FINE STRUCTURE OF POST-CAPILLARY VENULES IN MOUSE LYMPH NODES

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INTRODUCTION

The post-capillary venules in lymphatic tissue differ in some specific features from other blood vessels. They have a distinctive high endothelium through which lymphocytes migrate (Dawson and Masur, '29; Hummel, '35; Pirro, '54/55 and Sugimura, '62) and the endothelial cells are metachromatic and react strongly with the techniques for nonspecific esterase and succinic dehydrogenase (Smith and Hénon, '59). With the exception of Sugimura, the above authors considered that the lymphocytes moved into the venules from the surrounding lymphatic tissues, but in a recent study of the recirculation of lymphocytes, Gowans and Knight ('64) clearly showed that lymphocytes migrate from the blood to the lymphatic tissues.

At present, only a few electron microscope studies of the venules have been reported (Clark; '62, '63 and Marchesi and Gowans; '64). These investigations, however, are primarily concerned with migrating lymphocytes and reticulum, and there are no detailed descriptions of the fine structure of the endothelial cells. There is a question whether lymphocytes migrate by penetrating an endothelial cell or between the cells. Marchesi and Gowans stated that lymphocytes migrate by penetrating an endothelial cell but not pass through the intercellular junction.

In this paper, the authors furnish contrasting evidence that lymphocytes migrate between the endothelial cells of the venule, in addition to descriptions of the fine structures of the endothelial cells and the pericytes of the venules.

MATERIALS AND METHODS

The mandibular, superficial axillary, subiliac and cranial mesenteric lymph nodes of 11 dd strain mice were used. The names and locations of the lymph nodes were taken from JAP. J. VET. RES., VOL. 12, NO. 4, 1964
descriptions by KAWASHIMA, et al. ('64). The mice were of both sexes, ranging in age from 60 to 215 days and in weight from 21.5 to 37.5 g. Lymph node specimens were fixed for 1 to 1.5 hours and then dehydrated with gradedethanols. Fixation and dehydration were carried out at 0°C. The specimens were embedded in methacrylate or Epon 812 (LUFT). Sections were cut on a HITACHI UM-3 microtome using glass knives. After mounting on copper grids, the sections were stained with uranyl acetate (WATSON) or lead tartrate (MILLONIG) and examined with a JEM CHD-4 electron microscope at initial magnifications varying from 1,500 to 10,000. Some thicker sections were stained with toluidine blue for identification of the area to be sectioned for electron microscopy.

In addition, paraffin sections of lymph nodes from different locations were fixed in CARNOY's solution and then stained with hematoxylin-eosin, toluidine blue, pyronine-methyl green and PAS. Some paraffin sections fixed in ice-cold acetone were attempted to demonstrate alkaline and acid phosphatase.

OBSERVATIONS

With the optical microscope, post-capillary venules were observed in the dense, cortical lymphatic tissue (Figs. 1 and 2). The thick endothelium gradually became thinner as the veins traversed the medullary cords. The cytoplasm of the endothelial cells was pyroninophilic (Fig. 3) and stained metachromatically with toluidine blue, but was negative to the other procedures, such as PAS, acid phosphatase etc. Many lymphocytes were observed at several levels in the walls of venules, but with this magnification, it was impossible to determine whether the lymphocytes migrated into the endothelial cells or between them (Fig. 3). There were no smooth muscular cells in the wall of the venules.

With the electron microscope, the post-capillary venules were observed to consist of cuboid endothelial cells and slender pericytes.

1. Endothelial Cells

The venous lumen is covered by a single continuous layer of endothelial cells. These cells are usually cuboid, but they frequently are forced into irregular contours by the migrating lymphocytes (Figs. 4 and 5). The lateral cell surface has terminal bar-like junctions and interdigitations of the cell membrane (Fig. 14). The basal portions of the cells have irregular projections which differ from those usually observed in blood vessels, and which are surrounded by a basement membrane (Fig. 4).

The nuclei of the endothelial cells are forced into various shapes by the migrating lymphocytes. The nuclear membrane is triple-layered, at any point of which the nuclear membrane pores are visible (Fig. 9). The nucleoplasm consists of fine dense structures in a less dense matrix. These structures clustered around the periphery, forming a rim along the innermost nuclear membrane. Close to this nuclear membrane, there are one or two large nucleoli which are composed of nucleolonema. Another unknown body consisting of lower density particles is frequently found in the nuclei (Figs. 4 and 9).

On the free surfaces of the cell, there are a few microvilli and pinocytotic vesicles (Fig. 5). In comparison with other blood vessels, there are more organelles in the cytoplasm of the endothelial cells of the venules. There are many, usually oval or elongated, mitochondria.
with regular, plate-like cristae. Well developed Golgi complex, abundant, rough-surfaced endoplasmic reticula, and three types of inclusions seem to be characteristic of the endothelial cells. The Golgi complex occupies a large area of the cells and consists of smooth-surfaced vacuoles and many vesicles. Near the Golgi complex, there is the centrosome containing two centrioles (Fig. 10). There are many rough-surfaced endoplasmic reticula, which are usually cystic and which are closely related to the adjacent mitochondria, and also many free ribosomes in the cytoplasm of the endothelial cells (Figs. 14 and 15).

The inclusion bodies may be separated into three forms. The first type is a multivesicular body which is groups of small vesicles surrounded by a membrane (Figs. 5 and 9). This body appears to have been formed from the Golgi vesicles (Fig. 11). Sometimes the matrix of these bodies is of high electron density, and they closely resemble the second type (Figs. 12 and 13). Inclusions of the second type are round bodies of varying sizes, usually surrounded by a single thin membrane, which generally contains a homogeneous dense matrix, but which occasionally has a less dense matrix in which there are denser areas or myelin-like structures (Figs. 8, 9 and 15). Some of these bodies have no membrane (Figs. 9 and 21). Bodies of this second type are usually referred to as lysosomes, microbodies or segresomes. The origin of these bodies is unknown, but it may be the smooth-surfaced endoplasmic reticulum or the multivesicular body. Inclusion bodies of the third type contain a crystal and seem to be characteristic of the endothelial cells of the post-capillary venules. The bodies are spherical, resembling those of the second type, but they do not always have a distinct membrane. The matrices are of intermediate electron density and contain a cuboid crystal. Close to the sides of this crystal, there are either two or four highly dense structures (Figs. 4, 15 and 16). The origin of these third type of bodies seems to be an inclusion body of the second type.

No mitosis was observed in the endothelial cells.

2. Pericytes

The pericytes surround the endothelium of the post-capillary venule. They are separated from the endothelial cells by the basement membrane and an intercellular space. Usually there is at least one layer of these slender pericytes, and sometimes two or more (Fig. 15). There are only a few organelles in the cytoplasm, some scattered mitochondria, several rough-surfaced endoplasmic reticula, free ribosomes and vesicles (Figs. 6, 15 and 18). The nucleus of the pericyte is usually a long ellipsoid, the nucleoplasm is similar in appearance to that of the endothelial cells, but there are no prominent nucleoli. It was noted that there was a filamentous structure in the cytoplasm of these pericytes and a basement membrane surrounding them (Figs. 15, 18 and 21).

3. Reticular Cells

Reticular cells usually separate the pericytes from the lymphocytes of the cortex. There is an intercellular space between the pericytes and reticular cells. In some of the sections, there are gaps between the reticular cells in which there are small lymphocytes (Fig. 20). The reticular cells are large with irregular contours and cytoplasmic expansions. Some of the reticular cells have rich, rough-surfaced, tubular endoplasmic reticula but no inclusion bodies.
Other cells have many inclusion bodies and vesicles and none of the rough-surfaced endoplasmic reticula (Figs. 15 and 16). The nuclei of the reticular cells are ovoid and the nucleoplasm is of low density, similar to that of the pericytes, but they do not have basement membranes as the pericytes do (Fig. 17).

All of the previously described cells are separated each other by intercellular spaces containing a low density, amorphous substance and several filamentous structures, but not typical collagenous fibrils (Fig. 17).

4. Lymphocytes Migrating through the Venules

All of the lymphocytes migrating through the post-capillary venules are small lymphocytes with a thin cytoplasm containing fairly numerous free ribosomes, one or two tubular rough-surfaced endoplasmic reticula (Fig. 20) and scattered or grouped mitochondria (Fig. 11). Occasionally, poorly defined Golgi complex and centrioles were observed in the cytoplasm (Figs. 4, 6 and 8). The nuclei of the lymphocytes are usually rounded and rarely, there is a deep indentation in the nuclear membrane (Fig. 7). The nucleoplasm is dense with a small compact nucleolus. Frequently, one or more unknown bodies, resembling those previously described in the endothelial cells, are observed in the nucleoplasm (Figs. 6, 8 and 20). There were no other leucocytes in the walls of the venules.

The lymphocytes are located between the endothelial cells, and between the pericytes and the endothelial cells. Sometimes the lymphocytes seem to be in the endothelial cells but not between them (Figs. 4 and 6), but in many sections, the lymphocytes are located between the endothelial cells forcing and misshaping the adjacent endothelial cells. The cell membranes of both types of cells are always intact (Figs. 19 and 22). The lymphocytes seem to migrate between the junctions of the endothelial cells, although the direction of the lymphocytic migration cannot be determined from these observations.

One degenerated cell (lymphocyte?) was observed between the endothelial cells (Fig. 23).

DISCUSSION

Electron microscope studies of the blood vessels in the lymphatic tissue have been reported by many investigators (CLARK, '62 and '63; HOSOKAWA, '60; NAITO and ISOKAWA, '62; TÖRÖ, '63). The ultrastructure of the blood vessels in the lymphatic tissue is apparently similar to that of blood vessels in other tissues, with the exception of the sinusoids in the liver (HAMPTON, '64; SCHMIDT, '60), spleen (WEISS, '57, '61, '62; ROBERT and LATTA, '64) and bone marrow (WEISS, '61; ZAMBONI and PEASE, '61). On the other hand, only a few authors have described the ultrastructure of the post-capillary venules in the lymph nodes. CLARK ('62) stated that, in mice, the endothelial cells of the venule contained well developed ergastoplasm and some dense inclusions, as well as cytoplasmic vesicles and irregular microvilli along the luminal border, but he did not describe the pericytes. MARCHESI and GOWANS ('64) reported briefly that the endothelial cells in rat venules have organelles which are similar to these in other blood vessels. Their interest seemed to have
been focused on lymphocytic migration through the venules.

In the present paper, the authors have shown that the endothelial cells of the post-capillary venules have many mitochondria, well developed rough-surfaced endoplasmic reticula and numerous free ribosomes. In addition, the cytoplasm of the endothelial cells includes a prominent Golgi complex and three types of inclusions; a multivesicular body, a dense inclusion body without a crystal and an inclusion body with a cuboid crystal. The latter type of inclusion has never been observed in cells of other types of mouse lymph nodes. These features are characteristic of the endothelial cells of the venule which are different from those of the other blood vessels.

The endothelium is surrounded by pericytes. An intercellular space separates the basement membrane of the endothelial cell from these pericytes. The pericytes are also surrounded by a basement membrane. In the cytoplasm of the pericytes, there is a filamentous structure and poorly developed organelles. The pericytes of the post-capillary venule are much better developed than those of the arterial capillaries in the mouse lymph nodes, although HOSOKAWA ('61) reported that the arterial capillaries of the human lymph node are specific in having discontinuous, smooth muscular cells which correspond to the pericytes. In addition, NAITO and ISOKAWA ('62) described corresponding pericytes in the arterial capillaries of the human lymph node and called them "ROGET's cells" which are usually found only in arterial capillaries. From this point of view, it is possible that the post-capillary venules of the mouse lymph nodes may belong to the capillaries rather than to the venules. The pericytes may be active in contraction of specific venules.

Many earlier investigators considered the direction of movement of the lymphocytes through the walls of the venules to be to the venule from the surrounding lymphatic tissues (Dawson and Masur, '29; Hummel, '35; Pirro, '54/55; Smith and Hénon, '59), but GOWANS and KNIGHT ('64) clearly demonstrated that the direction was from the blood to the lymphatic tissue. Furthermore, Marchesi and GOWANS ('64) did electron microscope studies of the migration of lymphocytes through the endothelium of the venule in rat lymph nodes. They stated that all of cells in the wall of the venule are small lymphocytes, and that no other leucocytes are normally present. These small lymphocytes migrate across the vessel walls by entering the endothelial cells and traversing their cytoplasm, but not pass through the intercellular junction. In inflamed nodes, however, other leucocytes emigrate through the venules by penetrating between the endothelial cells.

CLARK ('62) stated that small lymphocytes could be observed at several levels in the walls of venules in mice lymph nodes. They lay between the endothelial cells in what appeared to be an intercellular space, beneath the endothelium in a space surrounded by an apparently split or reduplicated endothelial basement
membrane, and beneath the basement membrane among the reticular fibers.

The present authors found small lymphocytes between the endothelial cells in the majority of the sections, although, in some of the sections, the lymphocytes seemed to be in the cytoplasm of the endothelial cells. The lymphocytes produced irregularities in the outlines of adjacent endothelial cells. Small lymphocytes seemed to migrate by penetrating the junction between the endothelial cells but not by penetrating their cytoplasm. These findings agree with CLARK's description ('62).

The direction of the movement of the lymphocytes through the walls of the venules was not determined in this study. However, GESNER and GOWANS ('62) showed data indicating that lymphocytes recirculate from the blood to the thoracic duct, obtained in a study of the output of lymphocytes from the thoracic duct of unanaesthetized mice.

Certainly, small lymphocytes have a specific affinity for the endothelial cells of the post-capillary venules, but the present authors do not known the reason for this.

Recently, lymphocytes have attracted attention as "immunological competent cells" in homograft immunity and other immunological reactions (BURNET, '59, PRENDERGAST, '64). The results of the present studies indicate that the endothelial cells, in the cytoplasm of which there are a prominent Golgi complex, well developed rough-surfaced endoplasmic reticula and specific inclusions, but no mitosis, seem to produce any substance which would activate recirculating lymphocytes or be useful in their immunological function. But the actual functional relation between the endothelial cells and the small lymphocyte requires further investigation.

SUMMARY

The post-capillary venules of mouse lymph nodes were examined with optical and electron microscopes.

The endothelial cells of these venules contain many mitochondria, well developed, rough-surfaced endoplasmic reticula, numerous free ribosomes, a prominent Golgi complex and three types of inclusions; multivesicular bodies and dense inclusion bodies with and without a cuboid crystal. The prominent Golgi complex and the inclusion with a cuboid crystal are not present in the endothelial cells of other blood vessels in the lymph nodes of mice.

The endothelium of the venules is surrounded by pericytes which correspond to ROUSET's cells which are usually only observed in arterial capillaries.

All of cells migrating through the post-capillary venules are small lymphocytes and no other leucocytes are normally present. The small lymphocytes migrate by penetrating the junction between the endothelial cells but not by penetrating their cytoplasm.
One degenerated cell (lymphocytes ?) was observed between the endothelial cells of the venules.

REFERENCES

3) CLARK, S. L. JR. (1963): Ibid., 112, 1
ABBREVIATIONS TO PLATES

B : Unknown body in nucleoplasm
BA : Basement membrane
C : Centrosome
D : Degenerated cell
E : Endothelial cell
F : Filamentous structure
G : Golgi complex
IC : Inclusion body with a crystal
ID : Inclusion body without a crystal
J : Terminal bar-like junction
L : Lymphocyte
M : Mitochondria
MD : Multivesicular body with dense matrix
MU : Multivesicular body
N : Nucleus
NP : Nuclear membrane pore
NU : Nucleolus
P : Pericyte
PV : Pinocytotic vesicle
R : Reticular cell
RE : Rough-surfaced endoplasmic reticulum
SE : Smooth-surfaced endoplasmic reticulum

EXPLANATION OF FIGURES

Plate I

Fig. 1. Iliac node: Post-capillary venules in the cortex. The endothelial cells gradually flatten as the veins traverse the medullary cord H&E × 60

Fig. 2. Lumbar node: Post-capillary venule connected to a capillary. Many lymphocytes in the endothelium of the venule H&E × 1,000

Fig. 3. Iliac node: The cytoplasm of the endothelial cells is pyroninophilic. Lymphocytes at the level of the endothelial cells, and between the endothelial cell and perivascular sheath Pyronine-Methyl green × 1,000
Figures 4 to 23 are electron microscope photographs of sections embedded in Epon 812 and stained with uranyl acetate.

Plate II

Fig. 4. Mandibular node: In this low-powered view of the post-capillary venule, cuboid endothelial cells (E) cover the lumen of the venule. An endothelial cell contains an inclusion body with a cuboid crystal (IC). A small lymphocyte (L) has centrosome (C) and poorly developed Golgi complex. Basal border of endothelial cells (E) has irregular projections and basement membrane (BA). Intercellular space surrounding endothelial basement membrane contains amorphous substance and pericytes (P) × 8,100

Fig. 5. Mandibular node: Note two multivesicular bodies (MU) and prominent Golgi complex (G). On the free surface of the venule, pinocytotic vesicles (PV) and microvilli are observed. Small lymphocyte (L) between the endothelial basement membrane (BA) and the pericytes (P) has altered the shape of the endothelial cell × 12,300
Plate III

Fig. 6. Mandibular node: Two small lymphocytes appear to locate in cytoplasm of endothelial cell (E). The cell membranes of Loth types of cells are intact. A lymphocyte has a poorly developed Golgi complex (G) \( \times 13,000 \)

Fig. 7. Subiliac node: Part of the venule in the medullary cord. Venule is intermediate form between post-capillary venule and usual venule. Note flattened endothelial cells (E), poorly developed Golgi complex (G) and mitochondria. Perivascular space surrounding the basement membrane contains two layers of pericytes (P) \( \times 12,000 \)

Fig. 8. Mandibular node: Four small lymphocytes (L) appear to locate in endothelial cell \( \times 8,600 \)
Plate IV

Fig. 9. Mandibular node: Apical portion of the endothelium of the post-capillary venule. The nuclei of the endothelial cells (E) have one or two nucleoli (NU), unknown body (B) and nuclear membrane pore (NP). Terminal lar-like junction (J) between the endothelial cells, Golgi complex (G), multi-vesicular body (MU) and inclusion bodies (ID) are found \( \times 19,200 \)

Fig. 10. Mandibular node: Four lymphocytes (L) between endothelial cells. Note projections of endothelial cell between the lymphocytes (arrow). Prominent Golgi complex (G) and centrioles (C) are observed in the cytoplasm of an endothelial cell \( \times 11,500 \)
Plate V

Fig. 11. Subiliac node: Basal portion of the post-capillary venule. Note multi-vesicular bodies (MU) and prominent Golgi complex (G). In this figure, the multivesicular bodies seem to be formed from the Golgi vacuole and vesicles. Small lymphocyte (L) appears to lay between two or three endothelial cells × 14,000

Fig. 12. Mandibular node: Note multivesicular body with dense matrix (MD) near Golgi complex (G). Formation of this body seems to be related to the Golgi vesicles × 26,000

Fig. 13. Subiliac node: Multivesicular body with dense matrix (MD) × 26,000
Plate VI

Fig. 14. Subiliac node: Basal portion of the venule. Note terminal bar-like junction (J) and interdigitations of the cell membranes (arrows). In the cytoplasm of endothelial cells, rich mitochondria (M), well developed endoplasmic reticula (RE), abundant free ribosomes and prominent Golgi complex (G) are observed \( \times 16,000 \)

Fig. 15. Adjacent to Figure 14. In this figure, basal portion of endothelial cells, thin pericyte cytoplasm and reticular cells are observed. In the cytoplasm of an endothelial cell, there are three inclusion bodies with a clear membrane (ID) and an inclusion body with a cuboid crystal (IC). Note irregular basal border of the endothelium and basement membrane (BA). Pericyte (P) has filamentous structures (F) and basement membrane (BA). Intercellular space around the pericyte contains low density amorphous substance. Reticular cell (R) has numerous inclusions \( \times 15,500 \)
Plate VII

Fig. 16. Subiliac node: Adjacent to Figure 15. Note basal portion of endothelial cell (E), pericytes (P) and reticular cell (R). Reticular cell has variety of inclusions, but none containing crystals × 15,500

Fig. 17. Subiliac node: Basal portion of post-capillary venule. Reticular cell (R) separates the lymphatic tissue of the cortex from the intercellular space around the endothelium. Note well developed endoplasmic reticulum (RE) and prominent Golgi complex (G) in the cytoplasm of the reticular cell × 14,800
Plate VIII

Fig. 18. Subiliac node: Basal portion of venule. Note nucleus of pericyte with indentations of the nuclear membrane. The pericyte has poorly developed organelles, filamentous structure (F) and basement membrane (BA) × 16,500

Fig. 19. Subiliac node: Note small lymphocyte (L) between two endothelial cells, and terminal bar-like junction (J). The cell membranes of both types of cells are intact × 12,000

Fig. 20. Subiliac node: Lymphocyte (L) apparently located between three endothelial cells. Note projection of the lymphocyte through the basement membrane of the endothelium (arrow). Note small lymphocytes occluding gap in the perivascular sheath of the reticular cells (R) in the lower left and pinocytotic vesicles (PV) × 12,000
Plate IX

Fig. 21. Subiliac node: Small lymphocyte (L), located between endothelial cells (E) and a pericyte (P), seems to have amoeboid motion. Note filamentous structures (F) in the cytoplasm of pericyte \( \times 13,100 \)

Fig. 22. Subiliac node: Lymphocyte (L) located between two endothelial cells (E) indents the cell bodies. Note terminal tar-like junction (J) between the cell membranes of the endothelial cells \( \times 15,000 \)

Fig. 23. Subiliac node: Note degenerated cell (D) between endothelial cells and a pericyte (P) \( \times 10,500 \)