml of a 1–2 × 10^7 cells/ml suspension on 12-mm cover slips.

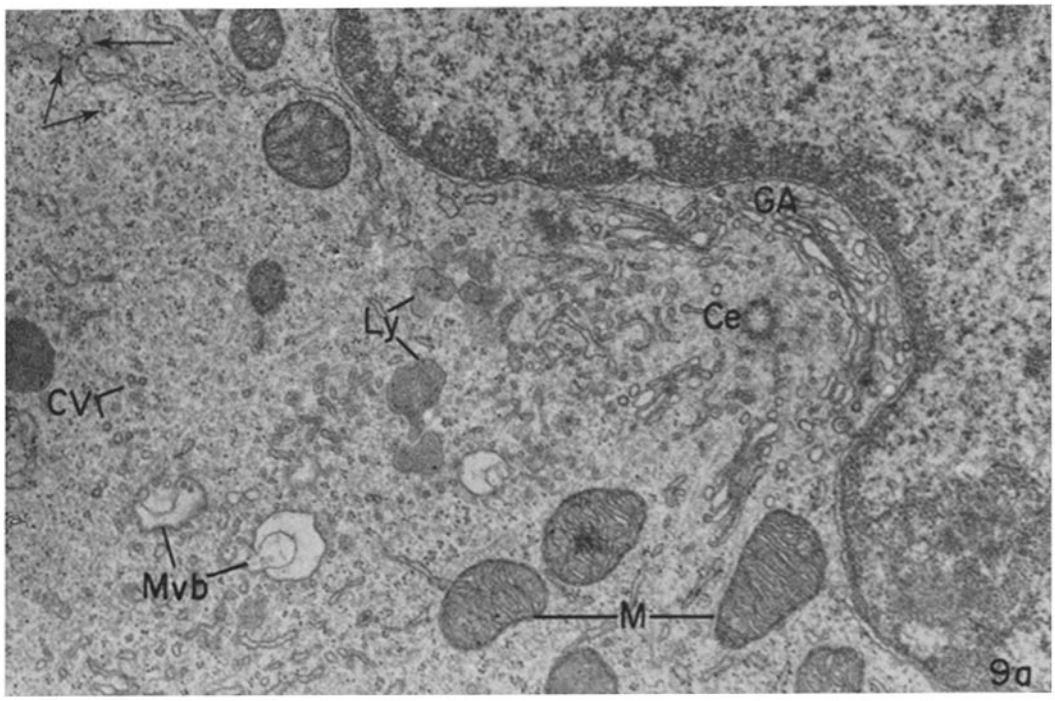
We therefore adopted a standard quantitation technique in which 0.1 ml of a 1 × 10^7 cells/ml suspension are plated on 12–13-mm cover slips for 1 h at 37°C in 10% FCS-medium 199. After washing, the cells are cultivated an additional ½ h to permit maximal extension of dendritic cell processes. Then the dendritic cells on 20–40 oil immersion fields (800 ×) were counted on triplicate cover slips. The standard deviation of six such triplicate determinations was 8.8%.

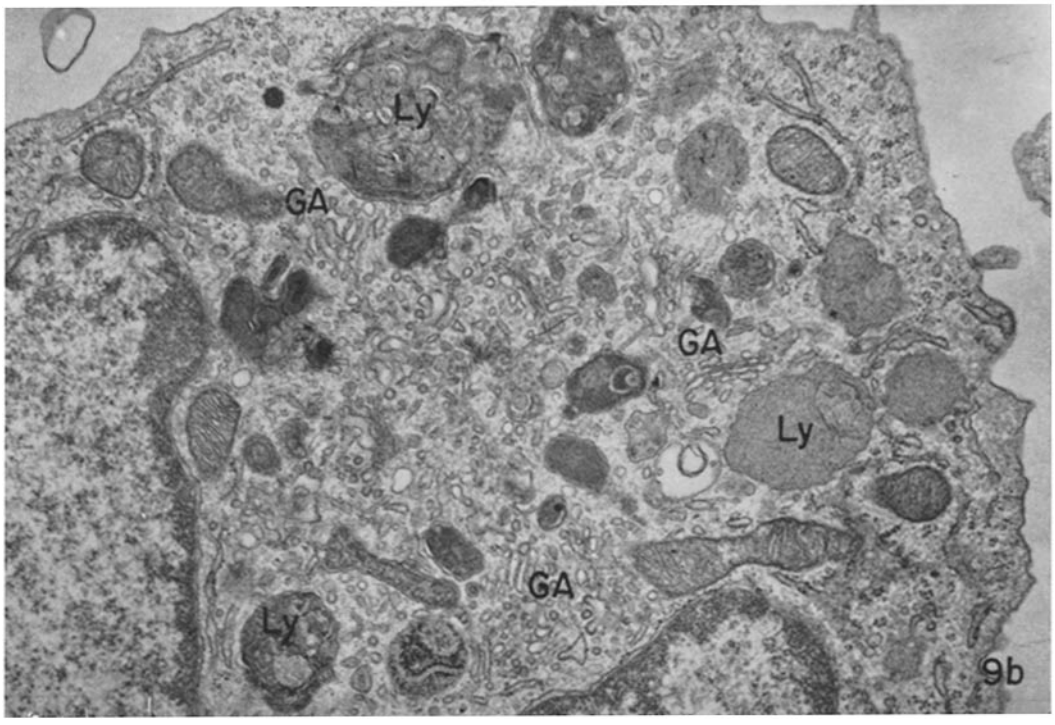
Tissue Distribution of Dendritic Cells.—The occurrence of dendritic cells, as defined by glass adherence and morphology, was quantitated in a variety of locations in the mouse as described above. In all cases, several concentrations of cells were cultivated and organs were dissected by both manual and enzymatic procedures.

In all strains of mice tested, the spleen contained similar percentages of dendritic cells, i.e., 1.0–1.6% of the total nucleated cell population (Table I). When we dissected out portions of red and white pulp before teasing out the single cell suspensions, the percentage of dendritic cells was consistently greater (1.5–6-fold) in white vs. red pulp. It is difficult, however, to separate red from white pulp cleanly because mouse spleen has relatively little of the former, and it is distributed in narrow strands along the white pulp nodules. It is conceivable that the entire population of spleen dendritic cells is restricted to white pulp. Similar percentages of dendriti ccells are found in male and female mice.

Dendritic cells were also found in lymph nodes and Peyer's patches, although generally in much smaller percentages than mouse spleen (Table I). In lymph nodes, dendritic cells appear to contain more refractile (lipid) granules than in spleen. In both of these organs, collagenase treatment is much preferred over manual dissection alone. The number of macrophages in the adherent cell

FIG. 9. A comparison of the perinuclear regions of splenic dendritic cells and macrophages. (*a*) In the dendritic cell, the Golgi apparatus (*GA*) and centrioles (*Ce*) are associated with relatively small numbers of lysosomes (*Ly*) and vesicles, both smooth and coated (*CV*). Multivesicular bodies (*Mvb*) are frequently observed. Large mitochondria (*M*) and scattered polysomes, both free and membrane bound (arrows) are evident. × 26,000. (*b*) The nuclear hof of this characteristic macrophage contains a well-developed Golgi (*GA*) associated with many vesicles and membrane-bound lysosomes (*Ly*) of varying content. × 20,000.





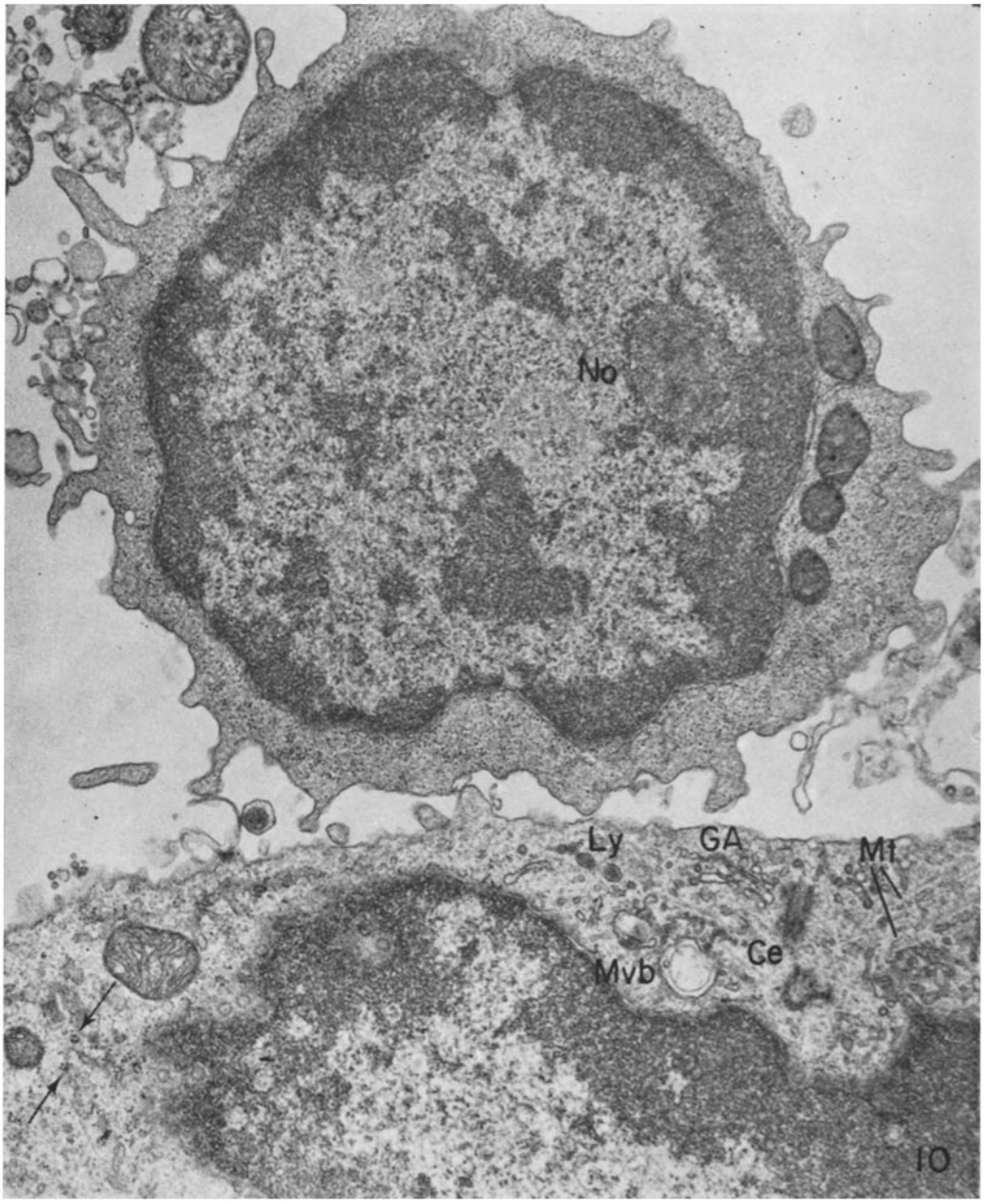


FIG. 10. The cytoplasm of a small lymphocyte is quite different from that of the dendritic cell. The lymphocyte contains many ribosomes and the cytoplasmic matrix material (more clearly seen in the short surface microvilli) is dense. The dendritic cell is sectioned through the Golgi-centriolar complex and exhibits the structures described in Fig. 9 *a*. The cytoplasm contains a few microtubules (*Mt*), scattered polysomes (arrows), and relatively electron-lucent matrix material. × 19,000.

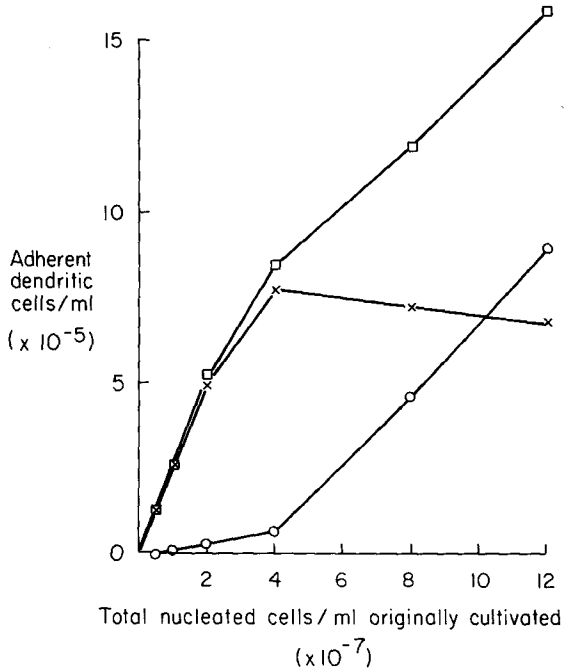


FIG. 11. The recovery of adherent dendritic cells from mouse spleen. 0.2 ml of varying concentrations of spleen cells were plated on 18-mm cover slips for 1 h. The nonadherent cells were removed by washing. The number of adherent dendritic cells per milliliter of each suspension was determined (×—×) and was found to increase linearly with cell concentration and then plateau. If the nonadherent cells are replated for 1 h at a concentration of 2×10^7 cells/ml, the number of additional adherent dendritic cells that are recovered (○—○) is related to the original cell concentration, and is negligible for concentrations of 2×10^7/ml or less. The total recovery of dendritic cells per milliliter of each starting cell suspension (□—□) is seen to be incomplete if high cell concentrations are used for the initial adherence step.

TABLE I

Distribution of Dendritic Cells

Organ	Dendritic cells (% total nucleated)
	%
Spleen	1.0–1.6
Mesenteric node	0.2–0.5
Axillary and cervical node	0.1–0.3
Peyer's patch	0.1–0.2
Thymus	0
Liver	0
Bone marrow	0
Intestine	0
Peritoneal cavity and exudate	0

Single cell suspensions of various organs were prepared as described in Materials and Methods. Cells were cultivated for 1 h in 10% FCS–Medium 199 on glass cover slips. After washing, the number of adherent dendritic cells were counted in triplicate. Each value comprises data from at least four mice.

preparations is greatly increased, and the yield of dendritic cells is much more consistent.

Dendritic cells were not found in thymus. In addition to large, well-differentiated macrophages, thymus adherent cells contained another elongate cell type (Fig. 12). Though similar in outline and nuclear morphology to the dendritic cell, its cytoplasm is different. The mitochondria are rod-like in shape, phase-dense lysosomes and phase-lucent vesicles are present, and the surface membrane possesses ruffles. Some of these cells may be macrophages, but some prob-

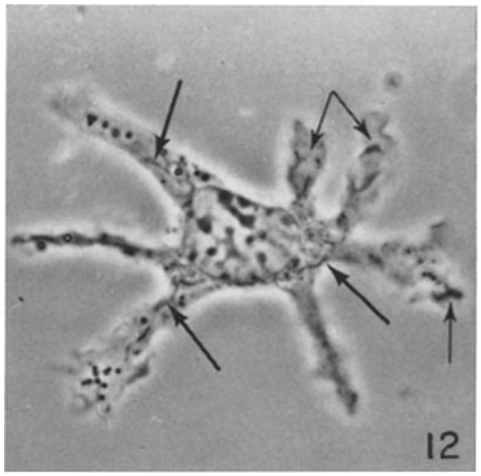


FIG. 12. A branching cell present in adherent cell populations of mouse thymus. Though similar in outline, this cell is not classified as a dendritic cell. The cell border has ruffles (small arrows), the mitochondria are filamentous in shape (large arrows), and there are many small phase-dense granules, probably lysosomes. × 3,200.

ably are not, as they lacked a "receptor" for immunoglobulin-coated particles (R. M. Steinman and Z. A. Cohn, unpublished observations).

Dendritic cells are not found in the adherent cell population of mouse bone marrow, intestine, and liver. The resident cells of the peritoneal cavity, as well as the 3 day exudate induced by thioglycolate, are also lacking in these cells.

Dendritic cells are present in the spleen and peripheral lymph nodes of athymic nude mice. The percentages in nude spleen are usually less than that found in nonnude littermates, but the total numbers are similar, because nude spleens generally contain larger numbers of total nucleated cells. We suggest that dendritic cells are confined to the white pulp of spleen, and that their decreased percentage in nude mice results from the relative paucity of white pulp as seen in tissue sections (R. M. Steinman and Z. A. Cohn, unpublished observations).

The spleen and lynph nodes of other mammals (rat, guinea pig, rabbit, cow) have been examined for the existence of dendritic cells similar to those described

in mice. Such cells do exist, but we have not made further efforts to quantitate and to characterize them in other species.

DISCUSSION

A novel type of adherent cell can be identified by phase-contrast and electron microscopy in peripheral lymphoid organs of mice. The most abundant source of these dendritic cells is spleen where they comprise some 1.0–1.5% of the total nucleated cell population. Smaller numbers are detectable in Peyer's patches and lymph nodes, but none are found in bone marrow, thymus, liver, intestine, or peritoneal cavity (resident and exudate cells). At this time, it is impossible to ascribe meaning to the absolute numbers of dendritic cells that are present in any given organ. Their identification depends on the capacity to dissociate the organ and to recognize the novel cell type after adherence to a surface. These features may vary from one location to another. However, our data strongly suggest that the dendritic cell is restricted in distribution to peripheral lymphoid organs.

In this paper the novel dendritic cell has been distinguished on the basis of morphological criteria after adherence and spreading on glass or plastic surfaces. Its salient features are its large, contorted, refractile nucleus and its long cytoplasmic processes, which contain many large, spherical mitochondria. Both nucleus and cytoplasm stain weakly with basic dyes, and the cell has none of the morphologic features of active endocytosis, i.e., membrane ruffling, pinocytic vesicles, abundant lysosomes, phagocytosed objects. In the living state in vitro, its cytoplasmic processes are continually elongating, retracting, and reorienting themselves, leading to a wide variety of cell shapes. The term, dendritic cell, would thus seem appropaiate for this novel cell in vitro.

The morphology of dendritic cells differs considerably from the other types of adherent nucleated cell present in mouse lymphoid organs, i.e., lymphocytes, granulocytes, and mononuclear phagocytes. By structural and staining criteria, the dendritic cell does fit the previously defined category of "reticular cell." The latter term is relatively nonspecific and has been applied to any cell that has long cytoplasmic processes, which do not stain with basic dyes, and a pale nucleus with margination of chromatin along the nuclear membrane.

The term, reticular cell, is a confusing one, however, because there may be four distinct types of differentiated cell that have the appropriate morphological features in thick sections of lymphoid and other organs. The term has been employed for the two different cells that are found in so-called reticuloendothelial organs, i.e., the macrophages and distinct sinus lining cells (1, 3, 9). Weiss (25) suggests that usage of reticular (reticulum) cell be restricted to the cell thought to be capable of synthesizing the extracellular protein, reticulin, found in lymphoid tissues. These fibroblast-like cells appear to contain moderate amounts of rough endoplasmic reticulum as well as bands of cytoplasmic filaments (18, 25). Both of these structures are infrequent in the dendritic cell described in this paper. The fourth and most recent use of the term, reticular cell, has been in

connection with the putative extracellular retention of antigens within secondary lymphoid follicles or germinal centers. It has been hypothesized that a dendritic reticular cell, or dendritic macrophage, retains antigens on its cell surface through the mediation of specific antibody (7, 15, 21, 22, 26). It is conceivable that the cell we have identified in vitro corresponds to this sort of reticular cell in situ. However, the idea that specialized, antigen-retaining, dendritic or reticular cells exist in lymphoid follicles still lacks considerable experimental support. There is as yet little detailed morphological evidence that a distinct type of reticular cell, i.e. different from phagocytic, connective tissue or vascular elements, exists in primary or secondary lymphoid follicles, or that this cell, and not others, is responsible for the phenomenology of follicular persistence of antigen.

Earlier workers also employed the term "primitive reticular cell" to denote a type of stem cell, again with a reticular morphology, which supposedly gives rise to the mature elements of the lymphoid, erythroid and/or myeloid systems (11, 12). Although this proposal was made decades ago, the identification of the precursor or stem cell in these various systems is currently a matter of considerable interest in cell biology. There is still no direct evidence that a reticular cell serves in a uni- or multipotent precursor fashion. Preliminary descriptions of candidate stem cells (14, 23) would suggest that these entities are morphologically dissimilar from the dendritic cell described in this paper. The availability of mouse dendritic cells in vitro should allow us to test the applicability of these various notions of reticular cell function.

In this paper, we have identified dendritic cells and distinguished them from other cell types on the basis of morphological features and tissue distribution. This analysis has been corroborated by studies of their properties in vitro and in vivo, and these observations will be the subject of future reports.

SUMMARY

A novel cell type has been identified in adherent cell populations prepared from mouse peripheral lymphoid organs (spleen, lymph node, Peyer's patch). Though present in small numbers (0.1–1.6% of the total nucleated cells) the cells have distinct morphological features. The nucleus is large, refractile, contorted in shape, and contains small nucleoli (usually two). The abundant cytoplasm is arranged in processes of varying length and width and contains many large spherical mitochondria. In the living state, the cells undergo characteristic movements, and unlike macrophages, do not appear to engage in active endocytosis. The term, dendritic cell, is proposed for this novel cell type.

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