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Ultrastructure of Small Intestine Submucosal and Serosal-Muscular Lymphatic Vessels

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Summary

Lymphatic vessels of small intestine submucosal and serosal-muscular layers in mice and bats have been studied by electron microscopy and tridimensional reconstruction of thin serial sections. Lymphatic endothelium has a continuous appearance, it lacks fenestrations and pores and it is encircled by a thick connective tissue layer. The endothelial wall shows intraendothelial channels, quite similar to those previously described in the lacteal vessels. The author believes that the above mentioned intraendothelial channels, together with pinocytotic vesicles, play a fundamental role in the transendothelial transport of fluids, proteins and macromolecules. Moreover, intercellular specialized junctional complexes do not appear to take any part in this process.

Introduction

In recent years several studies have been performed in order to understand the mechanisms of lymphatic transendothelial transport of fluids, proteins and macromolecules.

Palay and *Karlin* (1), *Ottaviani* and *Azzali* (2), *Papp* et al. (3), *Casley-Smith* and *Florey* (4), *Comparini* et al. (5) performed basic research work on the ultrastructure of the lymphatic vessels and the morphological modifications which take place in the lymphatic endothelial wall of several organs, such as the kidney (6, 7, 8), dental pulp (9), alveolar bone (10), diaphragm (11), dermis (12), testis (13), portahepatis (14), and small intestine lacteal vessels (15, 16, 17).

As regard to the transendothelial transport mechanisms, interpretation of various authors differ. *Ottaviani* and *Azzali* (18) and *Dobbins* and *Rollins* (19) believe that the primary pathways into the lymphatic lumen are represented by the pinocytotic vesicles and normal intercellular channels (8). On the other hand, *Leak* and *Burke* (20) and *Kalima* (21) ascribe a major role to the "open intercellular junctions" in the transendothelial transport. Other authors consider open junctions as morphological aspects of pathologic states (22, 23, 24, 25). Recently, *Azzali* (22, 26) found the presence of intraendothelial channels in the endothelial wall of the lacteal vessels, through which an open flow of lipids and other substances from the interstitium into the lymph is established.

Aim of the present study was to investigate under physiological conditions the morphological aspects of the endothelium in small intestine submucosal and serosal-muscular lymphatic vessels. These occupy an intermediate position between absorbing capillaries and collecting vessels (2).

Materials and Methods

A total of thirty animals were used in the present study. Seventeen were bats, of which five were caught during the winter-lethargic state and twelve during summertime. The other twelve animals were adult mice. Under ether anaesthesia the animals were perfused with a 2.5 % glutaraldehyde buffered with sodium cacodylate (0.1 M) at pH 7.4 for 30 minutes.

Electron microscopy: Specimens of intestinal wall (jejunum and ileum), four to five milli-

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meters in diameter, were fixed in 2.5% glutaraldehyde buffered with sodium cacodylate for three hours. After a bath in cacodylate buffer, the specimens were post-fixed in 1% osmium tetroxide and diluted in the same buffer for two additional hours. Following rinsing in bidistillated water for 30 minutes and dehydratation in acetone, specimens were embedded in Durcupan. One micrometer-thick sections were stained with 0.5% toluidine blue, whereas thin sections were stained with lead citrate (27) and examined by electron microscopy.

Tridimensional reconstruction: Thin sections of seven lymphatic vessels (four from the submucosal and three from the serosal – muscular lymphatic networks) have been serially photographed. Using the Werner technique (28), they were reconstructed with 0.8 mm thick waxed paper sheets, in order to get a tridimensional model. For each lymphatic vessel, 450 thin serial sections were employed. Each thin section was 700 Å thick. In order to show the intercellular junctions between adjacent endothelial cells, each cell of the lymphatic endothelial wall was stained with a different color, as suggest by *Casley-Smith* in the 7th International Congress of Lymphology held in Florence, 1979.

Results

In bats and mice the lymphatic vessels of the small intestine submucosal and serosal-muscular networks are layered by a continuous endothelial wall without fenestrations or pores. This wall is made up of 3–4 flat endothelial cells, each showing a cell body containing the nucleus and peripheral ondulated cytoplasmic processes. The latter connect with those of adjacent endothelial cells by means of end to end, overlapping or interdigitating contacts. Less often contacts are made with the abluminal surface of the same cell. Each intercellular contact shows specialized junctional complexes, such as maculae adhaerentes and occludentes. The abluminal endothelial wall is encircled by a thick layer of loose, fibrillary connective tissue. Limited areas of the abluminal cell surface are devoid of basal lamina.

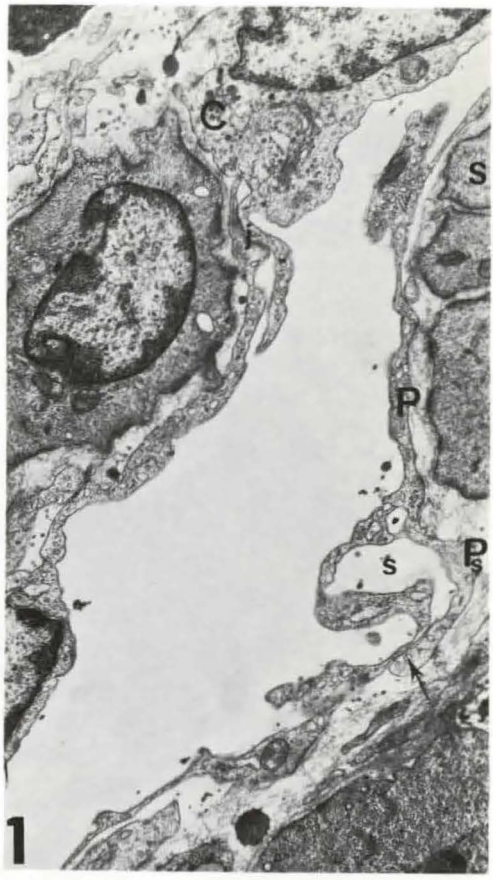

Fig. 1 Lymphatic vessel of mouse small intestine (jejunum) submucosal network. C = body of an endothelial cell showing well developed Golgi apparatus and rough endoplasmic reticulum. P = primary cytoplasmic process. Ps = secondary cytoplasmic process, which, after embracing a wide interstitial space (s), makes contact with the abluminal surface of an adjacent endothelial cell by means of a specialized intercellular complex (arrow). S = smooth muscle cell. X 15.000.

The endothelial cell body is made up of an abundant cytoplasm with a rather clear matrix, containing a prominent rough endoplasmic reticulum and Golgi apparatus, mitochondria, free ribosomes and a few small dense granules with an heterogeneous content. The matrix of the cytoplasmic processes discloses few mitochondria, rare dense granules and frequent micropinocytotic vesicles 300–400 nm in diameter.

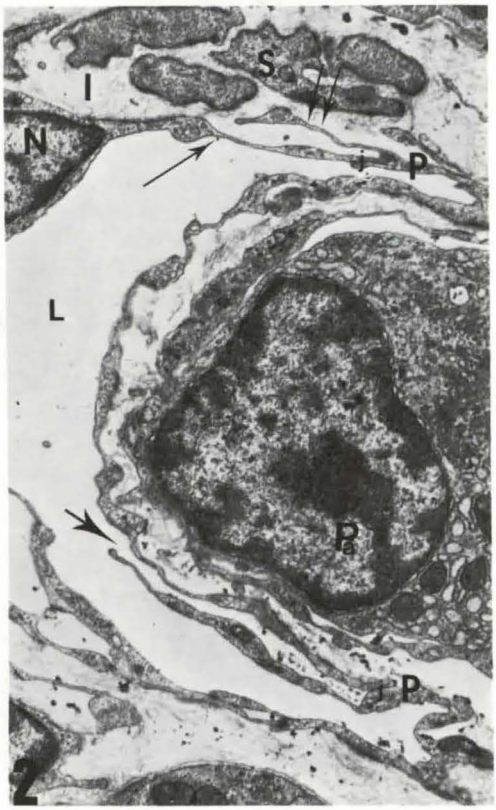

Fig. 2 Lymphatic vessel of bat small intestine (jejunum) serosal-muscular network. Top of the figure shows the lymphatic endothelial wall (arrow) and a secondary cytoplasmic process (double arrows) extending into the interstitium (I). Bottom of the same figure: luminal opening (large arrow) of the intraendothelial channel. P = primary cytoplasmic process near its bifurcation. J = Intercellular junctions. L = lymphatic vessel lumen. Pa = plasma cell in the interstitium. S = smooth muscle cell. N = endothelial cell nucleus. X 15.000

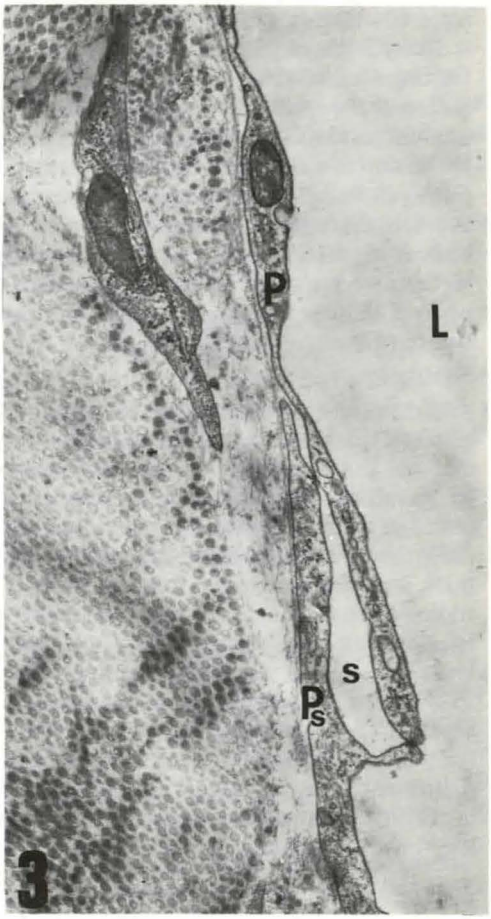

Fig. 3 and 4 (Fig. 4 see next side) Endothelial walls of an ileal, serosal-muscular network lymphatic vessel in the mouse. Fig. 3 shows intraendothelial channels (s) made up of primary (P) and secondary (Ps) cytoplasmic processes. L = lymphatic vessel lumen. X 25.000

At times cytoplasmic processes present a very thin wall. As a consequence, both the luminal and abluminal plasma membranes almost come in touch together. Often, the main cytoplasmic process bifurcates at its abluminal surface, thus originating a secondary cytoplasmic process. The latter extends into the interstitium toward the adjacent endothelial cell, joining its abluminal surface (Figs. 1, 2). At the contact site intercellular specialized junctional complexes are formed (Fig. 1, arrow). As a result of these ongoing changes in the endothelial wall, a circumscribed, ovoidal interstitial space is delimited (Figs. 2, 3). Its outer aspect is represented by the secondary cytoplasmic process and the inner aspect either by the primary cytoplasmic process of the adjacent cell or, sometime, of the same cell. The latter process, at

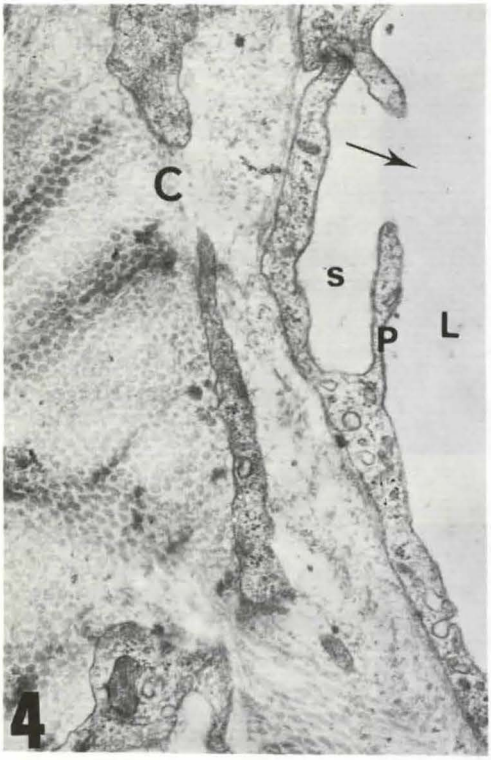

Fig. 4 The luminal opening (arrow) of an intraendothelial channel (s), due to the interruption of its primary cytoplasmic process (P). L = lymphatic vessel lumen. Intercellular junctions are not involved in the luminal opening formation. C = connective tissue fascicles. X 27.700

same point of its spatial extention, shows a discontinuity in its wall, which establishes an open communication with the lymphatic vessel lumen (Fig. 4).

The tridimensional reconstruction of thin serial sections (Figs. 5a, b) clearly demonstrates that the above mentioned space represents the lumen of a newly formed intraendothelial channel, extending through the endothelial wall for an average length of 6–8 μm (Fig. 5b). Near the lower edge of the intraendothelial channel, a discontinuity of its luminal surface is detected, and an opening connecting the cavity of the lymphatic vessel with that of the intraendothelial channel is formed. Between the top and the bottom of the intraendothelial channel a free communication of the interstitium with the lymphatic vessel lumen is then established (Fig. 5b, arrows). Through this communication substances primarily contained in the interstitium become integral part of the lymph. Site of discontinuity in the luminal surface of the intraendothelial channel and subsequent openings formation do not involve the specialized intercellular junctions.

During the evaluation of 4200 thin sections and 62 lymphatic vessels, nine morphological picture of lymphatic endothelial wall discontinuity have been noted. Four types were quite similar to those previously described as "open junctions' by *Leak* and *Burke* (20), or valve-like intercellular junctions by *Kalima* (21). The remaining five picture represent the consequence of traumatic breaks in the endothelial wall, due to preparation artifacts.

Discussion

Ultrastructural and tridimensional study of serial sections of small intestine submucosal and serosal-muscular lymphatic networks in bats and mice, on the whole confirmed the same cytological findings already shown to be present in other mammalian lymphatic vessels (1, 2, 22, 26, 29). The presence of a basal lamina, covering almost 95% of the abluminal endothelial surface, represents the only exception.

Moreover, in the lymphatic endothelial wall introendothelial channels have been shown to be very similar to those already described in the kittens and bats lacteal vessels by *Azzali* (22, 26).

These intraendothelial channels stress the fact that the lymphatic endothelium is capable to form canalicular structures not only at the beginning of the lymphatic system (lymphatic capillaries) but also in the submucosal and serosal-muscular networks, which are intermediate between the lymphatic capillaries and the collecting vessels.

It is therefore assumed that these portions of the small intestine lymphatic system reveal absorbing properties in spite of the presence

Fig. 5a, b Tridimensional reconstruction of a submucosal network lymphatic vessel, showing its abluminal surface (s). Opening of two intraendothelial channels are seen (arrows). Sections of the same reconstructed vessel (b) shows the intraendothelial channel and both its luminal (white arrow) and abluminal (black arrow) openings. The endothelial cells were stained with different stains to demonstrate the intercellular junctions.

of a cospicuous connective tissue layer around the abluminal endothelial surface. Our ultrastructural and tridimensional reconstruction of thin sections demonstrate that the "open intercellular junctions" reported by *Leak* (12) and other authors (11, 15, 20, 21) are the results of a tangential cut, involving both the abluminal and luminal openings of those intraendothelial channels which length does not exceed 2–3 μm.

This interpretation is supported also by the fact that open communication between interstitial space and lymphatic vessel lumen, when apparent, is visible in no more than 3–4 thin sections. Moreover, all the intercellular contacts are always tightened by intercellular specialized complexes and by a scanty number of open junctions, as already detected by *Dobbins* and *Rollins* (19).

Although the important role of intraendothelial channels in lymph formation has been stressed, still several questions must be answered: are these lymphatic intraendothelial channels temporary or permanent in nature? If temporary, which are the factors leading the lymphatic endothelium to make up such channels?

Certainly, different functional situations, chemical characteristics of the interstitial space content, as well as the different pressure gradients between interstitium and lymphatic vessel lumen must play an important role.

Our unpublished studies show that complete starving increases the number of intraendothelial channels. Future, appropriate research work is needed to explain the many complex aspects of this problem.

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