Normal Human Lymph Node Cells: An Electron Microscopic Study

By Robert E. Brooks and Benjamin V. Siegel

Although an extensive literature has accumulated on the electron microscopy of mammalian lymph nodes, the ultrastructure of normal nodes of human origin has received less attention. Bernhard and Granboulan and Granboulan have reported on the ultrastructure of human lymph node cells, and recently Bernhard and Leplus have published an atlas containing electron micrographs of cells from normal and pathological human lymph nodes. In addition to describing lymph node cells, Bernhard and Granboulan have presented a diagrammatic interpretation of cell development in the lymphocytic and plasma cell lines. Similar schemes for the development of the several lymphoid cell lines from a single primitive cell have been offered for the rat by Han, for the mouse by Moe, and for the human by Bernhard and Leplus. The present study was undertaken to provide further ultrastructural descriptions of the several cell types of normal human lymph nodes as a basis for comparison with pathologically altered lymph node cells.

Materials and Methods

Lymph nodes were obtained from adult patients undergoing surgery for conditions not related to lymphoid tissue disease. Nodes showing pathologic changes, when examined by light microscopy, were not included in this study. Lymph nodes from 15 individuals were prepared for light and electron microscopic examination. All nodes were abdominal, most from the lower abdomen. No attempt was made to correlate sex or patient age with lymph node fine structure.

Fresh nodes were fixed for electron microscopic study by cutting the tissue into approximately 1 cm. blocks while under a few drops of Caulfield’s osmium tetroxide-based fixative fluid. Following fixation at refrigerator temperature (about 5 C.) for 2 hours, the specimens were rapidly dehydrated at room temperature in absolute alcohol and propylene oxide and embedded in Epon 812 according to the method of Luft. Thin sections of the epoxy embedded nodes were cut with either a Servall or LKB microtome and examined in an RCA EMU 3-G electron microscope. Sections were usually stained with aqueous uranyl acetate for 5 minutes followed by Reynolds’ lead citrate stain for 30 seconds. Adjacent thick sections, about 1 micron in thickness, were stained with 1 per cent toluidine blue in 1 per cent aqueous borax and examined with the light microscope.

Observations

It was noted in the course of this study that the form and degree of nuclear chromatin aggregation provided useful features for identifying the normal...
Fig. 1.—Low magnification illustration of normal human lymph node cells. Reticular cells (RC), and lymphocytes (L) predominate in this section. The tip of a plasma cell (PC) is recognized by the characteristic abundance of rough-surfaced endoplasmic reticulum. An extravascular red blood cell (RBC) is present among the lymph node cells. \( \times 5500 \).

Lymph node cells. To take advantage of these identifying features, sections for electron microscopic examination were cut slightly thicker than usual, and the contrast of the nuclear material was further enhanced by double-staining the sections with uranyl acetate and lead citrate.

At relatively low electron microscopic magnifications, reticular cells, lymphocytes, plasma cells, and macrophages are distinguishable (Figs. 1-3). The reticular cells are primarily recognized by the irregular, usually elongated
shape of the cells and their nuclei, and by the thin band of chromatin at the nuclear margin. In contrast, the more rounded lymphocytic cells have nonmargined, denser nuclear chromatin. Plasma cells are recognized by the abundance of rough-surfaced endoplasmic reticulum in the cytoplasm, and by the wide and irregular band of nuclear chromatin at the periphery of the nucleus.

In addition to the above cells which comprise the majority of the lymph node cellular elements, hemocytoblasts (Fig. 4), sinus lining cells (Figs. 5 and
Fig. 3.—Low magnification illustration of normal human lymph node cells. A large macrophage (MAC) is surrounded by lymphocytes at various stages of maturity. The cytoplasm of the macrophage contains phagocytized materials of different sizes, shapes, and electron densities. × 5700.

6), and blood capillary endothelial cells (Fig. 7) are identifiable at low magnifications. The hemocytoblasts have large nuclei, prominent nucleoli, and a thin rim of chromatin at the edge of the nucleus. The remaining nuclear chromatin is evenly distributed. The abundant cytoplasm of the blast cells contains a large number of free ribosomes, but few mitochondria, and very little rough-surfaced endoplasmic reticulum. The sinus lining cells vary somewhat in appearance, depending upon their location. The cell shown in Figure 6 is a typical reticuloendothelial cell. The elongated nucleus and attenuated cytoplasm of this cell makes it similar in appearance to endothelium. The chromatin distribution of the reticuloendothelial cell is similar to that found in the reticular cell, and nucleoli are often large. A narrow, collagen fibril-containing space underlies the reticuloendothelial cells. Reticular cells lie adjacent to this space and together with the reticuloendothelial cells form a wall separat-
Fig. 4.—Low magnification illustration of normal human lymph node cells. A group of hemocytoblasts (HB) are surrounded by reticular cells (RC) and lymphocytes (L). The hemocytoblast is characterized by its large nuclear, nucleolar, and cytoplasmic size, and by the abundance of free ribosomes in the cytoplasm. × 5100.

...ing the nodular cells from those within the sinus. In the subcapsular region, the sinus lining cells differ somewhat from the above in that those immediately adjacent to the thick subcapsular connective tissue layer are closely joined, one to the other, and have a greatly attenuated, relatively empty cytoplasm. Trabecular cells, which bridge the gap between the subcapsular sinus lining cells and the reticuloendothelial cells, are intermediate in appearance. The trabecular cells, which may be one or two layers thick, follow a core of collagen fibers into the parenchymatous portion of the node. Neither the
Fig. 5.—Low magnification illustration of normal human lymph node cells.
A portion of a sinus is shown lined by a reticuloendothelial cell (RE). Within the lumen (LU) of the sinus, lymphocytes (L) as well as other cell types are found. A reticular cell (RC), oriented in the same direction as the reticuloendothelial cell, separates the parenchymal cells from the sinus lining. × 6800.

subcapsular lining cells nor the trabecular lining cells show much, if any, evidence of phagocytosis. The endothelial cells of the blood capillaries (Fig. 7) are separated from the surrounding lymphoid tissue by a continuous basement membrane and an adjacent, narrow connective tissue space containing collagen fibrils and fine cellular processes of fibroblasts. The endothelial cells occasionally contain dark pleomorphic material in the cytoplasm, suggesting that these cells are capable of phagocytosis.

Hemocytoblasts are shown in Figure 4 and at somewhat higher magnification in Figure 8. The hemocytoblast is a large cell of irregular outline. The
Fig. 6.—Low magnification illustration of normal human lymph node cells. A portion of a subcapsular sinus and lumen (LU) is shown lined by sinus-lining cells (SL) and trabecular (T) cells. The sinus-lining cells, adjacent to the subcapsular connective tissue space (SCT), appear very similar to capillary endothelial cells, while the trabecular cells appear more like reticuloendothelial cells. × 7700.

Large nucleus has marginated chromatin and nucleoli are prominent. The free ribosomes in the cytoplasm are numerous and tend to aggregate in groups. Few mitochondria or other cell organelles are present.

The lymphoblast (Fig. 9) is similar to the hemocytoblast, although it is a somewhat smaller cell having a more rounded shape. The nuclear chromatin is more dense than that of the hemocytoblast and shows little tendency towards aggregation. Nucleoli are usually prominent. The lymphoblast is similar to the hemocytoblast in that both cells have few cytoplasmic organelles.

Lymphocytes (Figs. 10 and 11) vary in size and appearance according to...
Fig. 7.—Low magnification illustration of normal human lymph node cells. An endothelial (EN) lined blood capillary, with a red blood cell (RBC) in the lumen (LU), is separated from the parenchymal lymphocytes (L) by a basement membrane and thin connective tissue space. × 7900.

...their maturity. The cell shown in Figure 10 is a medium-sized lymphocyte. It differs from the lymphoblast in that the cell is smaller in size, the nucleus is more irregular in shape, and the cytoplasmic organelles are grouped together at one side of the cell. In comparison with the medium lymphocyte, the cytoplasm and nucleoplasm of the small lymphocyte (Fig. 11) are noticeably...
more dense. Also, chromatin aggregation is more prominent in the small lymphocyte. A nucleolus is usually noted in both the small and medium lymphocyte. The nuclear membrane may be smooth or irregular and occasionally is markedly indented. Membranous and vesicular components of the Golgi apparatus, as well as centrioles, are found in these cells grouped in close proximity with the mitochondria. Little rough-surfaced endoplasmic reticulum occurs in the lymphocytes.

The mature plasma cell, (Fig. 12) is easily identified by the large amount of rough-surfaced endoplasmic reticulum arranged, for the most part, concentrically in the cytoplasm. The inner, frequently dilated, cisternae of the endoplasmic reticulum are filled with homogeneous gray material. A well-developed, perinuclear Golgi apparatus is typically found in this cell. Mitochondria
Fig. 9.—Lymphoblast. This cell differs only slightly from the hemocytoblast. The lymphoblast has a more regular cytoplasmic and nuclear outline. The electron density of the nucleoplasm has increased. Free ribosomes predominate in the cytoplasm. × 16,300.

are numerous in the peripheral portions of the cytoplasm and variable-sized dense round granules also occur in this location.

The mature reticular cell (Fig. 13) is irregular in shape, often elongated or stellate. Usually, part of the cytoplasm of the reticular cell surrounds or contacts a cord of collagen fibrils. The cytoplasm of the cell has a large amount of rough-surfaced endoplasmic reticulum, a well-developed Golgi apparatus, and occasionally small, dense, irregularly shaped granules.

The macrophage (Fig. 14) is a large cell, irregular in outline, which may or may not be in close contact with collagen fibrils. Long cytoplasmic processes of the macrophage extend between the various lymph node cells. The nucleus of the macrophage is similar to the reticular cell, being irregular in shape and showing margination of chromatin. The cytoplasm contains many mitochondria.
Fig. 10.—Lymphocyte. This medium-sized lymphocyte has an irregular nuclear outline, dense nucleoplasm, and a small nucleolus. Mitochondria (MIT) and Golgi apparatus (GA) are localized to one side of the cell. ×12,000.

dria, a moderate amount of endoplasmic reticulum, and numerous pleomorphic dense bodies, probably of lysosomal origin.

In addition to the blood cells, which will not be described here, mast cells frequently occur in the lymph nodes. As noted in Figure 15, the mast cell is readily distinguished by the large number of membrane-bounded, round granules of varying electron density that almost entirely fill the cytoplasm. Few mitochondria and little endoplasmic reticulum are seen in this cell, although there is a small Golgi zone. The round, centrally located nucleus contains large chromatin aggregations.

Cell to cell relationships are of interest in lymph nodes. One of the relationships which we have frequently noted, and which is illustrated in Figure 12, is the close association of plasma cells and macrophages to blood capillaries. Frequently, a capillary will be ringed entirely by mature plasma cells. In the illustration shown, the plasma cell is noted to abut directly on an incomplete basement membrane which lies slightly separated from the continuous endothelial basement membrane. The plasma cell shows many cytoplasmic protrusions on all sides which, together with the cell body, are in intimate contact with both macrophages and lymphocytes.

Cellular processes are particularly numerous in the lymph nodes. Such processes, labeled PR, are seen in Figures 2, 11, 14 and 15. Most of these processes contain few organelles. The major constituents are free ribosomes. Undoubt-
Fig. 11.—Lymphocyte. This small lymphocyte is similar to the medium lymphocyte, except both nucleoplasm and cytoplasm are more condensed. Mitochondria (MIT), Golgi apparatus (GA) and centriole (CN) remain at one side of the cell. Several cytoplasmic processes (PR) from other cells are seen adjacent to the lymphocyte. × 12,000.

edly, many of the processes are protrusions from adjacent cells, which, in section, appear as isolated bits of cytoplasm. Examination of serial sections tends to bear this out, since most processes can be traced back to a cell. However, it is possible that some of the processes may actually represent cytoplasmic blebs which have been pinched off from some of the lymph node cells.

DISCUSSION

The criteria utilized for cell identification in this report are based, in large part, on the electron microscopic identifications and schematic systems of lymph node development given by Han,4 Moe,5 Bernhard and Granboulan,1 and Bernhard and Leplus.3

We have obtained no evidence from our observations which would positively substantiate any theory of lymph node cell origin and development. We could not, with confidence, illustrate a primitive reticular cell. Han4 has illustrated several cells which he terms “nondifferentiated reticular cells.” These cells are stellate in outline and appear quite primitive. Moe5 has illustrated several similar cells, calling them primitive reticular cells. Since Han’s tissues were embedded in methacrylate and Moe’s in epoxy resins, it is difficult to compare the cells and assess the degree of similarity. Bernhard and Leplus3
Fig. 12.—Plasma cell. The mature plasma cell (PC) is characterized by the very extensive development of the rough-surfaced endoplasmic reticulum (ER). A perinuclear Golgi apparatus (GA) is typical in this cell. This plasma cell lies adjacent to an endothelial (EN) lined capillary, separated from the lumen (LU) of the capillary by basement membranes, a very narrow connective tissue space, and a thin layer of endothelial cytoplasm. A macrophage (MAC) and other cells are in close contact with the plasma cell. × 18,500.
Fig. 13.—Reticular cell. This mature reticular cell is irregular in shape, has an elongated nucleus, and a prominent nucleolus. The cell is close to a bundle of collagen fibrils (CF), partially enclosing the bundle by its cytoplasm. The abundant cytoplasm of this reticular cell contains rough-surfaced endoplasmic reticulum (ER), Golgi apparatus (GA) and centriole (CN). × 12,000.

have presented the best pictures of cells considered to be primitive reticular cells. However, the most clearly depicted of these cells contains a relatively large amount of rough-surfaced endoplasmic reticulum. Although this cell may not be a fully mature cell, nevertheless we would question whether it has not developed well along towards the reticular cell. From these published works and from our own experience we would tend to doubt that the primitive reticular cell has been positively identified at the electron microscope level. This conclusion is strengthened by the work of Bairati et al., who studied the reticular (collagenous) network of lymph nodes from several species of animals. These workers did not illustrate or mention a primitive reticular cell, although they did discuss the development of fibroblasts and macrophages from reticular cells. Moreover, Movat and Fernando, who have studied changes in the lymph node of rabbits following antigenic stimulation, saw only blast cells in the reactive nodes—no cells more primitive. Also, in a prior study,
Fig. 14.—Macrophage. The macrophage is a large cell of irregular shape with a large, often indented, nucleus. From the cell body, long, narrow cytoplasmic extensions pass between neighboring cells. The cytoplasm contains, in addition to mitochondria (MIT), pleomorphic, dense bodies, the lysosomes (LY), which are intimately involved in the process of phagocytosis. Processes (PR), or cytoplasmic extensions from other cells, are often partially or totally enclosed by macrophage cytoplasm. \texttimes 15,500.

the same authors\textsuperscript{11} could not identify a primitive reticular cell in normal rabbit lymphoid tissue.

Although the primitive reticular cell may present problems of identification, the mature reticular cell is usually easily recognized. This relatively large cell,
Fig. 15.—Mast cell. The mast cell is characterized by the numerous dense granules, about 0.5–1 micron in diameter, which fill most of the cytoplasm. The membrane-enclosed granules vary in electron density, shape, and internal structure. Few cytoplasmic organelles occur in the cell. The round nucleus shows appreciable chromatin clumping. Cytoplasmic processes (PR) are noted adjacent to the mast cell. × 12,000.

with large and irregularly shaped nucleus showing marginated chromatin and a large nucleolus, is typically irregular in shape. The cell may be elongated or stellate, but almost always has extensions of the cytoplasm which pass between other cells or which follow and surround cords of collagen fibrils. The mature reticular cell bears some resemblance to the fibroblast, but the reticular cell differs mainly in having more cytoplasm in the perinuclear zone. Both cell types show abundant, well-developed, rough-surfaced endoplasmic reticulum.

Although the normal human lymph node is an active organ and well-populated with many cell types, we have observed relatively few hemocytoblasts. The cells which we have illustrated as such are similar in appearance to those
shown by Bernhard and Leplus and are strikingly similar to what Movat and Fernando term "immunoblasts"—that is, cells which appear in response to antigenic stimulation. Both cell types are characterized by a large nucleus, large nucleoli, and an abundant, poorly developed cytoplasm containing mainly free ribosomes aggregated into small groups, or rosettes. Policard and co-workers have presented somewhat similar pictures of lymph node cells which appeared in response to antigenic stimulation. Bernhard and Leplus considered that the hemocytoblast develops from the primitive reticular cell, but Movat and Fernando were unable to make such a derivation.

The lymphoblast and medium and small lymphocytes are primarily identified by cell and nuclear size and shape, as well as by the relatively dense and uniform chromatin distribution. There appears to be general agreement among the various investigators as to the morphology of these cells.

The mature cells of the plasma cell series are very easily recognized by the abundance of rough-surfaced endoplasmic reticulum and by the characteristic chromatin aggregation in the nucleus. We have not illustrated an immature plasma cell (proplasmacyte, or plasmablast) because of the uncertainty of identification. Bernhard and Leplus showed reticular cells which have features they consider indicating that the cells are destined to develop into plasma cells. We think that this is a very difficult identification to make, since it is primarily based on the extent and form of endoplasmic reticulum, which occurs well developed in both the mature reticular cell as well as the mature plasma cell. Movat and Fernando failed to find this relation between reticular cells and plasma cells. On the contrary, they offered evidence and cited literature to indicate that the lymphocyte may give rise to the immunoblast, which in turn develops into a plasma cell via a plasmablast stage.

The remaining cells of the lymph node have been described by several workers with good agreement as to the morphology of the cells. The reticuloendothelial cell shows its typical properties of phagocytosis and endothelial-like lining mainly when it is within the parenchymatous portion of the node. Subcapsular sinus-lining cells at the capsular side show only endothelial characteristics. Trabecular cells resemble reticular cells, particularly in the manner with which their cytoplasm surrounds and follows the cords of collagen fibers. These cells have been studied and well-illustrated by Fresen, Yamada and Yamagishi, as well as by others.

The macrophages are, on the whole, larger cells than the reticular cells, although their numerous cytoplasmic processes tend to be thinner than those of the reticular cell. The macrophage is most easily identified by the lysosomes within the cytoplasm. These cytoplasmic organelles, whose function in the digestion and sequestration of phagocytized materials has been well-described, appear in these cells as pleomorphic granules of varying electron densities.

The mast cell is readily distinguished from the other lymph node cells by their different and distinctive granules. Blood cells, when they appear in the node, do not offer difficulties for identification.

The capillaries of the lymph nodes appear to be similar to those found in other organs. Endothelial pores are not noted. Endothelial cells may show
phagocytized materials in the cytoplasm. This is particularly true of larger, postcapillary vessels. Such vessels have been illustrated in the report on lymph node vessels by Policard and co-workers.\textsuperscript{17}

The large number of cytoplasmic processes which fill the interstices between parenchymal lymph node cells, has hitherto received little or no attention. Most of these processes appear to emanate from macrophages and reticular cells. A few originate from plasma cells. There is also the possibility that some of the processes are actually detached cytoplasmic blebs from one or more of the lymph node cell types. Such blebbing has been noted, in our own experience, in cell cultures of human lymph nodes. It is possible that these processes, or blebs, have some relation to the immunologic function of lymphoid tissue. In this connection, the frequently observed close spatial proximity of plasma cells to blood capillaries may indicate that antibodies are passed directly from the plasma cells into the vessels through the thin intervening connective tissue space and endothelium. Such transfer of material, however, has not yet been observed.

Summary

Lymph nodes, from 15 patients undergoing surgery for conditions not related to lymphoid tissue disease, have been examined with the electron microscope. The human lymph node cell types—including lymphocytic, reticular and plasma cells—have been described at low and medium electron microscopic magnifications, and the criteria for their identification are discussed. The characteristic features outlined for identification of these cell types provide a basis for comparison with pathologically altered lymph node cells.

Summary in Interlingua

Nodos lymphatic ab 15 patientes subjicite a chirurgia pro conditiones no relationate con morbo de tissu lymphoide esseva examine con le microscopio electronic. Le typos de cellulæ del nodos lymphatic human—incluse lymphocytes, reticulocytes, e plasmocytes—es describite in lor apparentia a base de intermedie magnificaciones de microscopio electronic. Es commentate le criterios pro lor identification. Le caracteristicas delineate pro le identification de iste typos cellular provide un base pro le comparation con pathologicamente alterate cellulæ de nodo lymphatic.

References

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ROBERT E. BROOKS and BENJAMIN V. SIEGEL