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Three-Dimensional Structure of Mucosal and Submucosal Lymphatics in Rat Small Intestine

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FUKUSHIMA, K., SASAKI, I., MASUDA, T., NAGURA, H., NAITO, H., FUNAYAMA, Y. and MATSUNO, S. *Three-Dimensional Structure of Mucosal and Submucosal Lymphatics in Rat Small Intestine.* Tohoku J. Exp. Med., 1998, **185** (1), 9-14 —— The organization of the lymphatic system in the rat ileum was investigated by the three-dimensional reconstruction using serial sections after dilatation of lymphatics. This method has an advantage to exclude possible artifacts in the conventional corrosion cast method. The villus possessed several lacteals and connected to each other by sinus. Two or three perpendicularly oriented lymphatics descended and led into the submucosal lymphatic network. In mucosa and submucosa, no valvular structure was detected. This study confirmed the voluminous lacteals and fine network structure of lymphatics in submucosa without valves, suggesting great potential to keep lymph and to drain soluble factors and cellular components. ———— lymphatic vessel; three dimensional reconstruction; small intestine

The function of lymphatic system of the gastrointestinal tract is the transportation of nutrient fluid, excess tissue fluid, and cellular components (Mayerson 1963; Benoit and Zawieja 1994). There are several reports demonstrating lymphatic involvement of human intestinal diseases. In particular, Crohn's disease is characterized by dilated lymphatic vessels and edema. The localization of lymphoid aggregates around them has been noted in this type of mucosal inflammation (Petras 1989; Mooney et al. 1995).

However, the investigation of individual intestinal lymphatic vessels is hampered because of the transparent nature of the lymphatics. This troublesomeness has often made it difficult to describe an anatomical structure of intestinal lymphatics. To overcome this methodological difficulty, the corrosion casts were

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made by the intraparanchymal puncture-injection of an acrylic casting materials, followed by detail observation using scanning electron microscopy (Ohtani 1987; Unthank and Bohlen 1988). This method, however, assumed that casting materials could spread through the lymphatics uniformly in all directions without any destruction of the structure by injection.

In this study, lymphatics were reconstructed three-dimensionally using serial sections by a graphic method. This approach clearly demonstrated the network of lymphatics without valvular structure in the mucosa and submucosa of rat small intestine.

Materials and Methods

Tissue preparation. Five Sprague-Dawley rats (250-350 g) were used in this study. The rats fasted for 12 hours with free access to water, and were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg) with supplemental injections as needed. One-third of the collecting lymphatics along vasa recta were ligated to dilate intestinal lymphatics in ileum as long as 5 cm. After 7 days the animals were killed and the ileal segments were removed, fixed with 10% buffered formalin, dehydrated, and immersed in 3% celloidin solution for 1 week. Finally, the tissues were embedded in paraffin.

Three-dimensional reconstruction of lymphatics using serial sections. Two sets of serial sections of 5 μm in thickness were prepared from each specimens. One consisted of sections cut in vertical manner to the sheet of intestinal wall, and another in horizontal. The sections were stained with Azan-Mallory method. This staining was better for identification of dilated lymphatics than hematoxylin-eosin staining. The lymphatics were projected onto a sheet of tracing paper under a magnification of 100 x using a profile projector (Model V-16, Nikon, Tokyo). Then, the pictures were overlapped by positioning obvious landmarks (blood vessels, etc.) and mucosal and submucosal lymphatics were traced by hand.

Results

Vertical section. In lunminal side three or four lacteals were generally found within a single villus (Fig. 1). The existence of a broad lymphatic sinus at the villous base was also noticed. From bottom of the sinus, two or three perpendicularly oriented lymphatics descended and led into the submucosal lymphatic plexus (Fig. 2).

Horizontal section. Lacteals were centrally located in the villus (Fig. 3A). The lacteals in each villus were anastomosed and formed a sinus at the level of the villus base (Fig. 3B). The lacteals in adjacent villi were interconnected by the large lymphatic at the villus base. The submucosal lymphatics consisted of a polygonal network of lymphatic vessels in a single layer (Figs. 3C and 4) and were not necessarily along the blood vessels (Fig. 3C). The distance between two

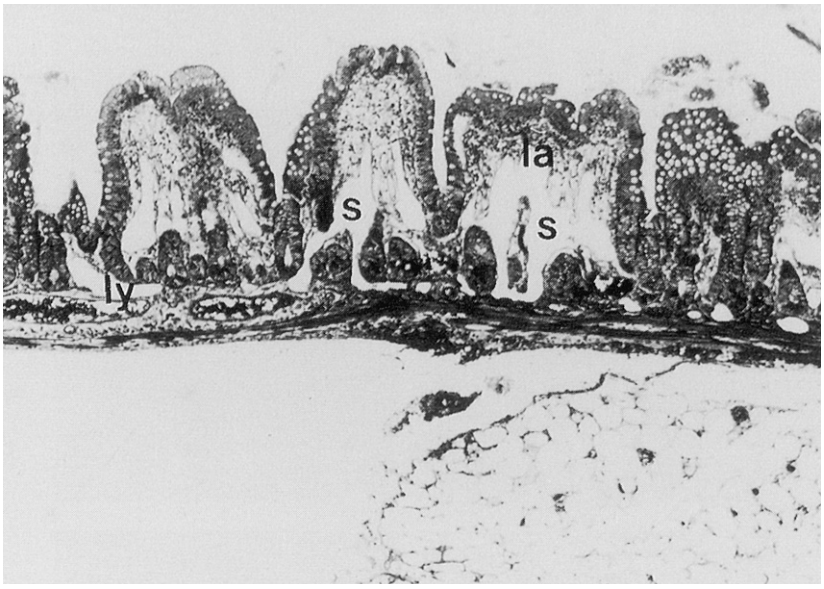

Fig. 1. Vertical section of ileum (×50). Remarkable dilatation of lacteal (la) and lymphatics (ly) were noticed. S, sinus.

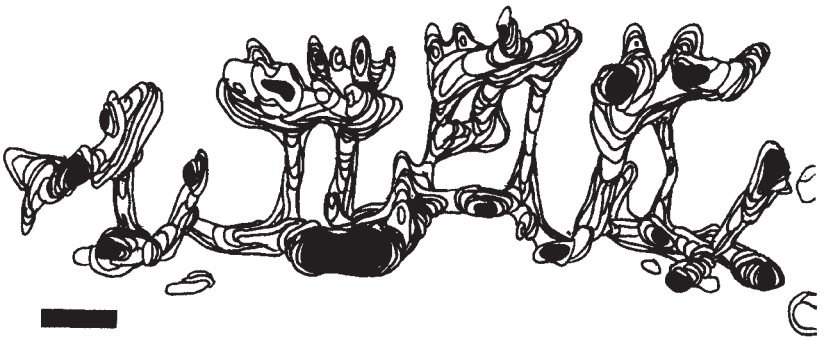

Fig. 2. Reconstruction of three dimensional structure of lymphatics in rat ileum. Bar, 100 μm.

adjacent intersections of the mesh averaged approximately 100–200 μm. The submucosal lymphatics gathered beside the vessels penetrating the muscle layer (Fig. 3D).

No valvular structure was identified in the mucosal and submucosal lymphatics.

Discussion

The corrosion cast method enabled to visualize the three dimensional organization of lymphatics in all layers of the intestinal wall (Ohtani and Ohtsuka 1985; Ohtani et al. 1986; Ohtani 1987; Unthank and Bohlen 1988). However, it has been pointed out that some artifacts or misinterpretations may have been introduced with the injection teqnique (Ohtani et al. 1986). For example, the interstitial tissue spaces are sometimes filled with the injection medium, and there is a possibility that all of the lymphatics are not filled with the injection medium (Ohtani 1987).

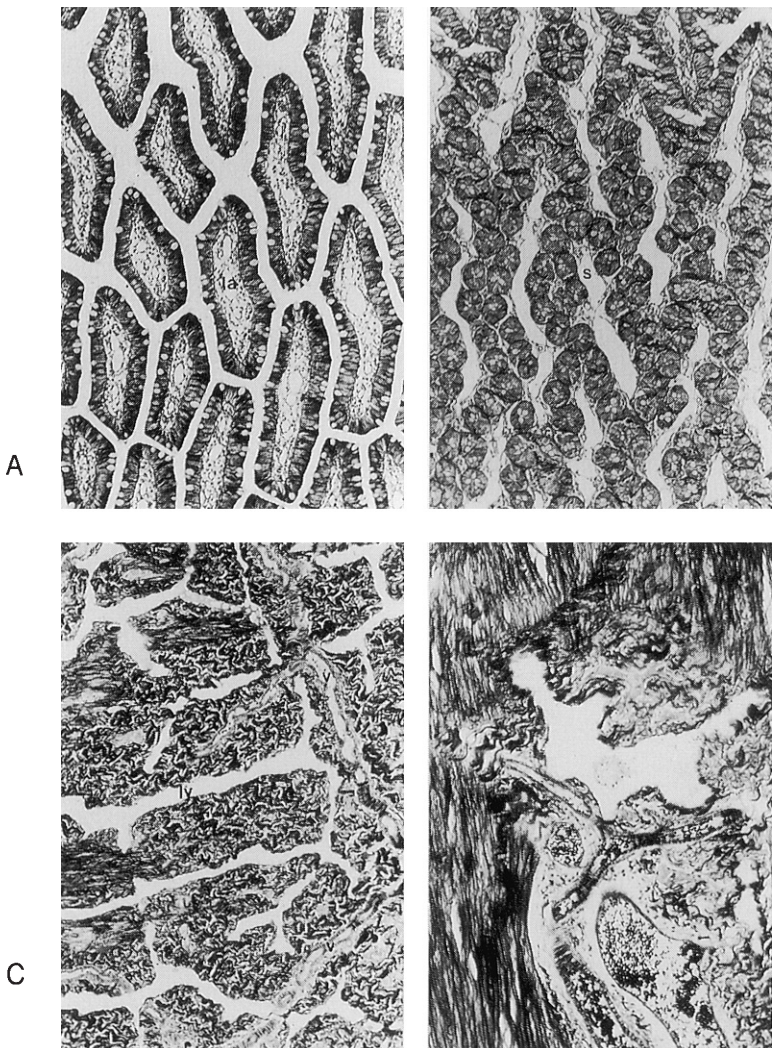

Fig. 3. A, Horizontal section of villus (×50); la, lacteal. B, Horizontal section at the level of villus base (×50); S, sinus. C, Horizontal section at the level of submucosal layer (×50); ly, lymphatics. D, Horizontal section at the level of border of submucosal and muscle layer (×50); V, blood vessel.

The present approach used in this study has several advantages. Dilated lymphatics after ligation could easily be identified among other important components such as villi, crypts and blood vessels. The layers in which lymphatics were present were easily recognized. Continuity of lymphatics was almost completely distinguishable by using 5 μm serial sections. On the other hand, lymph stasis or dilatation of lymphatics may have some influences on the lymphatic structure.

Nevertheless the lymphatic structure reconstructed by our graphic method was completely content with the data obtained from the corrosion cast method except for the lack of detection of valvular structure in the submucosa (Ohtani 1987). Freeze fracture and corrosion cast data reported the presence of valve-like structures within the submucosal lymphatic network (Ohtani 1987). The reason why valvular structure could not be found may derive from the dilatation of lymphatics in our experimental protocol. However, it is reported that dye

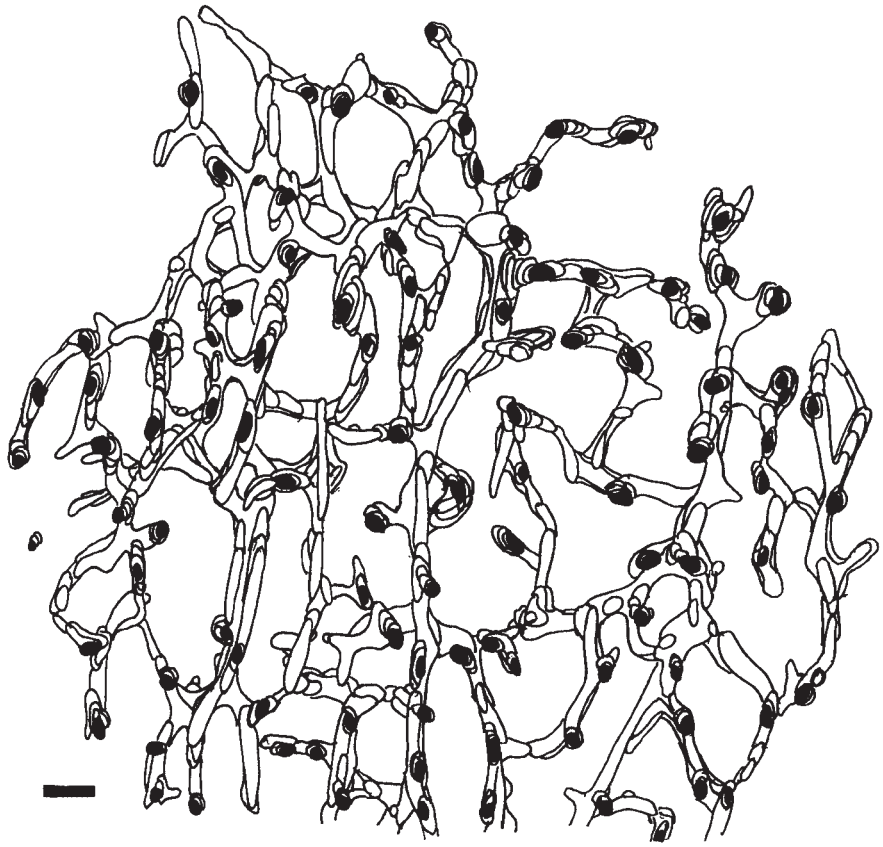

Fig. 4. Reconstruction of three dimensional structure of lymphatics in rat ileum. Bar, 100 μm.

injected into the submucosal lymphatic network rapidly appeared in the mucosal lymphatics (Unthank and Bohlen 1988). This data is interpreted as showing either the absence or lack of functional integrity of valves between the submucosal and mucosal lymphatic vessels (Benoit and Zawieja 1994).

In conclusion, this study confirmed the voluminous lacteals and fine network structure of lymphatics in submucosa without valves, suggesting great potential to keep lymph and to drain soluble factors and cellular components. Further investigation of human lymphatics in healthy and disease states may contribute to the better understanding of the role of lymphatics in the gut.

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