Lymphology 17 (1984) 69-72

# MORPHOLOGY OF LYMPHATICS IN THE CANINE LARGE INTESTINE

**Tadahisa Hirashima, Daisuke Kuwahara and Mitsumasa Nishi**

First Department of Surgery, Kagoshima University School of Medicine, Kagoshima, Japan

ABSTRACT:

*The finer distribution of lymphatics in the large intestine of adult dogs was morphologically examined by puncture injection of colored dyes, intra-arterial injection of silver nitrate mixed with India ink, and electron microscopy. In the lamina propria there was a double-layer of lymphatic channels — a shallow network (near the mucosal surface) and a deep compartment (just above the muscularis mucosae). Sparse lymph capillaries also extended vertically parallelling intestinal glands. Some lymphatics penetrated into the submucosa where they demonstrated prominent valves and formed collecting lymphatic trunks alongside blood vessels.*

The lymphatic system of the large intestine is important not only for absorption, metabolism and circulation, but also in the infiltration and dissemination of cancer cells. To date, however, the finer distribution of colonic mucosal lymphatics have not been fully delineated. In human large intestine, mucosal lymphatics generally are thought to be absent altogether, or to exist as blind lymphatic microvessels, or reach only to the base of the lamina propria. To reexamine this issue, structural studies on the finer distribution of lymphatics in the large intestinal wall were carried out in dogs after puncture injection of dye fluid, intra-arterial injection of a silver nitrate solution mixed with India ink, and electron microscopy.

## MATERIALS AND METHODS

The large intestine of 41 adult dogs were examined. Operations were carried out under sodium pentobarbital anesthesia.

### *Puncture Injection With Dye (15 Dogs):*

Colored dyes were instilled into the mucosal lamina propria and submucosally. Berlin blue and India ink solution (0.25 ml) were injected using a superfine needle (30 gauge) and mechanical injector into the subcutaneous tissue of the large intestine. To distinguish lymphatics from blood vessels, aberrant dye in arteries and veins was washed out with saline and refilled with barium sulfate (1). The resected segment of large intestine was fixed in 10% formalin, dehydrated with alcohol, and embedded in paraffin for 30$\mu$m thick sections. Optical microscopy was done after staining with Hematoxylin-Eosin (H-E).

### *Intra-Arterial Injection of Silver Nitrate Mixed With India Ink (23 dogs):*

Lymphatic channels within the mesentery of the large intestine were ligated to facilitate distention within the bowel wall. Except for blood vessels directly supplying the large intestinal segment to be studied, adjacent arteries were ligated. The equivalent of a 3.3% solution of sodium sulfate was then freely infused into the ab-

dominal aorta. Thereafter, a 0.7% silver nitrate solution diluted to 0.3% by addition of India ink, was also infused into the abdominal aorta (2). The resected intestine was fixed in 10% formalin, dehydrated with alcohol and embedded in paraffin for sections as described above. The advantage of this method was that removed samples turned yellow-brown by mere exposure to the sun without further staining. As a result it was relatively easy to differentiate within tissue layers between blood and lymphatic channels.

*Electron Microscopy (3 Dogs):*

The large intestine was resected, sliced, embedded in Epon and examined under the electron microscope. A 1μm slice was stained with methylene blue. Lymphatic-like tissue was detected by optical microscopy for final trimming. In addition, an ultrathin section was prepared and doubly stained with uranyl acetate and lead citrate.

## RESULTS

*Puncture Injection of Dye:*

Double-layered lymph capillaries were visible by optical microscopy. One layer traveled horizontally on the surface of the lamina propria (intramucosal shallow network) and the other traversed the bottom of the lamina propria (intramucosal deep lymph capillaries). These intramucosal lymphatics formed a rete vasculosum on the surface of the mucosae encompassing each glandular lacuna. Shallow lymph capillaries were visible just under the rete vasculosum. Structurally they had the characteristics of lymph capillaries — valveless, a string of beads appearance, and alternately wide and narrow diameters. Both the shallow and deep lymph capillary networks communicated by occasional lymph capillaries running vertically among the intestinal glands (Fig. 1).

*Intra-Arterial Injection of Silver Nitrate Mixed With India Ink:*

No ink particles were visible in lymph capillaries. Lymphatics were readily distinguished from blood vessels. A double layer of mucosal lymphatic capillaries were again observed (Fig. 2). Some lymphatics with valves penetrated into the submucosa (Fig. 3, 4), extended to form collecting

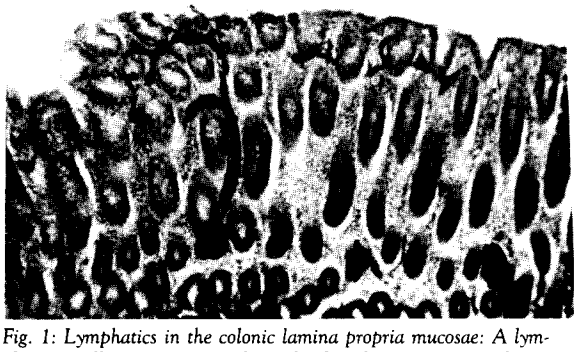


*Fig. 1: Lymphatics in the colonic lamina propria mucosae: A lymphatic capillary is seen just above the deep lamina propria. (Puncture injection of dye, 100x)*

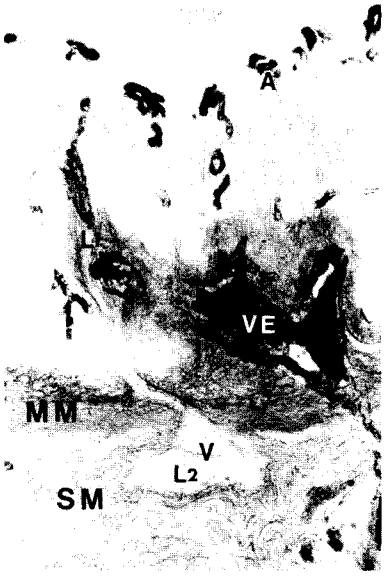
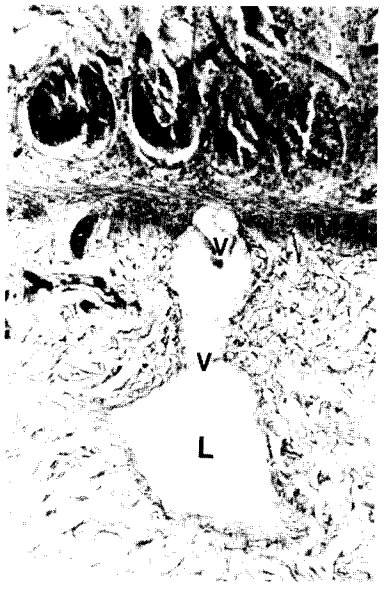



*Fig. 2: (right) Colonic lymphatic capillary (L1) entering submucosal layer (SM) from lamina propria mucosae through muscularis mucosa (MM). An intraluminal valve (V) is seen after penetrating through the MM. L: lymphatic lumen A: artery VE: venule (Intra-arterial injection of $AgNO_3$ solution; 100x)*



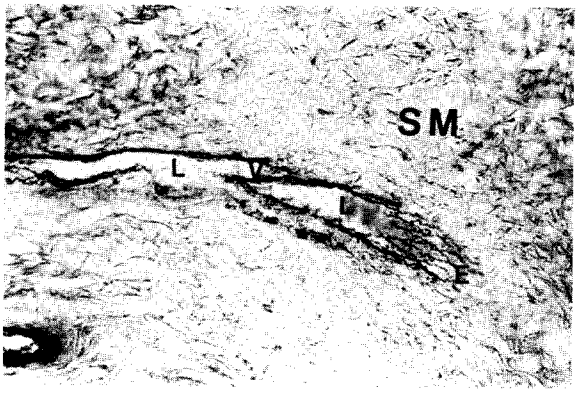




*Fig. 3: (left) Submucosal colonic lymphatic with two valves after penetration through the muscularis mucosae. L: lymphatic lumen (Intra-arterial injection of $AgNO_3$ solution; 200x)*

*Fig. 4: (above) Large intestinal lymphatic with submucosal valve. Note the special configuration of endothelial cells (E) and intralymphatic valve (V). L = lymphatic lumen (Intra-arterial injection of $AgNO_3$ solution; 200x)*

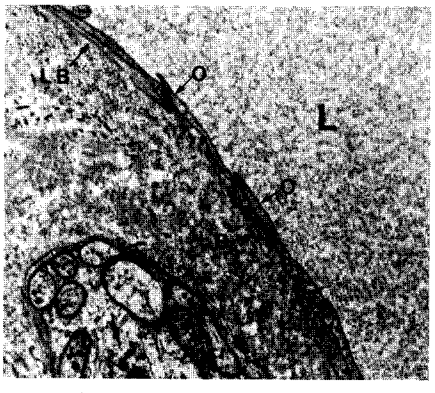
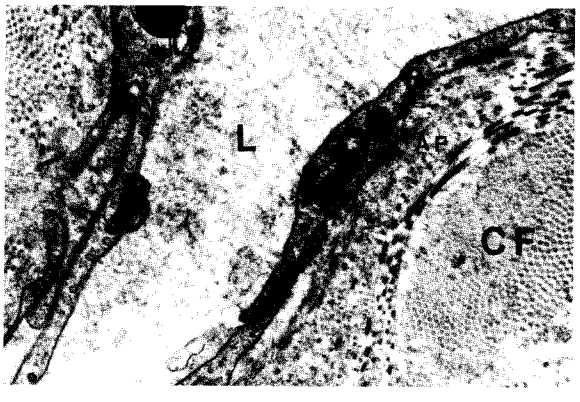
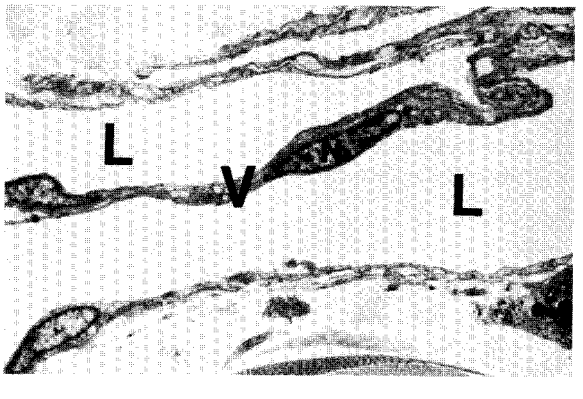



*Fig. 5: (above) Electron micrograph of endothelial cells in a colonic lymphatic. The endothelial cells (E) are thin, irregularly and loosely joined with notable overlapping (0). The lamina basalis (LB) is incomplete. L = lymphatic lumen; 5000x*

*Fig. 6: (above right) Electron micrograph of anchoring filament in large intestinal lymphatic. Anchoring filament (AF) is between endothelial cell (E) and collagen fiber (CF). L = lymphatic lumen; 10000x*

*Fig. 7: (right) Electron micrograph of large intestinal lymphatic and submucosal valve. Sides of valve (V) are covered with thin, irregularly and loosely joined endothelium with prominent nucleus (N). L = lymphatic lumen; 2000x*



channels parallelling blood vessels and re-emerged from the intestine after penetrating the muscularis externa.

*Electron Microscopy:*

Lymphatic-like lumens with valves were visible in the submucosa. Endothelial cells were thick, irregularly and loosely joined, with some separation. The lamina basalis was discontinuous (Fig. 5). Anchoring filaments were also evident (Fig. 6). Both sides of the valve were covered with thin irregular endothelium, and resembled that previously described in lymphatic walls (Fig. 7).

## *DISCUSSION*

Although the finer structure of intestinal lymphatics is potentially clinically important, anatomical delineation is difficult. Lymph capillaries are extremely slender and transparent thereby limiting macroscopic and microscopic observation. Moreover, lymphatic vessels contain powerful valves which limit or block backward infusion. Whereas lymphatics of the large intestine have previously been described particularly in the submucosa, the precise location of mucosal lymphatics has been less clear. Using 3 different techniques (colored dye injection, silver nitrate — India ink intraarterial infusion and electron microscopy) a dual layer network of intramucosal lymphatic capillaries (shallow and deep) were demonstrated which penetrate the muscularis mucosa to join submucosal lymphatic trunks.

(Presented in part at the 9th International Congress of Lymphology, Israel, 1983.)

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Tadahisa Hirashima, The First Dept. of Surgery, School of Medicine (M. Nishi, M.D., Director), Kagoshima University, 1208-1 Usuki-cho, Kagoshima 890, Japan