Increased Numbers of a Characteristic Type of Reticular Cell in the Thymus and Lymph Nodes of Leukemic Mice: An Electron Microscope Study

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SUMMARY
A characteristic type of reticular cell, the dense reticular cell, is described and illustrated by electron microscopy in the thymus and lymph nodes of mice. This cell is identified by its high nuclear and cytoplasmic density and by the presence of many ribosomes in its long, slender processes extending between lymphocytes (or thymocytes). The preservation of these processes requires special fixation procedures. Best results are obtained with Karnovsky's fixative, which combines paraformaldehyde and glutaraldehyde. Although the dense reticular cell shares some features with macrophages and epithelial reticular cells of the thymus, it should be recognized as a distinct cell because of its characteristic morphology and its occurrence not only in thymus but also in lymph nodes. Remarkably, the number of these cells, very low in normal tissue, is greatly increased in leukemic AKR mice.

INTRODUCTION
Two types of thymic reticular cells have been observed by electron microscopy: epithelial reticular cells and mesenchymal reticular cells or macrophages (5-7, 11, 13, 14, 33). Three kinds of reticular cells are known in the lymph nodes and considered as mesenchymal: reticular cells associated with fibers, macrophages, and undifferentiated reticular cells (2, 4, 12, 21). In this report, we describe another variety of reticular cell present in the mouse thymus and lymph nodes: the dense reticular cell (DRC), the number of which is greatly increased in leukemic mice. It is characterized by high electron density and by long, slender cytoplasmic processes containing many ribosomes (15). The preservation of these processes requires special care in fixation, which is therefore the essential condition for the description of this cell. Although it was possible to observe them with the fixatives used routinely in electron microscopy, the best results were obtained with Karnovsky's fixative, which combines glutaraldehyde and paraformaldehyde (16).

The object of this report is to describe this cell, to discuss its relationship with other reticular cells, and to indicate a hypothetical significance in leukemia.

MATERIALS AND METHODS
Normal thymuses and lymph nodes were obtained from mice of both sexes from strains with a low incidence of leukemia (C3Hf/Bi, I, BALB/c, 6 weeks to 10 months old) and from mice aged 6-8 weeks from the high-incidence AKR strain showing no histologic evidence of leukemia.

The neoplastic material consisted of the thymuses and lymph nodes of AKR mice aged 6-8 months with macroscopic and histologic signs of leukemia. Paraffin sections of all tissue samples were examined under the light microscope.

For electron microscopy, Karnovsky's fixative was most frequently used. Two gm of paraformaldehyde powder were dissolved in 25 ml of water by heating to 60-70°C and stirring. One to three drops of 1 N NaOH were added until the solution cleared. The solution was allowed to cool, 5 ml of 50% glutaraldehyde were added, and the volume increased to 50 ml with phosphate buffer pH 7.4-7.6.

The formaldehyde obtained by dissolving paraformaldehyde in water "penetrates faster than glutaraldehyde and stabilizes structures which are subsequently more permanently stabilized by glutaraldehyde" (16). Moreover, this formaldehyde is methanol-free (25).

The tissues were generally fixed from 1 to 5 hours at room temperature. However, some samples were fixed at 0°C and others at 37°C. They were then washed in phosphate buffer (20), postfixed in 2% buffered osmium tetroxide (25), 1 or 2% glutaraldehyde, and osmium tetroxide (27), and fixed by phosphate-buffered osmium tetroxide alone. All samples were dehydrated in alcohol and embedded in Epon (17).

The contrast was enhanced with uranyl and lead. For light microscopy, sections approximately one micron thick were stained with methylene blue (26) and photographed under oil immersion at × 800 or 240 magnification.

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OBSERVATIONS

Morphology

The DRC were stellate. Long processes containing ribosomes extended between neighboring lymphocytes (or thymocytes).

The Nuclei (Figs. 4-8) were polygonal, ovoid, or lobulated but rarely spherical. In sections, their area was often smaller than that of the nuclei of the surrounding cells. Both the heterochromatin and euchromatin were very dense. Nucleoli frequently contained a conspicuous “pars amorpha” without any “nucleolonema” (Figs. 4, 6). No mitotic figures were observed. Nuclear pyknosis was very rarely seen.

The Perikaryons were narrow. They contained mitochondria, lipid droplets (Fig. 5), endoplasmic reticulum profiles (Fig. 8), free ribosomes (Figs. 4, 5), and occasionally a Golgi complex (Fig. 5). The hyaloplasm was uniformly dense and granular (Figs. 4, 8). In some DRC the perikaryon was larger and contained phagosomes of various sizes and contents (Fig. 10). Moreover, a few DRC showing phagocytic activity were clumped together without any visible separating cell membrane.

The long cytoplasmic processes of these cells extended between lymphocytes (or thymocytes). In cross section, they were frequently triangular or rectangular (Figs. 4, 6-8). These processes were characterized by a dense cytoplasmic matrix containing many ribosomes (Figs. 11-13). While a delimiting membrane was frequently seen separating the processes from the surrounding cells, some processes, particularly the narrow ones, did not show a clearly visible membrane (Figs. 9, 13). Often they were in close contact with a distinctly different type of process. The latter structures were clear and round and contained ribosomes, a scattered granular material, and no other cytoplasmic organelles (Figs. 4, 7, 13). The origin of these clear processes was not determined.

The DRC were barely visible in paraffin sections but easily identified in “thick” sections (Figs. 1-3). Their stellar shape and the network made by their long, slender processes were conspicuous. Nuclei, perikaryons, and processes were deeply stained by the basic dyes.

Occurrence and Special Features

Normal Lymph Nodes. In both the low-leukemic strains and the young AKR mice, DRC were very uncommon. These few DRC which were present tended to aggregate in small groups (Fig. 4).

Lymphoma. In the leukemia AKR mice, numerous DRC were observed, frequently in clumps (Fig. 5). Many processes, which were particularly large and rich in ribosomes, completely surrounded the lymphocytes. Some were close to capillaries.

Normal Thymus. Both in low-leukemic strains and in young AKR mice with histologically normal thymuses, typical DRC were very rare (Figs. 1, 6, 7). They were located both in the cortex and the medulla, frequently near capillaries. Nevertheless, many forms intermediate between DRC and epithelial reticular cells were found. Some were characterized by a dense nucleus and cytoplasm as in the DRC, but their larger size and their nucleoli resembled those of epithelial reticular cells. They had both large processes with cytoplasmic organelles and slender processes filled with ribosomes. Larger and clearer intermediate forms, more similar to the epithelial reticular cells, were also observed.

In the same sections, typical epithelial reticular cells with large, clear nuclei, conspicuous nucleolonema, clear cytoplasm with tonofilaments, and desmosomes were seen. Long cytoplasmic processes containing ribosomes were never found extending from these cells.

Thymoma. The enlarged thymus of leukemic AKR mice contained more DRC than that of nonleukemic ones (Figs. 2, 8). However, the number of these cells was not so remarkably increased here as in the leukemic lymph nodes. Many thymocytes, occasionally in mitosis, were surrounded by the dense processes (Fig. 9). Under the light microscope, the striking length of some processes was clearly visible (Fig. 3).

Some DRC showed signs of phagocytic activity (Figs. 2, 10). Yet they were easily distinguished from the conventional macrophages observed in the same material, which did not show the electron density of the DRC and contained a clear nucleus and cytoplasm. Forms intermediate between DRC and epithelial reticular cells were less common than in normal thymus.

Various Aspects of Fixation

Karnovsky’s fixative, combining paraformaldehyde and glutaraldehyde, and used at room temperature before osmium tetroxide fixation, preserved the close contacts between the different cell types of the thymus or lymph nodes, thus permitting demonstration of the long slender processes of the DRC (Figs. 4, 7).

The temperature of the fixative appeared to be a critical factor. At 4°C, Karnovsky’s fixative produced shrinkage of all the lymphatic cells and retraction of the dense reticular processes. In contrast, at 37°C, cell contacts were maintained and the reticular processes were visible.

The only observed disadvantage of this fixation has been slight widening of the perinuclear cisternae, swelling of some mitochondria and extraction of lipid droplets (Fig. 5).

With other fixatives, paraformaldehyde and osmium tetroxide, glutaraldehyde and osmium tetroxide, or osmium tetroxide alone, the nuclei and perikaryons of the DRC were visible (Fig. 2) but their processes were preserved only occasionally (Figs. 2, 9). The lymphatic cells frequently shrunk, resulting in widening of the extracellular spaces (Fig. 2). It was more difficult under these conditions to recognize the DRC, the processes of which probably retracted or were inadequately fixed.

DISCUSSION

Discussion will be confined to problems of fixation, characterization of the DRC, and their possible function.

To our knowledge the DRC have not been described previously (2, 5, 6, 11-14, 18, 21, 29, 28, 33, 36). In Burkitt’s lymphoma, Aehong and Epstein observed “intra cellular spaces filled by an amorphous matrix which, in some areas, contains cell debris,” possibly corresponding to the processes of the DRC described here (1).

The difficulty in observing these cells is probably related to the special requirements of fixation. Although it is possible to
The DRC are characterized by nuclear and cytoplasmic density and by the presence of many ribosomes in their long processes. They appear to be morphologically the same in the thymus and the lymph nodes of normal and leukemic mice.

The presence of many ribosomes correlates with the deep basophilia of the DRC in light microscopy. However, in electron microscopy, the density of the cells is not explained solely by the presence of ribosomes. There is also a general density of the hyaloplasm demonstrable after Karnovsky’s fixation as well as after other fixation procedures.

The plasma membranes limiting the processes are not always visible. This is particularly true with very narrow processes. In this case, the presence of a DRC process between the lymphocytes is indicated only by the occurrence of ribosomes in the dense cytoplasmic matrix characteristic of the DRC. One explanatory hypothesis might be that tangential incidences of sectioning give too faint a picture of the membrane. The three-dimensional shape of the processes might correspond to pyramids with blunt points, giving numerous possibilities of tangential sectioning near the end of the processes. It is unlikely that the ribosomes were scattered in the extracellular spaces as a result of cell disruption because: (a) the fixation of our samples was entirely satisfactory and disrupted plasma membranes were never seen, and (b) ribosomes seen in this situation were within a dense cytoplasmic matrix and did not appear on a clear background, as would be the case if they were dispersed in extracellular spaces.

The processes of DRC run between lymphocytes (or thymocytes) and, in some instances, form a complex network which may surround them. Therefore, the term “reticular” seems to be particularly suitable for the DRC.

The presence of DRC is not restricted to thymuses and lymph nodes; some have been observed in spleens. Moreover, they have been found in the thymus of rats (I. Weisblum, personal communication), of guinea pigs, and in canine lymphoma (unpublished observations).

The processes of DRC are frequently the satellites of a clear, round process. The exact origin of these clear processes was not seen. However, we suggest that they might be the pseudodopodia of phagocytic cells in ameboid locomotion which are known to contain only ectoplasm without cytoplasmic organelles (9).

What are the differences observed between DRC and other reticulocytes of the thymus and lymph nodes after identical fixation?

The conventional macrophages of the lymphatic tissue have a different morphology from DRC. They are round cells, contain a large, clear nucleus, a clear cytoplasm, an abundant Golgi complex, and many rough endoplasmic reticulum profiles. Their processes, if any, are short. In addition, when DRC express some phagocytic activity, they retain their individual features: dense nucleus and cytoplasm, and processes rich in ribosomes.

In lymph nodes, DRC are different from the reticular cells associated with fibers, since they are never surrounded by any fiber. They are also distinct from “stem cells” or “undifferentiated reticular cells,” which are characterized by a large clear nucleus and poorly differentiated cytoplasm (2).

Apparently, a close relationship between DRC and epithelial reticular cells is suggested by the observation of many intermediate forms of variable size and density in the same sections. These intermediate forms are not easy to classify as DRC or epithelial reticular cells, suggesting transformation of one cell type into the other. However, if such transformation takes place, it is difficult to ascertain in what direction it occurs. Are the DRC the precursors of epithelial reticular cells or vice versa? This question cannot be answered at this time. Even if DRC and epithelial reticular cells have a common origin, the DRC appear to form a separate family for the following reasons: (a) morphologic differences generally permit us to distinguish these two cell types; (b) DRC are also present in lymph nodes, which are not of lymphoepithelial origin and do not contain epithelial reticular cells.

Therefore, although DRC share some features with macrophages and epithelial reticular cells, they can be considered as a separate cell entity. Moreover, they do not represent “dark cells” already described in other tissues, particularly the liver. In pathologic liver, “dark cells” and “light cells” have been observed (23, 30). The hepatic “dark cells” were deeply stained by methylene blue in thick sections and their hyaloplasm was very dense under the electron microscope, but their cytoplasmic organelles were the same as in “light cells.” To explain the difference in density, a modification in hydration or a modulation in metabolic activity of these cells was suggested (23, 30). One can hardly imagine how such metabolic modulation of another type of reticular cell could give rise to the unique appearance of DRC, which are distinguished not only by their density but also by their long cytoplasmic processes containing numerous ribosomes. In any event, these DRC are entirely different from the dark necrotic cells described by Weinberger and Banfield (35).

The role of these cells is difficult to evaluate. Do they have any similarity to the “lymphocyte-like reticular cell” recently described in the regenerative thymus by Blackburn and Miller (3), or to the “lymphocyte-like reticular cell” observed by Swartzendruber in antigenic-stimulated spleen (32)?

In the normal thymus, where they are frequently situated near capillaries, do they separate some thymocytes from the circulating blood? Do they participate with epithelial reticular cells in a “functional barrier” (22) preventing thymocytes from being stimulated by circulating antigens?

So far, the most important observation concerning the role of the DRC is that their number is considerably increased in leukemic tissues. In neoplastic thymuses and lymph nodes, their phagocytic activity is increased and their processes completely surround many lymphocytes (or thymocytes), some of them in mitosis. Metcalf et al. (19) observed an increased number of phagocytic cells surrounded by lymphocytes with high mitotic activity in AKR lymphoma. They suggest that,
in addition to removing debris from lymphoma tissue, these phagocytic cells might be essential for the continuous proliferation of lymphoma cells (19). Evidence concerning the role of DRC in the phagocytosis of leukemic or pyknotic cells and in the regulation of lymphopoiesis in both normal and neoplastic tissues might emerge from further studies of these characteristic reticular cells.

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REFERENCES

Figs. 1-13. Unless otherwise indicated, all samples were fixed in Karnovsky's solution at room temperature, postfixed in osmium tetroxide and posttreated in uranyl acetate at 4°C. DRC, dense reticular cell(s).

Fig. 1. Normal thymus, 6-week-old AKR mouse. DRC and its processes (→). X 6,400.

Fig. 2. AKR thymoma. Fixation in glutaraldehyde and osmium. DRC and their processes (→). DRC with phagocytic activity (P). The cells are slightly shrunken and the extracellular spaces widened (→). X 2,400.

Fig. 3. AKR thymoma. DRC with very long processes. X 760.

Figs. 4-13. Electron micrographs from sections contrasted with uranyl and lead.

Fig. 4. Normal lymph node, C3Hf/Bi mouse. DRC and their processes (→). The dense processes are frequently in contact with clear processes of unknown origin (*). X 7,500.

Fig. 5. AKR lymphoma. Three clustered DRC and their processes (→). In the cytoplasm: mitochondria (M), lipid droplets (Ld) Golgi complex (G), free ribosomes. The micrograph shows some slight disadvantages of the Karnovsky's fixation: widening of perinuclear cisternae, swelling of mitochondria, extraction of lipid droplets. X 20,000.

Fig. 6. Normal thymus, C3Hf/Bi mouse. DRC and processes (→) extending between thymocytes. X 7,500.

Fig. 7. Normal thymus, 2-month-old AKR mouse. Karnovsky's fixation at 37°C. DRC and processes (→). Clear process of unknown origin (*) in close contact with the dense processes. X 7,500.

Fig. 8. AKR thymoma. DRC containing rough E.R. cisternae. Thymocytes (T). X 17,500.

Fig. 9. AKR thymoma. Glutaraldehyde and osmium tetroxide fixation. Cell in mitosis completely surrounded by dense processes containing ribosomes (→). X 16,500.

Fig. 10. AKR thymoma. DRC with phagocytic activity. Dense nucleus (N). Dense hyaloplasm. Phagosomes (P). Processes (→). X 33,500.

Fig. 11. Normal AKR mouse. Dense process containing ribosomes in close contact (or in continuity) with a process containing tonofilaments (t). The cell membrane of the process and the surrounding thymocytes is clearly visible on the left (→) and not visible on the right (→). X 17,000.

Fig. 12. AKR thymoma. Dense process. Two "unit membranes" are visible on both sides (→). X 39,000.

Fig. 13. AKR lymphoma. Processes of DRC. The density and granular aspect of the hyaloplasm are easily seen. Two visible "unit membranes" on the left (→), only one on the lower right (→), none on the upper right (→). A clear process of unknown origin in the center (*). X 39,000.
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