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

IV. Identification and Distribution in Mouse Spleen*

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A novel class of cells has been identified in single cell suspensions prepared from mouse peripheral lymphoid organs. In vitro, these dendritic cells have been distinguished from other established cell types by a variety of morphologic and functional properties (16–18). Although they represent a small fraction (1% or less) of isolated spleen cells, dendritic cells can be enriched by a combination of floatation on bovine serum albumin columns followed by glass adherence (17). The cytologic features of dendritic cells in such enriched populations are uniform and, when taken together, are quite distinctive from previously described cell types obtained from lymphoid organs.

In this paper, we identify cells in situ which are structurally identical to the cells we have characterized in vitro. In addition, dendritic cells can be distinguished from phagocytic and connective tissue elements. Though all three cell types may be very irregular, branching, reticular, or dendritic in shape, they each possess distinct cytologic features that are presumably related to their functions.

We have chosen mouse spleen for our initial in situ work, since it is currently the most abundant source of dendritic cells for in vitro analysis. We have examined both germfree and nonaxenic mice. The latter exhibit several morphological features of antigenic stimulation, e.g. numerous large lymphocytes, plasma cells, and germinal centers. Since work currently in progress suggests that dendritic cells may associate with stimulated lymphocytes, we felt that germfree tissue would provide a more appropriate baseline.

Materials and Methods

Mice. 10- to 12 wk old, 21–25 g, germfree, outbred, female mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Nonaxenic mice were purchased from the same source, and from the specific pathogen-free NCS colony of The Rockefeller University, New York.

Light microscopy. The spleen was removed and 1.5- to 2-mm transverse slices were made in 10% formalin in 0.1 M sodium cacodylate buffer, pH 7.4. After fixation overnight, the tissues were washed in several changes of buffer over several hours, dehydrated in 70% and 100% ethanol, and embedded in glycol methacrylate (10). 1- to 2-μm sections were stained with 0.3% toluidine blue in 0.1 M phosphate buffer, pH 6.0, or with methyl green-pyronin (10).

* Supported by grants AI 07012 and AI 01831 from the U. S. Public Health Service.

‡ Leukemia Society of America Scholar.

Electron microscopy. The spleen was sliced transversely as above in 2% paraformaldehyde-2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature. 30–45 min later, the periphery of the slices was trimmed away and small portions (0.5–1.0 mm on largest edge) of red or white pulp were dissected out. Alternatively, initial fixation was performed by perfusing fixative beneath the splenic capsule using a no. 27 gauge needle. 0.5- to 1.0-mm pieces of tissue were then dissected out. In both cases, the portions of spleen were fixed for an additional 3 h in cold fixative, rinsed in buffer, and postfixed for 90 min in cold 1% osmium tetroxide in 0.1 M sodium cacodylate, pH 7.4. The blocks were rinsed in normal saline, stained en bloc for several hours in 0.5% magnesium uranyl acetate (K and K Chemicals, Plainview, N. Y.) in normal saline, pH 5.0, rinsed in saline, dehydrated in alcohol, and embedded in araldite or epon. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I (Siemens Corp., Medical/Industrial Groups, Iselin, N. J.) or Zeiss EM 9 microscope (Carl Zeiss, Inc., New York.).

Results

General Approach. This study is primarily concerned with the minority of cells in spleen that have extended or branching cell shapes. The majority of cells in splenic white pulp are small lymphocytes, and in red pulp, marrow elements. The structure of these cells is well known (e.g. 14, 23, 27) and need not be reviewed. In general, they have circular or oblate shapes, and their cytological features are easily distinguished from the cell types to be described here.

Characterization of the irregularly shaped cells in spleen was first attempted in germfree animals and sought to answer two questions. The first was whether cells could be found which were cytologically identical to the dendritic cells described in vitro. This must be approached by electron microscopy since dendritic cells cannot be reliably identified in 1-μm plastic sections of spleen. They possess no distinguishing cytoplasmic structure, e.g. a special granule, pyroninophilia. The staining properties and shape of their nuclei in thick sections are also insufficient to consistently distinguish them from other cell types, i.e., cells which in thin sections are cytologically quite different from the dendritic cells we have characterized in vitro. The second question is whether criteria exist to identify cells, which though extended in shape, are functionally distinct from dendritic cells. For example, considerable histologic work has shown that there are numerous macrophages and connective tissue cells in spleen (e.g. 14, 26), and that these may be highly branched in appearance. In vitro, dendritic cells lack the basic functional properties of these cells, viz, active endocytosis and synthesis of collagen-like molecules. However, phagocytic and fiber-forming cells are not readily identified in single cell suspensions of mouse spleen and additional evidence was required on their recognition in situ.

Dendritic Cells. The white pulp of spleen contains a minor population of irregularly shaped cells that would appear to be the counterparts of dendritic cells in vitro. The nucleus is helpful in detecting these cells at lower magnifications (Fig. 1). It is large, irregularly shaped (often several portions of the nucleus are seen in a single section—Figs. 2 and 3) and contains a continuous, rather uniform band of peripheral heterochromatin (Figs. 1–5). The nucleolus is small and generally absent in thin sections. In contrast, lymphocytes have small generally round nuclei, with abundant heterochromatin and more readily apparent nucleoli (e.g. Figs. 1, 2, 4, 13, and 14).

Dendritic cells in situ exhibit the same number and kinds of cytoplasmic organelles that were expected from in vitro studies (Figs. 2–5 and 11). There are

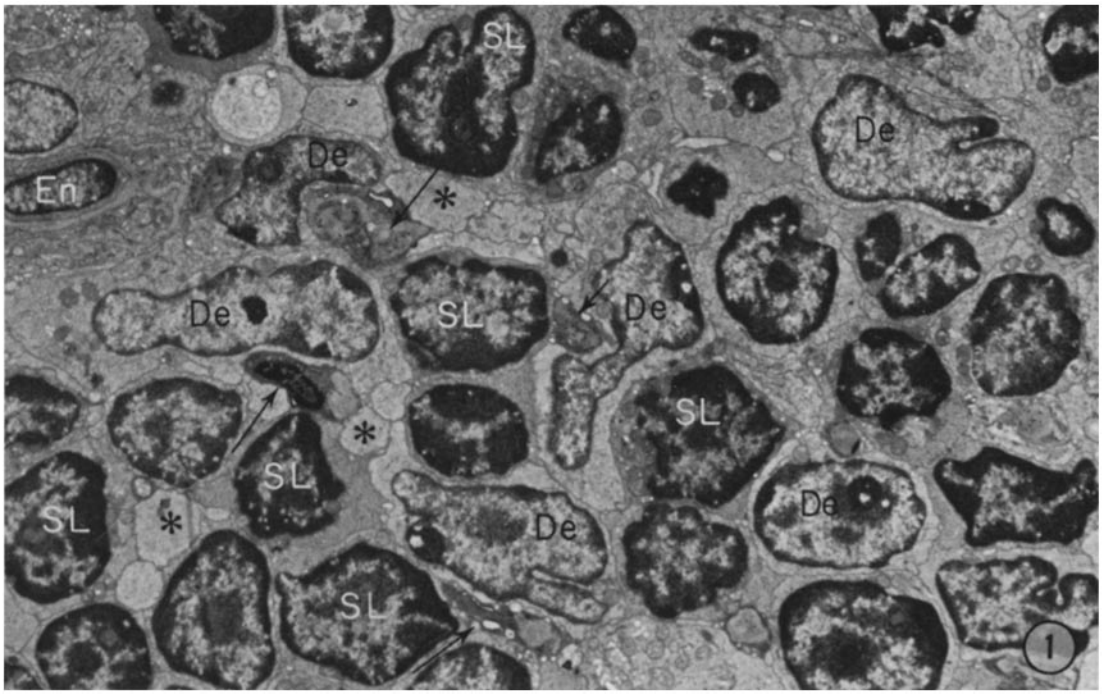
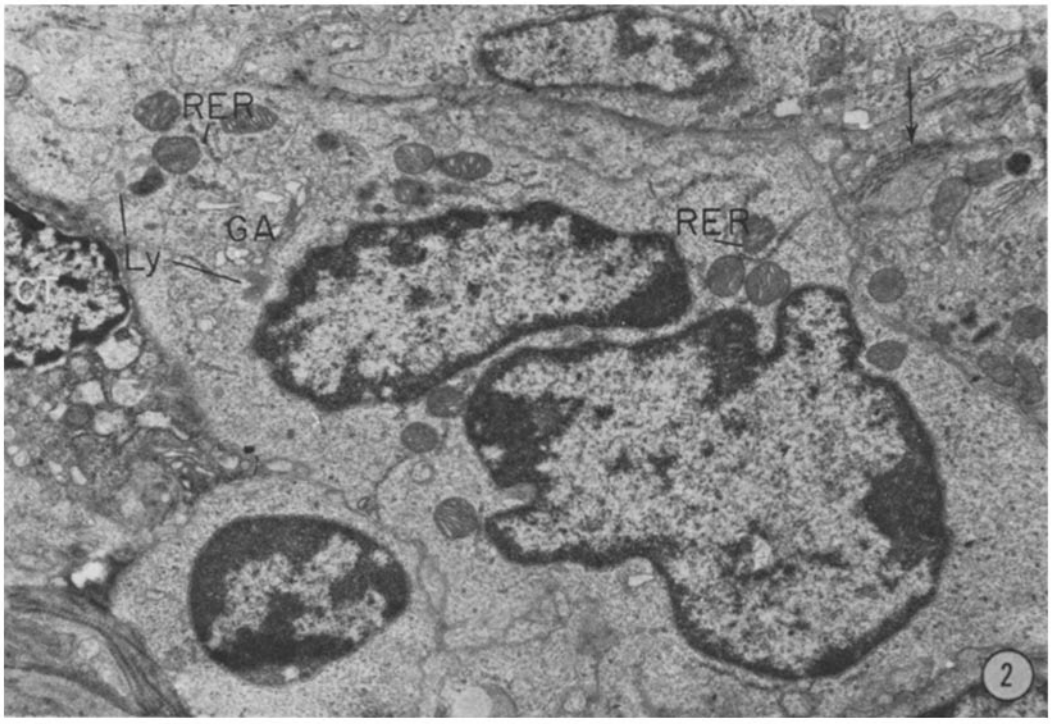


FIG. 1. A low power view of a white pulp region containing many dendritic cells (De). They have irregularly shaped nuclei with peripheral bands of heterochromatin, and nucleoli are infrequently seen. The nuclei of small lymphocytes (SL) are more circular in shape and contain abundant heterochromatin and readily detectable nucleoli. Dendritic cell cytoplasm contains few organelles and has a characteristic electron lucent matrix relative to the small lymphocytes. In many instances, electron lucent cell processes (*) do not connect with any of the cell bodies in this thin section, but additional sections through similar fields show that most emanate from dendritic cells. This group of dendritic cells is close to a small penetrating arterial vessel, the endothelium (En) of which is cut in grazing section. The dendritic cells are also frequently juxtaposed with connective tissue cell processes and/or reticulin deposits (arrows). ×4,250.

scattered mitochondria and short slips of rough endoplasmic reticulum, and usually the two organelles are situated next to each other. The mitochondria often occur in groups so that large pseudopods of the dendritic cells may either contain many or very few of these organelles. A single Golgi region is present and is associated with a few small lysosomes. Multivesicular bodies were prominent in dendritic cells in vitro, and are found near the Golgi, and elsewhere in the cytoplasm, in situ. The cytoplasmic matrix is relatively electron lucent and contains few polysomes or microfilaments. The cytoplasm of the more abundant

FIGS. 2 and 3. Two typical dendritic cells in which the Golgi region is seen to good advantage. The Golgi apparatus (GA) itself is small and is associated with a few small membrane-bound granules, or lysosomes (Ly), and multivesicular bodies (MvB). Mitochondria are frequently associated with small portions of rough endoplasmic reticulum (RER). The nucleus is large, irregular in shape, and has a peripheral band of heterochromatin. The nucleolus is small and not often seen in thin section. Dendritic cells are often adjacent to connective tissue cells (CT) and/or extracellular reticulin deposits (arrows). Fig. 2, ×10,000; Fig. 3, ×12,500.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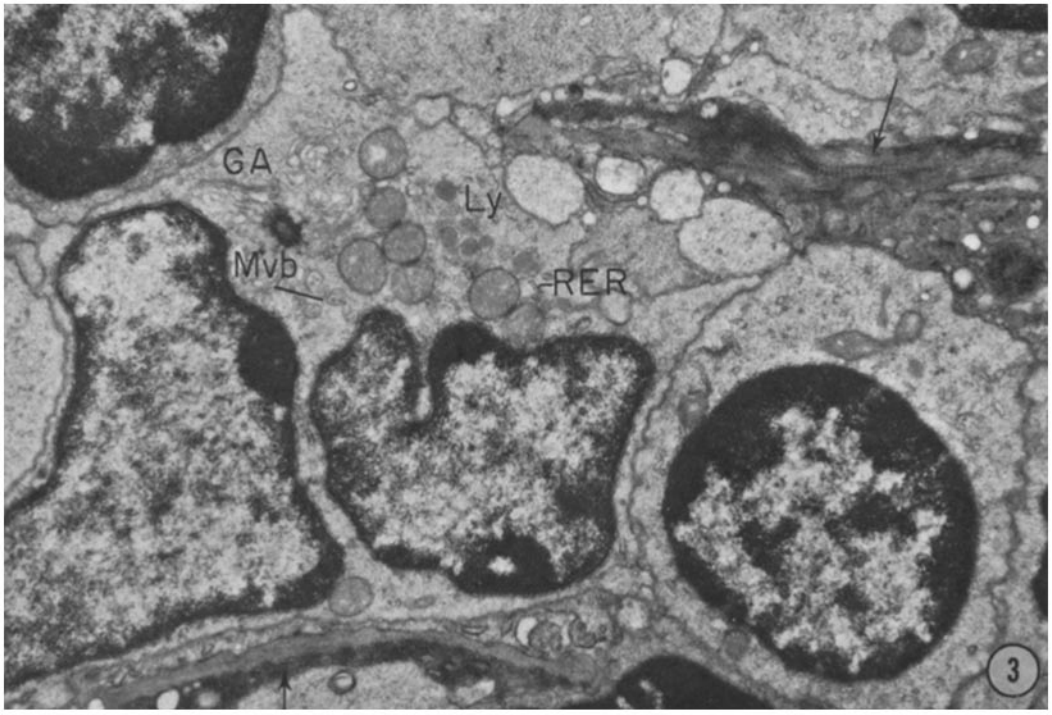
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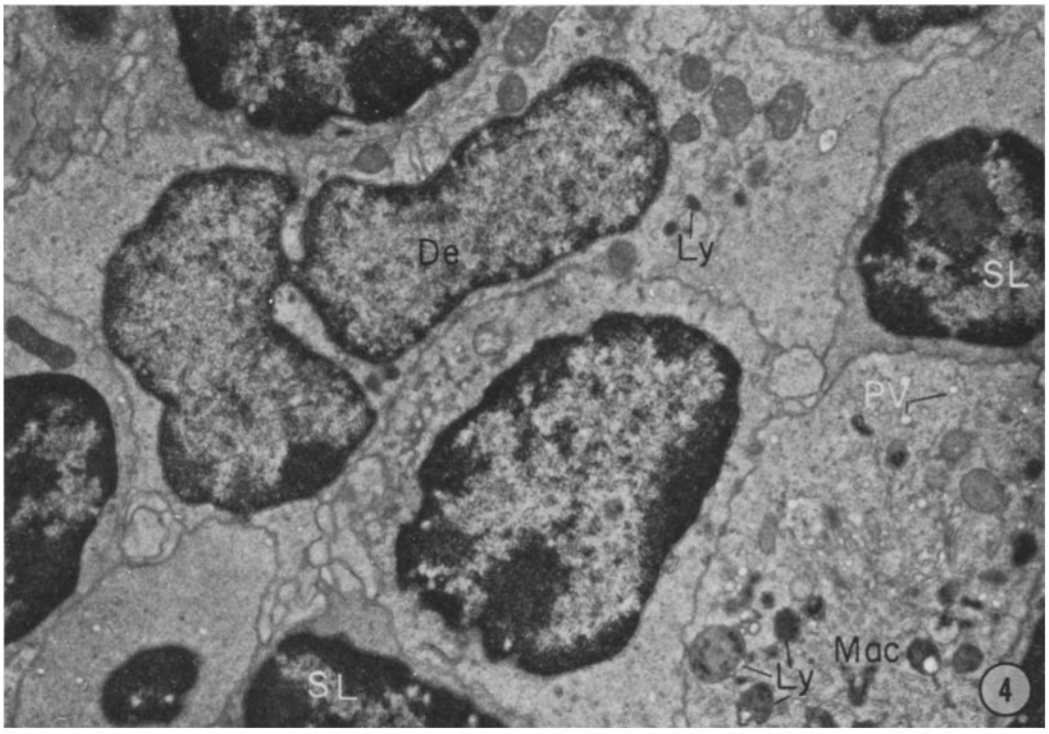
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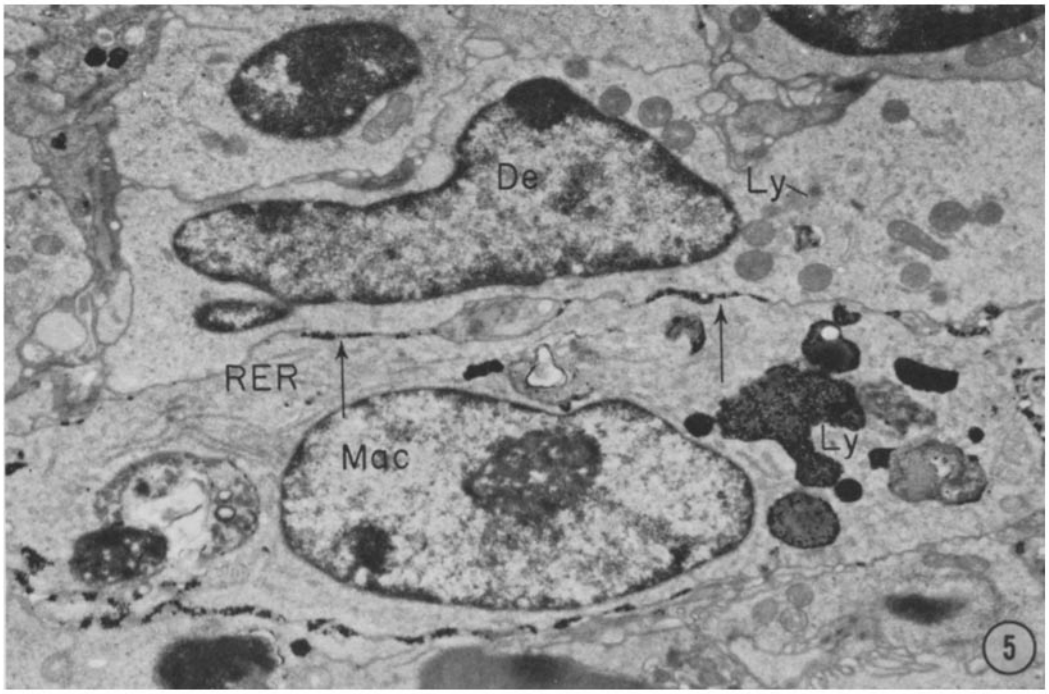
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small lymphocytes is quite different from dendritic cells in that there are many more particles, presumably ribosomes, and the cytoplasmic matrix is more electron dense (Figs. 1, 2, 4, 13, and 14).

The overall shape of dendritic cells could not be readily determined in single thin sections. Generally it was clear that cells with the nuclear and cytoplasmic features outlined above were extended in orientation. In serial sections, many broad processes, as well as a few tiny offshoots, were apparent. The processes often made sharp turns around connective tissue cells or extracellular connective tissue deposits.

Dendritic cells are found only in white pulp. We were not able to distinguish between thymus-dependent and thymus-independent areas in our preparations, so that it is not known if dendritic cells have a predeliction for one or the other. Dendritic cells were frequently found in groups, and in these cases, a small blood vessel was nearby (Fig. 1). In other instances, the cells were identified close to connective tissue cells (Figs. 1–5, and 11), though conceivably many of the latter were in turn associated with small penetrating vessels. Dendritic cells did not form specialized intercellular junctions with each other or with other cell types.

It was not possible to obtain accurate data on the number of dendritic cells in thin sections. One limitation was the difficulty in identifying dendritic cells cut on edge. Another was that groups of dendritic cells could be found in one region, while other areas contained few if any. However, it was evident from examining a large number of specimens, that dendritic cells constitute only a small fraction (less than 1%) of the total cellular elements of spleen, as was suspected from in vitro work in germfree mice (18).

Adoptive transfer experiments have shown that nonadherent spleen cells could repopulate the adherent dendritic cell population of irradiated mice (18). However, we have not identified a presumptive dendritic cell precursor by morphologic criteria.

To test the endocytic capacities of various cell types in situ, we administered colloidal thorium dioxide (thorotrast) intravenously before sacrifice (26). Shortly (3 h) after injection, particles are widely distributed throughout the intercellular spaces of the white pulp. However, at all times studied (1–24 h), dendritic cells contained relatively little of the endocytic marker (Figs. 5 and 11), especially when compared to other cell types (videinfra).

Macrophages. This designation is given to cells containing the numerous

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FIGS. 4 and 5. Dendritic cells (De), macrophages (Mac), and small lymphocytes (SL) are present in these micrographs of white pulp. The cytologic features of dendritic cells are similar to those mentioned in previous figures. The macrophage in Fig. 4 contains many lysosomes (Ly) as well as pinocytic vesicles (PV). The macrophage in Fig. 5 has interiorized large amounts of colloidal thorium dioxide particles (Ly) administered intravenously 3 h before sacrifice. Dendritic cell lysosomes (Ly) contain very little even though colloid is present in the intercellular space between the two cells (arrows). The macrophage nucleus is typical of white pulp phagocytes—it is oval in shape, contains little heterochromatin, and has a large nucleolus. The rough endoplasmic reticulum is well developed (RER). Fig. 4, ×8,750; Fig. 5, ×9,500.

membrane-bound vesicles and dense bodies which are typical of differentiated phagocytes (Figs. 4, 5, 6, and 10). These structures are enriched in the perinuclear region, but are also evident throughout the cell's cytoplasmic processes. The dense bodies or lysosomes assume an array of sizes, shapes, and content. Most contain a uniform electron dense content in which are embedded small very dense granules, presumably hemosiderin (Fig. 6). Another type of lysosome contains degenerating cells or "tingible bodies," (21) but these are quite infrequent in germfree animals.

Cells exhibiting an extensive development of the lysosomal system may have other distinctive cytological features, when compared to dendritic cells. The nucleus is often oval in shape, smooth in outline, and contains relatively little heterochromatin (Fig. 5). The nucleolus is more frequently noted and may be quite large (Fig. 5). The cytoplasm is replete with organelles including mitochondria, rough endoplasmic reticulum, and many stacks of Golgi saccules.

Within an hour of an intravenous dose of thorotrast, the macrophages of the marginal zone and red pulp had interiorized large amounts of colloid. With the dosage employed, uptake also occurred in white pulp macrophages (Figs. 5 and 10), though this often was evident only 3–8 h after the start of the experiment.

The distribution of macrophages in germfree spleen was quite different from that of the dendritic cells. In white pulp, they are less numerous than dendritic cells, but in red pulp and at the junction of white and red pulp, macrophages were found in abundance.

Connective Tissue Cells. Spleen contains a large number of elongate- and/or stellate-shaped cells that are invariably situated next to deposits of typical collagen fibrils and/or the more amorphous reticulin (Figs. 6–10, 12, and 13). Often the reticulin masses invaginate deeply into the cell body. The presence of prominent rough endoplasmic reticulum and Golgi regions are consistent with the notion that these cells secrete the connective tissue with which they are associated.

Connective tissue cells (fiber-forming cells, fibrocytes) have a number of other distinctive structural features. In most (Figs. 6, 7, and 12), the electron density of the heterochromatin and nucleoplasm is much greater than that seen in other cell types. The nuclear shape can be extremely irregular, and the nucleolus is often

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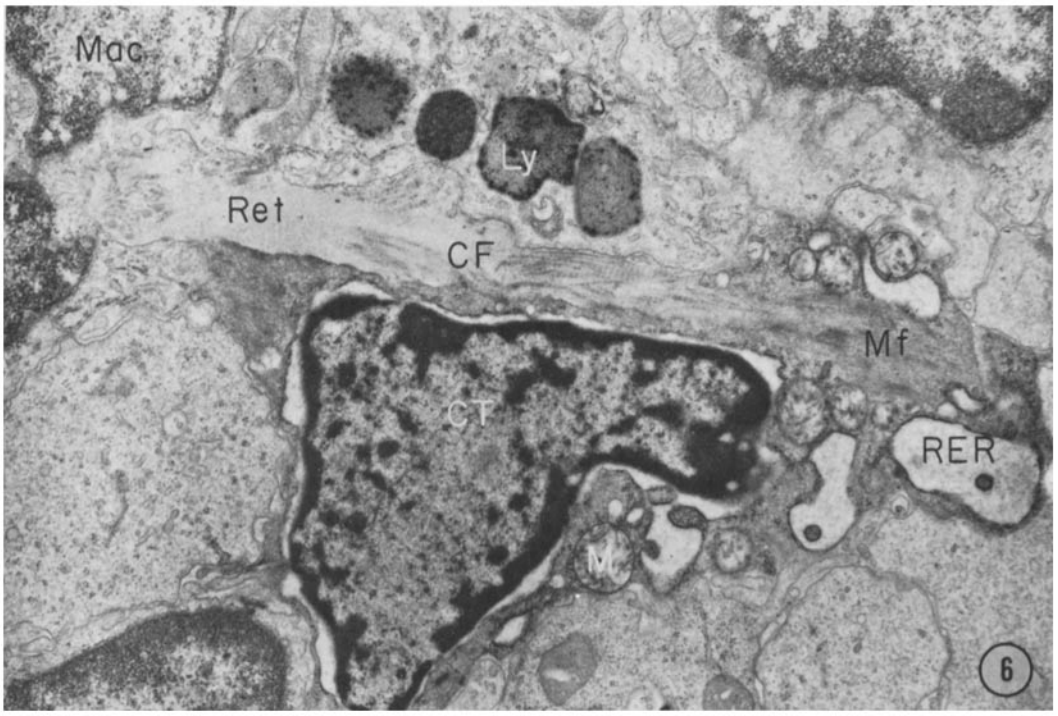
FIG. 6. A typical "dark" connective tissue cell (CT). These highly branched cells are found throughout red and white pulp and are associated with extracellular deposits of collagen fibrils (CF) and amorphous reticulin (Ret). The nuclear chromatin and nucleoplasm are both more electron dense relative to other cells. The rough endoplasmic reticulum (RER), including the perinuclear cistern, is dilated and the mitochondria (M) are often vesicular. The cytoplasm contains large bundles of microfilaments (Mf). A portion of a macrophage (Mac) is seen. The large membrane-bound lysosomes (Ly) contain electron dense granular deposits, presumably hemosiderin. ×13,000.

FIG. 7. Connective tissue cells (CT) extend and insinuate throughout the white pulp nodule and are especially abundant at the periphery shown here. Electron dense colloidal thorium dioxide particles may persist extracellularly for relatively long periods (8 h in this case) after intravenous administration. The colloid appears to be trapped in the reticulin deposits, e.g., at arrows. ×3,100.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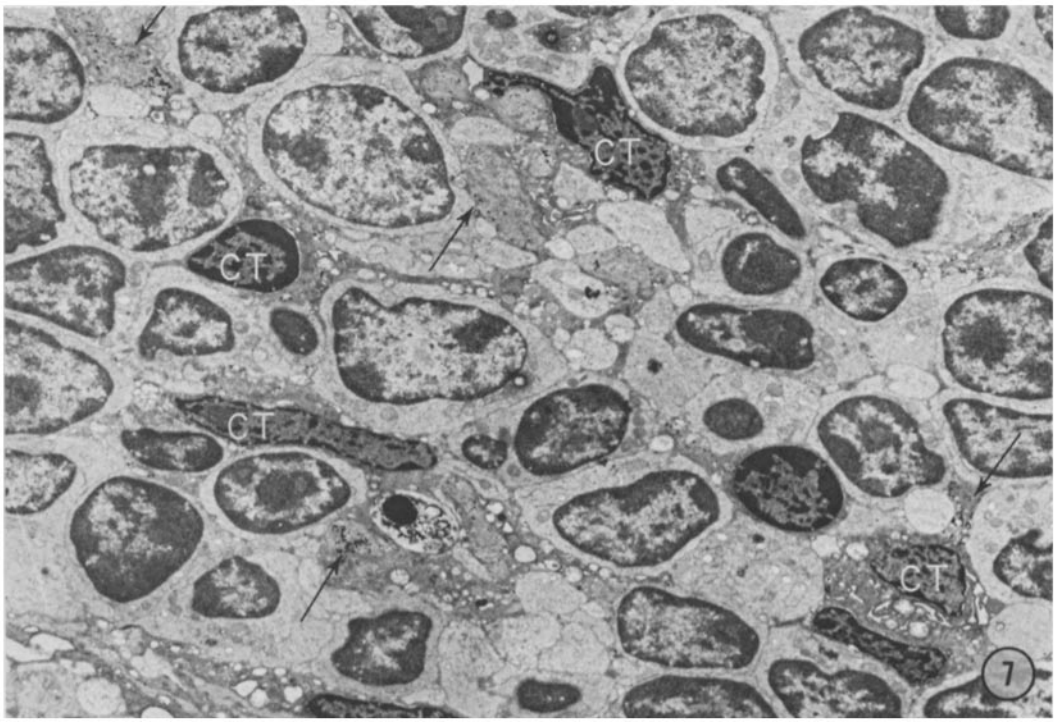
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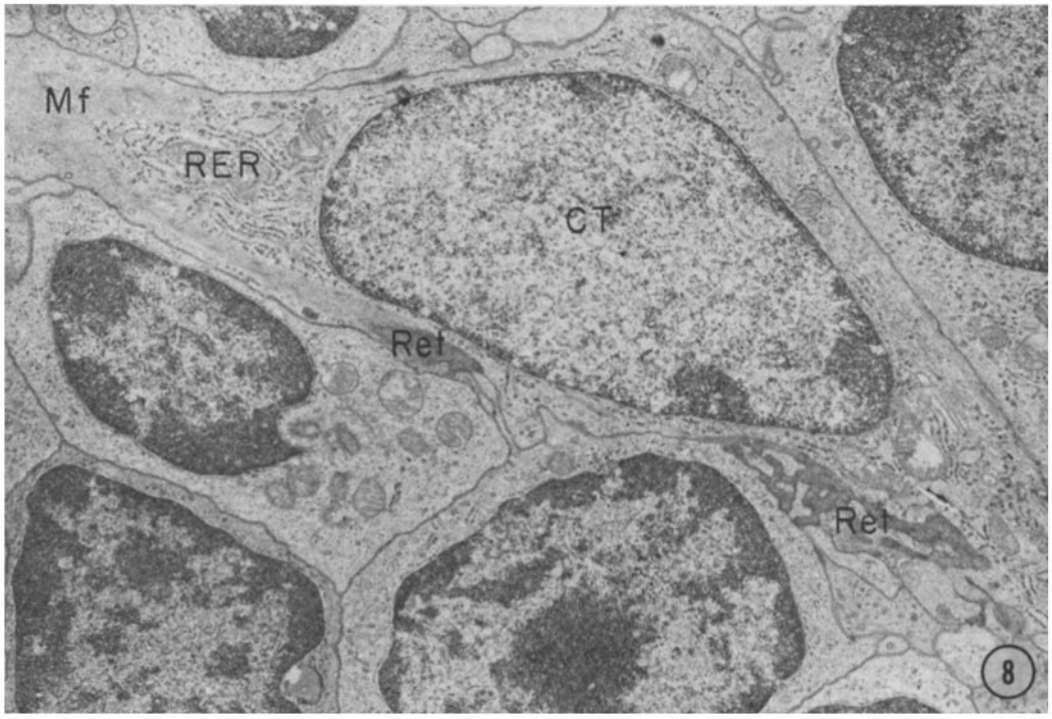
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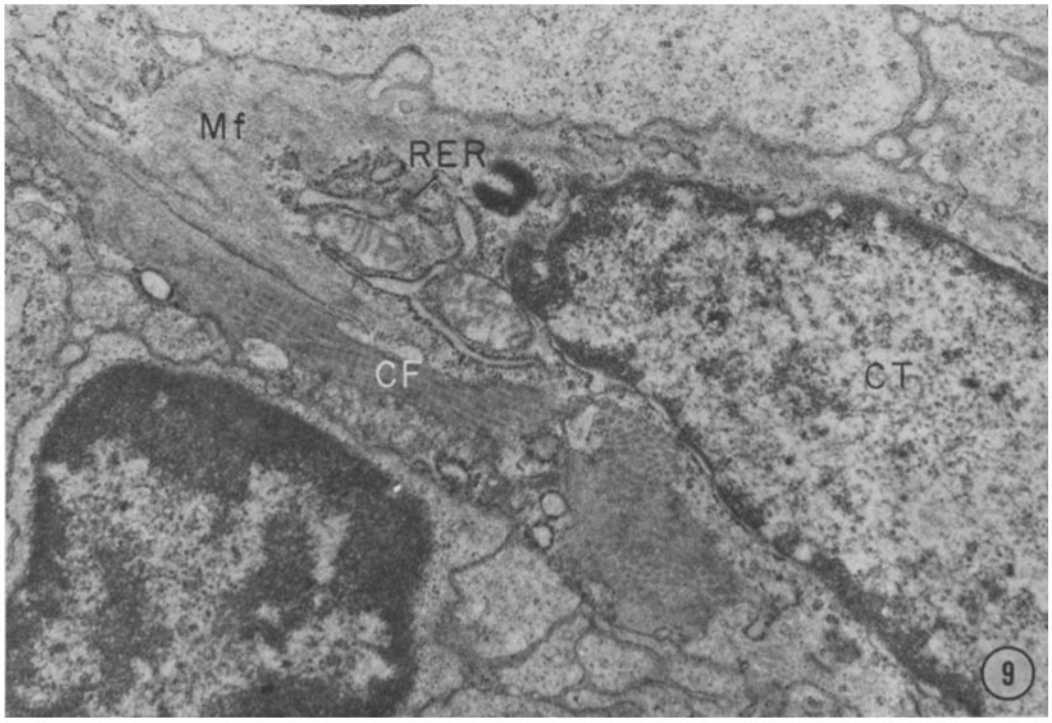
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seen in thin sections. The cytoplasmic matrix is also very electron dense, and contains prominent bundles of microfilaments. The rough endoplasmic reticulum is extremely dilated and the mitochondria often vesicular in appearance. Membrane-bound granules are variable in number, but can be numerous (Fig. 12). These "dense" or "dark" connective tissue cells are generally highly ramified in shape and extend many fine processes throughout the white pulp (Figs. 1 and 7).

Some of the connective tissue cells, i.e. those that contain abundant rough endoplasmic reticulum and are located close to connective tissue deposits, have different morphological features than that described above (Figs. 8 and 9). The cell shape is more fusiform than stellate. The nucleus contains less heterochromatin and is smoother in outline. Rough endoplasmic reticulum is abundant but not as dilated. The cytoplasmic matrix is more electron lucent, but microfilament bundles are readily apparent. Cells with these features may be younger and/or more active stromal elements.

Connective tissue cells are found throughout the spleen. In white pulp they are more numerous than dendritic cells or macrophages, while in red pulp, they are found in numbers similar to that of phagocytic elements. Most are easy to recognize in thick plastic sections because their cytoplasm stains strongly with basic dyes like toluidine blue.

Cell Types in Nonaxenic Mice. Mice reared under nonaxenic condition exhibit all three extended or branching cell types described in germfree animals. Their responses to intravenously administered colloidal thorium dioxide are similar. An additional, extremely ramified cell—which can be distinguished cytologically from the dendritic, phagocytic, and connective tissue cells described above—is found in nonaxenic mice, primarily in association with germinal centers. This cell type will be described in detail in a subsequent report.

Dendritic cells are more numerous in nonaxenic mice, as occurred when single cell suspension from the two types of animal were compared in vitro (18). In addition, the cell surface of the dendritic cells may be more irregular (Figs. 13 and 14), i.e. there are more small processes, the plasma membrane can form deep infoldings into the cell body, and sometimes cell processes from adjacent cells interdigitate with the dendritic cell.

Discussion

Cytologic criteria have been used to identify cell types in mouse spleen, particularly the white pulp. Although most white pulp cells have the morphologic features of small lymphocytes, we have concentrated on the minority which exhibit highly irregular cell shapes. These have been the subject of considerable

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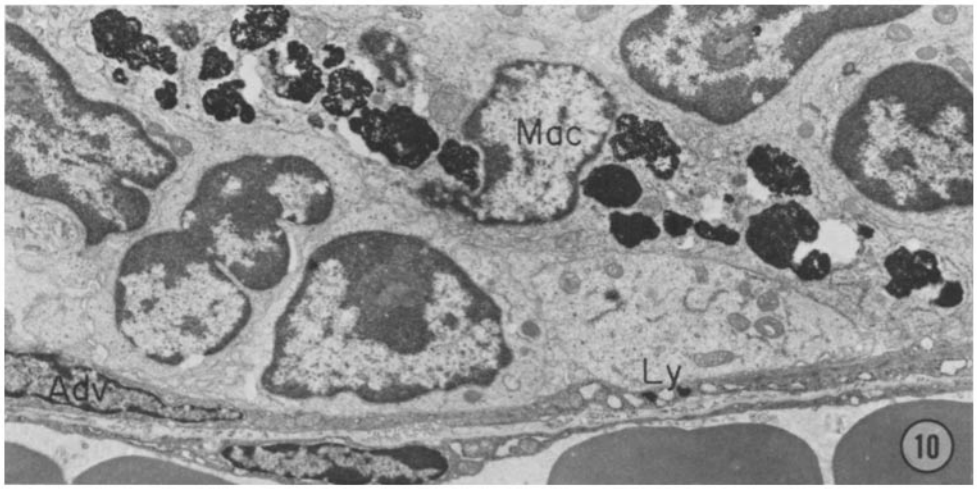
FIGS. 8 and 9. A minority of presumptive connective tissue cells (CT) exhibit a more electron lucent or "light" appearance, in both nucleus and cytoplasm. These cells are closely associated with extracellular matrix deposits (CF and Ret) which may invaginate deeply into the cell bodies. Rough endoplasmic reticulum (RER) is abundant but less dilated, and bands of microfilaments are prominent. Fig. 8, ×13,800; Fig. 9, ×18,200.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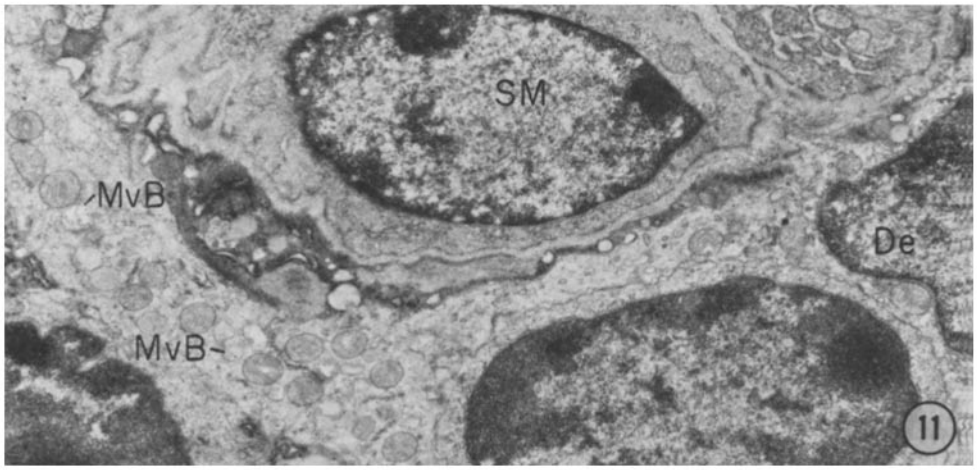
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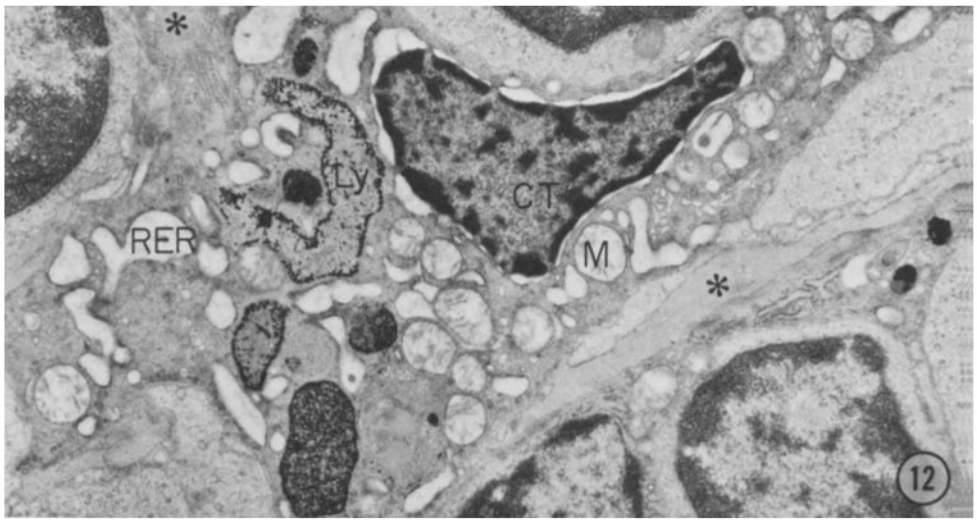
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12

previous work, but there is still no consensus on the sturcture, nomenclature, or function of this group of cells.

A priori, we suspected that at least three functionally distinct, irregularly shaped cell types must be present in germfree spleen, and that is what we found. (*a*) Dendritic cells, cells which were originally identified in vitro and which form many cytoplasmic processes after adherence to glass (16, 17). In vitro, they do not actively endocytose or synthesize collagen-like proteins, but their function is not yet known. The term dendritic cell was and is here employed only in reference to this in vitro entity, as it is not established if these cells have any relationship to previously described "dendritic" cell types in situ (dendritic macrophages and dendritic reticular cells, e.g. 4, 11, 15, 22, 28). (*b*) Phagocytic cells, i.e. cells capable of active endocytosis, particularly of immunoglobulin-coated particulates. These have been known to exist in abundance in spleen, primarily since the work of Aschoff (1). He administered vital dyes and then identified cells that actively stored these markers, many of which were irregular or highly branched in shape. (*c*) Connective tissue cells, i.e., cells responsible for the secretion of extracellular matrix materials. In lymphoid organs, the latter are termed "reticulin" deposits, probably because they too constitute a fine network. Light microscope studies of lymphoid organs have never clearly delineated connective tissue from phagocytic, or potentially phagocytic cells (e.g. 1, 6). In vitro, however, it is known that mononuclear phagocytes do not secrete collagen (3). In contrast, primary and continuous fibroblast tissue cultures lack the ability of macrophages to bind and interiorize immunoglobulin-coated particles (13, 20). Several electron microscope studies of spleen have demonstrated the existence of a network of cells which are associated with reticulin deposits, but which lack the morphologic features of active phagocytic cells (9, 12, 14, 26). They have been termed "reticular" or "reticulum" cells through this term has been used with a variety of other connotations (19 and videinfra).

We were able to find cells which were cytologically identical to the dendritic cells recognized in vitro. They were restricted to white pulp nodules, had irregular shapes, contained the expected nuclear and cytoplasmic features, and did not actively endocytose intravenously administered colloidal thorium dioxide. They were found in greatest frequency near white pulp blood vessels. There is very little previous work pointing to the existence of these dendritic cells in tissue sections. Clearly they lack a single unique cytologic feature that would have

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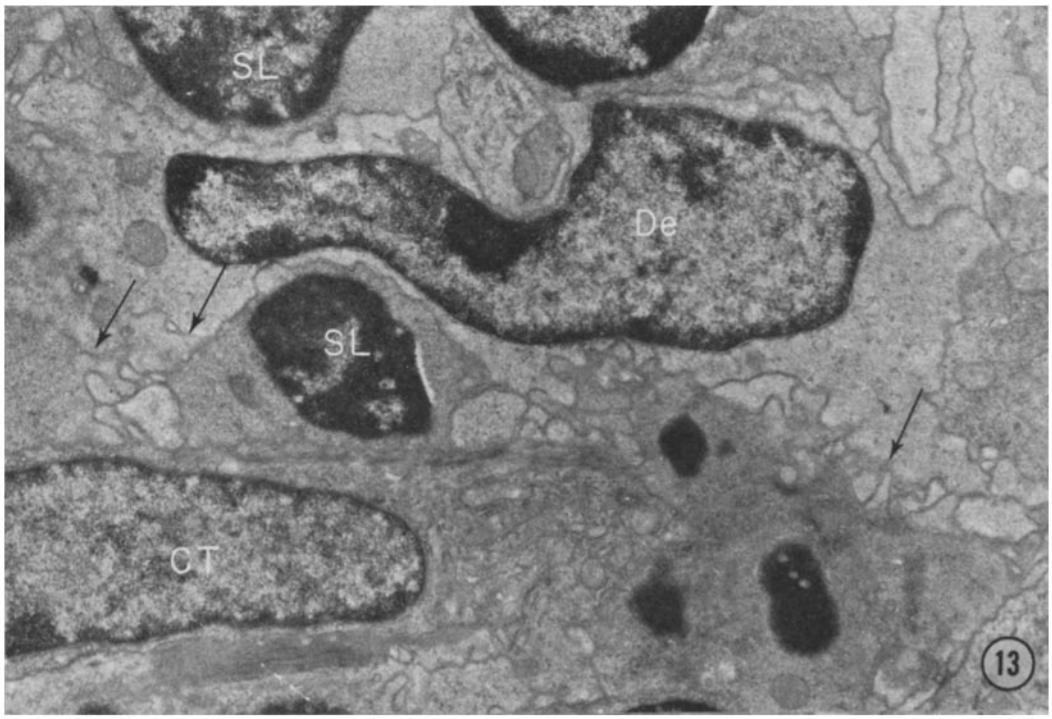
FIGS. 10–12. White pulp cells 24 h after intravenous administration of thorium dioxide particles.

FIG. 10. A periarterial macrophage (Mac) is full of colloid. The adventitial cell (Adv) is identical to the so-called dark connective tissue cells and has a few lysosomes containing colloid (Ly). ×4,700.

FIG. 11. A dendritic cell (De) encircling a blood vessel (SM, smooth muscle or media cell) contains little colloid. The extended cytoplasm has many mitochondria and multivesicular bodies (MvB). ×9,200.

FIG. 12. Some connective tissue cells (CT) may contain large, thorotrast-filled lysosomes (Ly) though in most cells the lysosomes are few in number and small in size (e.g., at arrows). This cell is classified as a connective tissue cell rather than a macrophage because of its typical rough endoplasmic reticulum (RER), vesicular mitochondria (M), and matrix deposits (*). ×13,800.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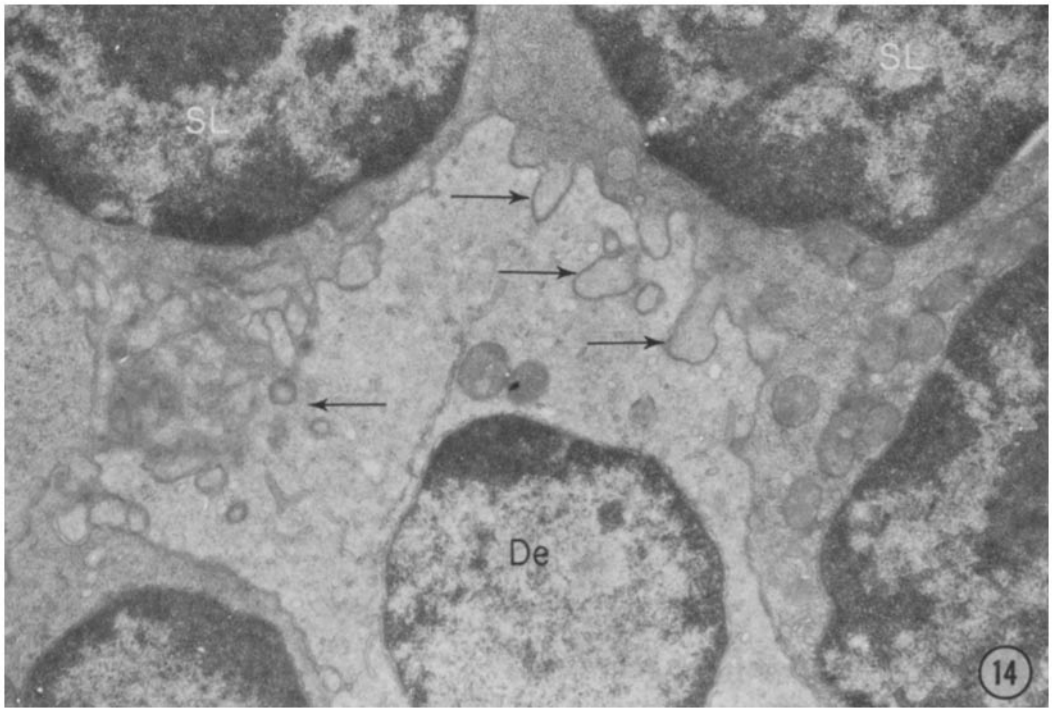
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permitted their identification. It was prior in vitro work that suggested to us the presence of this distinct class of cells and that provided a group of cytologic criteria that has enabled their description in situ. Certainly cells that might be classified as dendritic cells can be recognized in previously published micrographs, though they have been described noncommitally as mononuclear cells, large lymphocytes, or reticular cells. Veldman (25) has cited the existence of two sorts of irregularly shaped cells in rabbit lymph node—interdigitating cells and dendritic cells—that "could without exception easily be distinguished from reticulin-fiber-associated reticular cells (fibrocytes) and phagocytic reticular cells (macrophages)." Hoefsmit (5) and Veerman (24) have also called attention to interdigitating cells in thymic-dependent areas of rat node and spleen, respectively, and have distinguished them from a more highly ramified "dendritic" cell described by Hanna and Szakal (4, 22). Our current impression is that the dendritic cells we have described in vitro and in this study in vivo are similar to the "interdigitating cells." The resemblance becomes more striking in nonaxenic or in antigen-stimulated germfree mice (R. M. Steinman and Z. A. Cohn, manuscript in preparation), where the cells we are describing exhibit a much more irregular and interdigitated surface. Hopefully the nomenclature of these morphologically distinct, extended cell types can be simplified when further functional information becomes available.

Phagocytic cells were identified in this study using the same criteria previously employed by others (2, 9, 25, 26), i.e. they have abundant lysosomes and interiorize large amounts of appropriate endocytic markers, such as colloidal thorium dioxide particles. Additional features distinguishing macrophages from dendritic cells were their predominence in red pulp, nuclear structure, content of electron lucent pinocytic vesicles, and elaborate Golgi apparatus.

Those cells which were closely associated with reticulin deposits, and which contained abundant rough endoplasmic reticulum were titled connective tissue cells. Fiber-forming cells, fibrocytes, and fibroblasts are equally appropriate terms. The cytology of most connective tissue cell nuclei was also distinctive in that both the chromatin and nucleoplasm were markedly electron dense. These features were not evident in some previous studies involving tissues fixed solely in osmium tetroxide (9, 26). Cytoplasmic bands of microfilaments were another distinguishing feature of connective tissue cells. These have been noted previously in spleen (9, 12, 26) but not in other tissues. It is conceivable that the connective tissue cells perform other functions than secretion of the matrix deposits. The cytoplasmic microfilaments may enable them to influence the extensive migration of lymphocytes that occurs through the white pulp, e.g., by providing a

FIGS. 13 and 14. Dendritic cells (De) from mice reared under nonaxenic conditions often exhibit a more irregular cell surface than those seen in germfree animals. There are more fine processes, the plasmalemma forms invaginations of varying depth (arrows, Fig. 13), and adjacent lymphocytes (SL) may send interdigitating processes into the dendritic cell (arrows, Fig. 14). The small lymphocytes contain abundant nuclear heterochromatin and cytoplasmic ribosomes, and their nucleoli are often evident in thin sections. A young connective tissue cell (CT) is present in Fig. 13. Fig. 13, ×11,700; Fig. 14, ×16,000.

skeletal framework or by exerting a contractile function. In addition, splenic connective tissue cells endocytosed colloidal thorium dioxide particles, sometimes in considerable amounts relative to lymphocytes. The significance of this pinocytic activity is unclear. Where fibroblasts have been studied in vitro, it is known that they are capable of pinocytic activity, though they are less active than macrophages (20).

Distinguishing cells on the basis of morphologic criteria does not prove that they are in fact functionally different, or that one cell cannot assume the morphologic features and functional properties of another. With respect to dendritic cells, an in vitro analysis is possible. At this stage, it appears that dendritic cells definitely lack the functional properties of phagocytic and connective tissue cells (17, 18) and our current work shows that they do not transform into either cell type during prolonged cultivation in vitro.

A traditional histologic term we have avoided is that of reticular or reticulum cell. We feel this term is losing its usefulness as a functional entity. The morphologic criteria implied by it are too vague to denote a single class or groups of cells, and it is best replaced by more specific designations. Some authors emphasize the literal meaning of reticular, i.e. any cell with a network of branching processes, but it seems that at least three functionally distinct cell types—dendritic cells, macrophages, and connective tissue cells—may have this property. Others equate reticular cells with cells producing the connective tissue network of reticulin, but this entity is complicated by the notion that the reticular cells may be primitive or multipotential, i.e. capable of giving rise to hematopoietic and lymphocytic elements (6, 8). There is little evidence for this possibility, and it would seem unlikely. Single cell suspensions of mouse spleen, in which we cannot identify the connective tissue cells described in situ, are fully capable of reconstituting the hematopoietic and lymphocytic systems of lethally irradiated mice (R. M. Steinman, unpublished observations). Finally, other authors have stressed certain nuclear cytologic features of reticular cells, i.e. large size and/or relative lack of heterochromatin (7). This too is nebulous since large lymphocytes, macrophages, dendritic cells, and fibroblasts may meet these criteria, especially in light microscopic preparations.

We regard these morphologic observations on mouse spleen as a baseline for future work. We are now studying germfree spleen after administration of sheep red blood cells as a defined antigenic stimulus. Within 2 days, dendritic cells appear in relatively large numbers in areas of a developing plasma cell response, and there exhibit even more irregular cell shapes. These observations will be the subject of a future report.

Summary

White pulp nodules of mouse spleen contain a minor population of cells with morphologic features that are identical to those of dendritic cells, a cell type recently described in vitro. They have characteristic large, irregularly shaped nuclei with distinctive chromatin patterns and small nucleoli. The cytoplasm is extended in processes that contain relatively few organelles. These presumptive dendritic cells can be distinguished from other cell types that are known to exist in spleen including those that have irregular or branching cell shapes. In particular, dendritic cells do not contain the large number of lysosomes seen in

phagocytes, and do not actively interiorize intravenously administered colloidal thorium dioxide particles. They also lack the well developed secretory apparatus (rough endoplasmic reticulum and Golgi zone) and microfilament bundles that are noted in connective tissue cells. These morphologic observations, combined with previous in vitro work, substantiate the existence of a novel class of cells in mouse lymphoid organs.

The authors benefited from many helpful discussions with Dr. Robert North and Jon Deissler, The Trudeau Institute, Saranac Lake, N. Y.

Received for publication 12 December 1974.

References

1. Aschoff, L. 1924. Das Reticulo-Endotheliale System. Ergebn. d. inn. *Med. u. Kinderh.* **26**:1.
2. Burke, J. S., and G. T. Simon. 1970. Electron microscopy of the spleen. I. Anatomy and microcirculation. *Am. J. Pathol.* **58**:127.
3. Green, H., B. Goldberg, and G. J. Todaro. 1966. Differentiated cell types and the regulation of collagen synthesis. *Nature (Lond.).* **212**:631.
4. Hanna, M. G. Jr., and A. K. Szakal. 1968. Localization of ^{125}I-labeled antigen in germinal centers of mouse spleen; histologic and ultrastructural autoradiographic studies of the secondary immune reaction. *J. Immunol.* **101**:949.
5. Hoefsmit, E. C. M. 1975. *In* Mononuclear Phagocytes. R. van Furth, editor. Blackwell Scientific, Philadelphia, Pa. 2nd edition. In press.
6. Marshall, A. H. E. 1956. An outline of the Cytology and Pathology of the Reticular Tissue. Oliver & Boyd Ltd., Edinburgh and London, England.
7. Marshall, A. H. E., and R. G. White. 1950. Reactions of the reticular tissues to antigens. *Br. J. Exp. Pathol.* **31**:157.
8. Maximow, A. A. 1932. The macrophages or histiocytes. *In* Special Cytology, Vol. II., E. V. Cowdry, editor. Paul B. Hoeber, Inc., New York. 709.
9. Movat, H. Z., and N. V. P. Fernando. 1964. The fine structure of lymphoid tissue. *Exp. Mol. Pathol.* **3**:546.
10. North, R. J. 1971. Methyl green-pyronin for staining autoradiographs of hydroxyethyl methacrylate-embedded lymphoid tissue. *Stain Technol.* **46**:59.
11. Nossal, G. J. V., A. Abbot, J. Mitchell, and Z. Lummus. 1968. Antigens in immunity. XV. Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. *J. Exp. Med.* **127**:277.
12. Pictet, R., L. Orci, W. G. Forssmann, and L. Girardier. 1969. An electron microscopic study of the perfusion-fixed spleen. I. The splenic circulation and the RES concept. *Z. Zellforsch. Mikrosk. Anat.* **96**:372.
13. Rabinovitch, M. 1969. Uptake of aldehyde-treated erythrocytes by L2 cells. *Exp. Cell Res.* **54**:210.
14. Rhodin, J. A. G. 1974. Histology. Oxford University Press, New York.
15. Sordat, B., M. Sordat, M. W. Hess, R. D. Stoner, and H. Cottier. 1970. Specific antibody within lymphoid germinal center cells of mice after primary immunization with horseradish peroxidase: a light and electron microscopic study. *J. Exp. Med.* **131**:77.
16. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, and tissue distribution. *J. Exp. Med.* **137**:1142.

17. Steinman, R. M., and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J. Exp. Med.* **139**:380.
18. Steinman, R. M., D. S. Lustig, and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. III. Functional properties in vivo. *J. Exp. Med.* **139**:1431.
19. Steinman, R. M., and Z. A. Cohn. Dendritic cells, reticular cells, and macrophages. In *Mononuclear Phagocytes*. R. van Furth, editor. Blackwell Scientific, Philadelphia, Pa., 2nd edition. In press.
20. Steinman, R. M., J. M. Silver, and Z. A. Cohn. 1974. Pinocytosis in fibroblasts: quantitative studies in vitro. *J. Cell Biol.* **63**:949.
21. Swartzendruber, D. C., and C. C. Congdon. 1963. Electron microscopic observations on tingible body macrophages in mouse spleen. *J. Cell Biol.* **19**:641.
22. Szakal, A. K., and M. G. Hanna, Jr. 1968. The ultrastructure of antigen localization and virus-like particles in mouse spleen germinal centers. *Exp. Mol. Pathol.* **8**:75.
23. Tanaka, Y., and J. R. Goodman. 1972. Electron Microscopy of Human Blood Cells. Harper & Row, Publishers, New York.
24. Veerman, A. J. P. 1974. On the interdigitating cells in the thymus-dependent area of the rat spleen: a relation between the mononuclear phagocyte system and T-lymphycytes. *Cell Tiss. Res.* **48**:247.
25. Veldman, J. E. 1970. Histophysiology and electron microscopy of the immune response. Ph.D. Thesis. State University of Groningen, Groninger, The Netherlands.
26. Weiss, L. 1964. The white pulp of the spleen. *Bull. Johns Hopkins Hosp.* **115**:99.
27. Weiss, L. 1972. The Cells and Tissues of the Immune System. Prentice-Hall, Inc., Englewood Cliffs, N. J.
28. White, R. G., V. I. French, and J. M. Stark. 1970. A study of the localisation of a protein antigen in the chicken spleen and its relation to the formation of germinal centers. *J. Med. Microbiol.* **3**:65.