Intestinal mucosal lacteal in transport of macromolecules and chylomicrons\textsuperscript{1,2,3}

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The lacteal occupies a central position in the lamina propria of intestinal villi (Fig. 1). Arterioles, veins, and capillaries tend to occupy a more peripheral position in the lamina propria. The lacteal has a blind ending at the tip of the villus, while proximally villous lacteals empty into submucosal lymphatics that anastomose to drain via the mesentry to the thoracic duct. In man, the villous lacteal can be identified rather infrequently, regardless of whether the tissue is obtained by suction biopsy or surgery. The lacteal in the human intestinal mucosa is more easily identified following a fat meal, but again its presence is often difficult to detect (1). In contrast to the villous lacteal, the submucosal lacteal appears to remain distended in both the fasting and fed state and is generally very easily detected at the light microscopic level (1).

Even when detected at light microscopy, the villous lacteal cannot be distinguished with certainty from adjacent capillaries, although an educated guess as to their distinction can be made with considerable accuracy. The submucosal lacteal is easily distinguished from capillaries by light microscopy (1). Electron microscopy is required in order to be absolutely certain of the distinction of a lymphatic channel from a blood channel, although this degree of certainty is rarely necessary. Capillaries of the intestinal mucosa are characterized by the following findings (2): a) They possess a well-developed basal lamina (Fig. 2). b) The endothelium is fenestrated, that is, there are numerous window-like openings in the endothelium closed by thin diaphragms (Figs. 2, 3). c) Occasionally present are well-defined pericytes, smooth muscle-like cells distinguished by their characteristic location between leaflets of the basal lamina (Fig. 2). d) The capillary has a smooth, rounded outline when cut in cross section. e) Cell junctions between endothelial cells appear to be predominantly “tight” (zonulae occludens) (Figs. 2 and 4A and B). f) Erythrocytes are frequently seen within the lumen of capillaries (2).

Physiological studies indicate that capillary permeability can be explained by postulating two pore systems, a small-pore system and a large-pore system (2). These systems are considered to be open, water-filled channels along which molecules move by diffusion or by hydrodynamic flow. There are reasonable morphologic data indicating that pinocytotic vesicles of cardiac and skeletal muscle are the structural equivalent of the large-pore system and that endothelial fenestrae of blood capillaries of the intestinal mucosa are the morphologic equivalent of both systems (2). Endothelial junctions do not appear to play a major role in capillary permeability, although there is considerable controversy concerning this (2).

The structure of the lacteal, the initial point of entry of chylomicrons into the lymphatic system, has been well defined (3–11). Certain aspects of lacteal structure and some differences between lacteals and other small lymphatics, as reported in the literature and as studied in normal man, rat, guinea pig, and mouse in this laboratory, are described in detail here.

In general, lacteals occupy a central posi-
tion within the lamina propria of intestinal villi of mammalian species (Fig. 1). Apically, the lacteal is a "blind" pouch whereas proximally the lacteal communicates with submucosal lymphatics (6, 12). Occasional lymphocytes may be seen within the lumen of lacteals. The central lacteal consists of an attenuated endothelium without fenestrae (Fig. 5). There is often considerable overlap of and luminal projection of endothelial processes, especially at junctional complexes (Figs. 5 and 6). The endothelium is widened at the site of nuclei, which generally project with their surrounding cytoplasm into the lumen of the lacteal (1, 6). Cell organelles, other than for pinocytic vesicles, are similar to those of most cells. Pinocytic vesicles are prominent, tend to be oblong rather than rounded in shape, average 1,600 Å in diameter and occupy 15% of the cytoplasmic volume as determined by quantitative methods (13). There is a considerable tendency for pinocytic vesicles to fuse, thus accounting in part for their greater apparent diameter than that of blood capillary vesicles, which are about 750–1,000 Å in diameter (2, 14). The vesicles are surrounded by a membrane of the same density and thickness as that of the plasma membrane. Often the vesicles communicate freely with both the tissue front and the luminal front of the lacteal endothelium, the communication taking place through a stoma with a slightly narrowed neck (Fig. 5). Occasionally a thin diaphragm ~50 Å thick stretches across the opening of vesicles to the outside environment (Fig. 5). A thickened "knob" can be observed rarely in the center of these diaphragms, similar to
Fig. 2. Electron micrograph of intestinal mucosal capillary of man. Capillary endothelium (E) facing absorptive cells (A) is quite attenuated and is punctuated by numerous fenestrae (arrows) that have been shown to be permeable to large molecules such as peroxidase (mol wt, 40,000) and may represent both the small- and large-pore system of intestinal capillaries. There is a prominent basal lamina (bl) about the capillary endothelium and below the absorptive cells. Junctional complexes (j) of the capillary endothelium are "tight," that is, there is fusion of the outer leaflets of adjacent plasma membranes. Red blood cells are present within the capillary lumen and a pericyte (pc) can be seen between leaflets of the basal lamina. Fixed in osmium tetroxide, embedded in Epon, double stained. × 12,000.
that described for capillary vesicles (2) (Fig. 3). “Coated” vesicles are sometimes observed. A poorly defined, often discontinuous basal lamina is present, consisting of a fine fibrillar material (Figs. 5–8). Adjacent to the basal lamina are scattered filaments of ~150 A diameter with an apparent hollow profile similar to, though slightly larger than, lymphatic anchoring filaments described by Leak and Burke (15). However, in contrast to the findings of Leak and Burke in ear lymphatics, it is unusual to find evidence of attachment of these filaments to the endothelial plasma membrane of villous lacteals (Fig. 5). Submucosal lacteals do have prominent anchoring filaments that commonly attach to the endothelial plasma membrane. Elastic fibers are occasionally seen to mouse endothelium and, in all species examined in our laboratory, collagen fibers sometimes appear to extend from the surface coat of adjacent smooth muscle to endothelial basal lamina and microfilaments. Other than for the usual close association of smooth muscle cells with the lactal endothelium, pericytes, similar to those seen in blood capillaries (2, 14), are not present. There are no junctional contacts between smooth muscle cells and lactal endothelium.

Intercellular junctions of endothelial cells are remarkable for their complexity. Usually, there is considerable interlocking and overlapping of endothelial processes at junctional sites (Figs. 5 and 6). Tight junctions (zonulae occludens), defined as close apposition of 10 A or less of outer leaflets of plasma membranes of adjacent endothelial cells, are often present at points along the junctional complex (Fig. 6). Close junctions with ~40-A gaps as defined by Karnovsky (16) are infrequent. Desmosome-like attachments (maculae adherens) are demonstrable as areas of increased density in cytoplasmic segments of adjacent plasma membranes. The intercellular space between these dense cytoplasmic plaques is often so dense that the width of the junctional gap cannot be clearly measured. The most common spacing between adjacent membranes of the junctional complex is ~100–200 A (zonula adherens), but except for a rare, wide open junction, the majority of junctional complexes have areas of one or more tight or close junctional complexes or areas in which the junction spacing cannot be measured because of tangential sectioning of the apposed membranes or because of the presence of obscuring intercellular densities (Fig. 6, A).

Macromolecules and chylomicrons have been presumed to enter lacteals through open gaps at endothelial junctions as well as by pinocytotic transport across the endothelium. It is generally considered that entry via open gaps is the more important route (3–7, 9, 11, 17–26). Our data using peroxidase (mol wt 40,000; diameter 50 A), ferritin (mol wt 500,000–1,000,000; diameter, 110 A), and chylomicrons (range in size, 1,000 A–1 μ) as
Fig. 4. A. Electron micrograph showing junctional complex (j) of guinea pig capillary endothelia. B. The fusion of the outer leaflets of the adjacent membranes is shown in this enlargement (arrows). This junction is a "tight" junction and is probably impermeable to most substances except very small molecules such as water and electrolytes. Numerous pinocytotic vesicles (pv) are present. Basal lamina (bl). Lumen (L). Osmium fixed, Epon embedded, double stained. A. × 50,000. B. × 200,000.

tracers, suggest that pinocytotic transport is the more important route (13) (Fig. 7). Entry of macromolecules into lymphatic capillaries via open endothelial gaps is far easier to demonstrate following injury (8, 17, 18). It is always difficult to exclude injury as a cause of the open gaps even in the most careful studies of "normal" states. Only six open gaps were observed in our study following identification of over 300 lacteal junctional complexes (13, unpublished data). It is quite possible that all six of these open junctions resulted from inadvertent trauma during the processing of the tissues. Ten of the junctions showed 40-A gaps; 112 were tight with either apparent fusion of outer leaflets of adjacent
Fig. 5. Electron micrograph of lacteal endothelium (E) of fasting guinea pig illustrating marked complexity of junctional complex (j) with considerable interlocking and overlapping of endothelial processes. There are several desmosome-like (d) points of attachment and a probable tight junction (t). Pinocytotic vesicles (pv) are prominent, and one appears to have a diaphragm (arrow) stretching across its stoma. The basal lamina (bl) is quite inapparent and can hardly be distinguished from precipitated proteins in the lumen (L) of the lacteal and in the extracellular spaces. A portion of smooth muscle (sm) can be seen adjacent to the lacteal endothelium. Anchoring filaments (af) may attach lacteal endothelia to adjacent smooth muscle. Osmium fixed, Epon embedded, double stained. × 50,000.
membranes or no greater than a 10-A gap between the outer leaflets, and the remaining junctions were classified as indeterminate (Fig. 6, A).

It may be that tight junctions are the morphological equivalent of the small-pore system of lymphatics (16, 27). The occasional close junction is likely to function similarly. Casley-Smith (28) has shown that ions and molecules of molecular weight less than 1,000 can readily pass through endothelial cell junctions of lymphatics, whereas molecules of molecular weight greater than 20,000 cannot do so. There are a number of reports showing tight junctions in peripheral lymphatics (8, 10, 17, 18, 24, 26, 28, 29) and lacteals (3, 5, 6, 8, 11, 21). Most of these reports emphasize that tight junctions in lacteals and lymphatics are not common, though tight junctions are quite common in submucosal lymphatics and in less peripheral lymphatics (23, 26), that is, in lymphatics found in relatively motionless areas.

Our findings suggest that macromolecules of a mol wt >40,000 enter lacteals predominantly by pinocytotic transport and that pinocytotic vesicles represent the large-pore system of lacteals. This conclusion is based on the assumption that the tight junctions demonstrated were zonulæ occludens and not zonulæ adherens, and that the indeterminate junctions were also zonulæ occludens. It is possible that the tight junctional complex demonstrated in lacteals is merely an occasional sporadic contact of two adjacent membranes. This is probably true for the tight junctions seen between luminal cytoplasmic projections. However, most tight junctions are seen at the abluminal side of lactal endothelia, and these latter complexes remain intact even when the lacteal is massively distended with fluid (Fig. 8) (30). Luminal and abluminal cytoplasmic projections appear to permit great changes in lacteal volume according to functional state, whereas junctional complexes remain intact. When there is little fluid transport, the lacteal tends to be collapsed and has numerous cytoplasmic projections. When there is active fluid absorption, the lacteal is distended and cytoplasmic projections are no longer present, presumably having been utilized to allow for elongation of lactal cytoplasm.

Lacteals possess a basal lamina that is usually inapparent and often discontinuous. The basal lamina, when present, consists of fine filamentous-like structures similar to those seen about capillaries (2, 31), and some larger filaments. At no time does the basal lamina of lacteals appear to be a significant barrier to movement of chylomicrons and other macromolecules (13).

If the observation that most lacteal junctions are at least zonulæ adherens and are probably zonulæ occludens, then the mode of entry of macromolecules into lacteals is presumably that of vesicular transport (13). Apparent pinocytotic uptake of large ions (28), macromolecules such as thiorhum dioxide, ferritin, colloidal carbon and latex particles (8, 17, 18, 25, 26, 29), and lipoproteins and chylomicrons (1, 3–5, 8, 11, 20, 22) has been demonstrated in lacteals. Studies in our laboratory demonstrate the presence of ferritin, peroxidase, and chylomicrons within pinocytotic vesicles of lacteals (13). These substances have been seen within vesicles opening to the abluminal side, within vesicles apparently free within the cytoplasm, and within vesicles opening to the luminal side, all necessary criteria to demonstrate vesicular transport (32, 33). It was far easier to demonstrate vesicular “uptake” of peroxidase and ferritin while chylomicrons were less frequently seen within either large or small pinocytotic vesicles. The rarity in which chylomicrons could be demonstrated in either pinocytotic vesicles or in open endothelial junctions has been commented upon previously (1, 3, 4, 21, 22). Nevertheless, it seems likely that chylomicron transport across lacteal endothelium is largely vesicular. Casley-Smith (8) has clearly demonstrated that increased lymphatic flow following trauma is related to opening of lymphatic endothelial junctions, and he has suggested that this is also true during “physiologic trauma” such as that of chylomicron absorption. The conflicting data (8, 13) require resolution in the future.

It is probable that chylomicron release from the intestinal absorptive cell is that of “reverse” pinocytosis in which intracellular membranes surrounding chylomicrons fuse with the plasma membrane, releasing the chylomicrons into extracellular spaces. If, as
Fig. 6. A. Electron micrograph of lacteal endothelium (E) of fed guinea pig illustrating typical junctional complex. There are at least two points of fusion of outer leaflets of adjacent membranes (arrows), better illustrated at high magnification in B. A portion of the junction is classified as indeterminate (I) because the type of junctional gap cannot be determined due to tangential sectioning of the adjacent membranes. It is likely that this “indeterminate” portion would represent a tight junction if sectioned appropriately (13). Lumen (L). Basal lamina (bl). Pinocytotic vesicles (pv). Portion of unidentified cell (U). Osmium fixed, Epon embedded, double stained. A. × 50,000. B. × 200,000.
Fig. 7. Electron micrograph of lacteal endothelium (E) of fed guinea pig illustrating pinocytotic “transport” of chylomicrons (arrows). Lumen (L). Basal lamina (bl). Portion of unidentified cell (U). Osmium fixed, Epon embedded, double stained. \( \times \) 50,000.

...seems likely, the chylomicrons traverse the absorptive cell within a membranous system, it is not too farfetched to suggest that they are similarly transported across lacteal endothelium, albeit in a different membranous system.

...Often chylomicrons appear within overlapping portions of endothelial processes.
Fig. 8. Electron micrograph of endothelial junction of massively distended guinea pig lacteal showing that the junctions remain "tight" (t). Overlapping endothelial luminal processes (LP) probably play no role in this junctional complex. Basal lamina (bl). Fibrocyte (F). Osmium fixed, Epon embedded, double stained. × 50,000.

This could represent flow through an open junction, the open junction itself being outside the plane of section, or it could represent macromolecules that have been transported by pinocytosis across one endothelial process and subsequently are to be transported into the lumen by vesicular transport across the overlapping endothelial process. Because open junctions are so rarely identified in carefully processed tissues, the latter postulate seems to be correct.

If the formation of pinocytotic vesicles re-
quired energy, then metabolic inhibitors would be expected to interfere with pinocytotic transport but would be unlikely to affect flow through open endothelial junctions. However, Jennings and Lord Florey (34) were unable to demonstrate an inhibitory effect on vesicular transport in capillaries of in vitro hearts using a variety of oxidative, metabolic, and glycolytic inhibitors. In view of these findings, some observers have suggested that Brownian movement could account for pinocytotic vesicular movement across endothelia (8, 35). Bruns and Palade (32) have pointed out that available evidence does not allow a conclusion as to the energy requirements, if any, for pinocytotic transport. Ryser (36) has shown, using labeled proteins in mammalian mononuclear cells, that pinocytotic transport proceeds at low rates, requires little energy, and is enhanced by polybasic compounds. He has found that cells favor cationic macromolecules of large molecular weight and that large macromolecules are taken up more readily than small ones (36). Similar functions may eventually be established by quantitative methods for endothelial pinocytotic transport.

Pinocytotic transport is not likely to be selectively directed in either direction across the endothelium. Pinocytotic transport presumably proceeds equally in both directions and direction of transport depends upon concentration gradients and luminal flow. The proximal flow of lymph should result in a lower luminal concentration of macromolecules and, hence, the ultimate clearance of macromolecules from extracellular spaces of the intestinal lamina propria is similar to passive diffusion, a mechanism dependent upon concentration gradients alone.

Studies of the lacteal in disease states are limited in number. The disease in which intestinal lymphatics are most prominently involved is that known as primary lymphangiectasia (37, 38). Patients with this disease usually present with "idiopathic hypoproteinemia." That is, hypoproteinemia occurs in the absence of hepatic, renal, endocrine or other obvious gastrointestinal disease. Intestinal biopsies in these patients show marked dilatation of mucosal and submucosal lymphatics. It is thought that there is a congenital malunion of lymphatics draining the intestine and thus obstruction to outflow of lymph. In two patients with primary intestinal lymphangiectasia in which electron microscopic studies of the mucosal lymphatic have been performed in our laboratory, there are some electron microscopic differences from that of the normal (37, unpublished observations). In lymphangiectasia, the basal lamina, supporting cells, and collagen fibers appear to be much more prominent than in the normal lacteal, and lymphatic endothelial cells contain unusually prominent intracellular filaments. This increase in extracellular and intracellular filaments may represent a form of work hypertrophy. Because there is marked protein loss in these patients, one would expect to find open gaps in endothelial cell junctions through which protein might be lost. However, open cell junctions have not been observed. Rather, cell junctions appear to be tight. Chylomicrons appear to be trapped within the lumina of the lymphatics and large lipid droplets are seen within the cytoplasm of the endothelium. Massive numbers of chylomicrons can be seen in extracellular spaces of the lamina propria. The mechanism of fat malabsorption in these patients would appear to be merely that of mechanical blockade to lymphatic flow. The mechanism of intestinal protein loss is not quite so clear but again is undoubtedly related to the mechanical block to lymph flow. In view of the peroxidase studies cited above (13), it would be reasonable to speculate that there is an excessive loss of extracellular protein via the cell extrusion zone. Documentation of this has not yet been obtained.

Lymphangiectasia may also be acquired when inflammatory lesions or neoplasms involve and obstruct intestinal lymphatics. One patient with apparent primary hypo-beta-lipoproteinemia was found to have intestinal lymphangiectasia that appeared to be secondary to obstruction related to excess lipid storage within abdominal lymph nodes and within macrophages of the intestinal lamina propria (39). Microscopy showed that considerable numbers of chylomicrons were retained within the extracellular spaces of the lamina propria and intestinal absorptive cells were massively packed with lipid, a finding similar to that seen in patients with a-beta-lipoproteinemia (patients in whom intestinal
lymphatics are morphologically normal). The lacteals in this patient appeared to be normal except for the finding of numerous chylomicrons outside the lymphatic and within overlapping endothelial cell processes of the lymphatics. Lactal junctions were tight. The chylomicron retention was presumably a manifestation of secondary blockade to lymphatic flow, blockade due to the infiltration of abdominal lymph nodes by lipid-containing macrophages.

Dilatation of intestinal and abdominal lymphatics has been a prominent finding in most reports of patients with Whipple's disease (40). This dilatation is presumably related to infiltration of abdominal lymph nodes and the lamina propria of the intestinal mucosa by periodic acid-Schiff-positive macrophages. This infiltration by macrophages, similar to that by lipophages in the patient mentioned above, appears to result in obstruction to lymphatic flow. Also the massive number of macrophages within the lamina propria may well impede the passage of chylomicrons toward the lacteal. In addition, actual bacterial invasion of the endothelium of lacteals in these patients and the presence of numerous bacterial-containing lysosomes within the endothelia have been noted (40). Open cell junctions of the lacteals can be identified through which polymorphonuclear leukocytes and other cells appear to enter the lacteal. Malabsorption in Whipple's disease appears to be related to lymph outflow obstruction, possibly to bacterial invasion of lymphatic endothelium with resultant inhibition of pinocytotic activity, and more importantly to bacterial invasion of intestinal absorptive cells (40). Protein loss from the gastrointestinal tract in patients with Whipple's disease may be promoted by the finding of occasional open junctions in the lacteals of these patients.

Lymphatics of the intestinal mucosa in patients with the following disease states have been examined and found to be normal (unpublished observations): a) five patients with cirrhosis of liver (lacteals are dilated), b) two patients with Zollinger–Ellison syndrome and with gastric metaplasia of the intestine, c) three patients with neomycin-induced steatorrhea, d) two patients with celiac-sprue, and e) one patient with congenital beta-lipoprotein deficiency.

Summary

The central lacteal of intestinal villi transports chylomicrons, macromolecules, and fluids. The normal structure of lacteals is reviewed here and briefly compared to the structure of intestinal mucosal capillaries. Pinocytotic vesicles of lactal endothelium have been shown to occupy 15% of cytoplasmic volume and to average 1,600 A in diameter. Apparent pinocytotic transport of macromolecules such as peroxidase, ferritin, and chylomicrons is easily demonstrated. When the intestinal mucosa is examined using great care to avoid trauma to tissues, the great majority of lactal junctional complexes are too narrow to permit passage of macromolecules. Thus, by inference, it would appear that these substances gain entry into the lacteal by pinocytotic transport rather than by entry through endothelial cell junctions. Just as intestinal capillary fenestrae may represent both the small- and large-pore system postulated by physiologists, the lactal pinocytotic vesicles appear to be the morphologic equivalent of the small- and large-pore system of lacteals. Lactal junctions also represent the small-pore system.

Interestingly, the lactal junctional complex remains tight in primary intestinal lymphangiectasia, a disease state in which intestinal lacteals are massively distended. This further suggests the major role of pinocytosis in lacteal endothelial transport of macromolecules. Open lactal junctions in inflammatory disease states (such as Whipple's disease) may be the route by which some cells and even bacteria gain entry to the circulation.

References


