Lymphology 36 (2003) 52-61

LYMPHATIC VESSELS IN THE COLONIC MUCOSA IN ULCERATIVE COLITIS

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ABSTRACT

In the normal colonic mucosa, lymphatics are found only in a narrow band associated with the muscularis mucosae and are absent from the rest of the mucosa. This study examined whether this arrangement of lymphatics is also valid in ulcerative colitis. Histological sections of colon from 15 long-standing cases were investigated with antibodies against CD 34 (negative for lymphatics; positive for blood vessel endothelium) and, in selected cases, podoplanin (positive for lymphatic endothelium; negative for blood vessel endothelium). Whereas inflammation of the mucosa was not associated with changes in lymphatics, an increase in intramucosal lymphatics was seen when the pathological changes included widening of the muscularis mucosae or penetration of the mucosa by muscle fibers, filiform changes in the mucosa, and hyperplasia of the mucosa-associated lymphoid tissue (MALT). In specimens with epithelial dysplasia, an association between the dysplastic epithelium and ectatic and quantitatively increased lymphatics was observed. With superimposed carcinoma, no relationship between the malignant tumor and lymphatics was identifiable. Nevertheless, pre-existing lymphatics in the muscularis mucosae were involved in lymphatic tumor spread. The immunohistochemical findings demonstrated that lymphatics occurred in all areas of the mucosa in ulcerative colitis (or, in effect, at sites which were not normally found under physiological conditions) and in regions that favored lymphatic tumor dissemination. Whether these lymphatics were actually involved in metastasis remains to be defined.

Ulcerative colitis is associated with a markedly increased risk of colon adenocarcinoma, with susceptibility increasing with duration of the colitis. The colon with carcinoma develops chronic persistent and/or recurrent inflammation and various grades of epithelial dysplasia, accompanied by various cytogenetic changes, such as activation of oncogenes and inactivation of tumor suppressor genes. This dysplasia carcinoma sequence occurs within the fairly short interval of 3-13 years, which is of shorter duration than ~10-15 years estimated in those cases in the absence of inflammatory bowel disease (1,2).

Previously, we showed that chronic inflammatory bowel disease is associated with hyperplasia of the submucosal lymphoid tissue, which may in exceptional cases lead to the formation of lymph nodes (3). This study deals with the issue as to whether the colonic mucosa, which is generally accepted not to contain lymphatics, exhibits lymphatic angiogenesis in ulcerative colitis. If so, neolymphatics would likely facilitate tumor metastasis, and perhaps dictate earlier colonic resection with advanced dysplasia in patients with ulcerative colitis.

MATERIAL AND METHODS

The investigation was performed on preserved tissue from 15 patients with long-standing (as far as is known, 8-16 years) ulcerative colitis, in whom colectomy was performed. The indications for resection were severe persistent inflammation (7 patients), dysplasia (5 patients), and adenocarcinoma (3 patients). Six patients exhibited marked filiform polyposis, with its typical finger-like polypoid mucosal changes (4), and, in most cases, pronounced atrophy of the submucosa.

Sections and paraffin blocks were retrieved from the archives of the Department of Pathology for the previous three years. In each case, 8-20 H&E-stained sections of paraffin-embedded tissue were available, and 1-3 sections were cut for immunohistochemical investigation from areas of mild or severe inflammation, submucosal fibrosis, and mild or moderate dysplasia. A few regional mesenteric lymph nodes were also immuno-stained. Sections from seven areas of colon with no pathological changes (three of which were from cases of diverticulosis) were also investigated and acted as controls. Immuno-staining was performed by the avidin-biotin-peroxidase complex method (5) with antibodies against CD34 (QBEND10, diluted 1:500), sm-actin (diluted 1:500), and desmin (diluted 1:100), all obtained from Dako, Hamburg, Germany. Sections from three of the cases were also stained for podoplanin in the laboratory of Professor Dr. D. Kerjaschki and Priv.-Doz. Dr. S. Geleff (Institute of Clinical Pathology, Vienna General Hospital).

RESULTS

The H&E-stained tissues were divided into three main groups on the basis of the pathological changes:

1. Inflammation of the mucosa predominant
2. Structural changes (mucosa and/or submucosa)
3. Precancerous and cancerous changes

Inflammation ranged from mild to severe, and both acute and chronic changes were seen. Structural changes involving the colonic mucosa consisted of erosions, ulcers, polypoid or filiform structures, and hypertrophy of the muscularis mucosae, with or without projection of muscle fibers (positive for sm-actin and desmin) into the mucosa. A further structural change observed was hyperplasia of the mucosa-associated lymphoid tissue (MALT), which was within the mucosa and topographically related to the muscularis mucosae. The three different types of histological changes seen in the submucosa consisted of fibrosis, narrowing (which made the mucosa appear to lie directly over the muscle layer), and increased lymphoid tissue with the formation of large lymph follicles and lymphangiectasia.

The third group consisted of epithelial dysplasia and frank invasive carcinoma.

When the inflammatory changes were confined mainly to the colonic mucosa and no structural derangements were evident, no CD34- lymphatics were seen in the upper and middle layers of the mucosa, although the number of CD34+ blood vessels was markedly increased. A few CD34- lymphatics running parallel to the muscularis mucosae were seen between its muscle fibers and on its mucosal and submucosal borders. This latter finding is normal as it was noted in the noninflamed colon, in sections of normal colon, and in specimens from cases of diverticulosis. Very rarely vessels that were weakly positive for CD34 were noted between the muscle fibers, but it was not clear whether these were lymphatics or blood vessels.

When the muscularis mucosae was widened, i.e., hypertrophied, the lymphatics in and around this layer were markedly increased in number and usually dilated. In particular, there was an increase in CD34- vessels on the mucosal side. These vessels representing lymphatics were completely unreactive, or at most only weakly reactive, for CD34, in contrast to the intensely CD34+ blood vessels. Where muscle fibers radiated

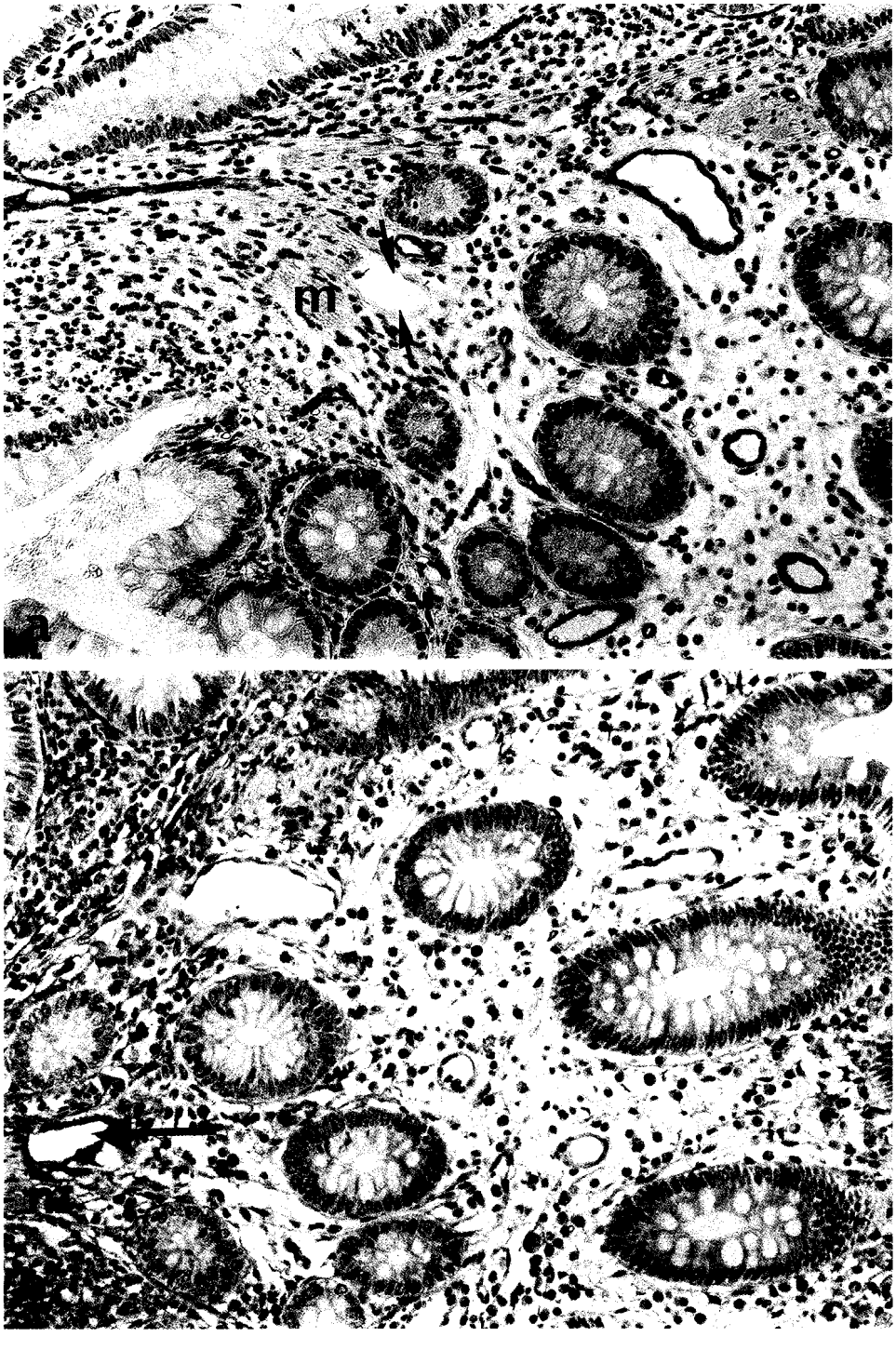

Fig. 1. Ulcerative colitis. The middle part of the colonic mucosa with (a) a CD34-negative lymphatic (arrows) in close association with muscle fibers (m), which are projections of the muscularis mucosae (not shown). (b) shows the same vessel, with staining for podoplanin (arrow), and podoplanin-negative blood vessels. (a) and (b) 280x

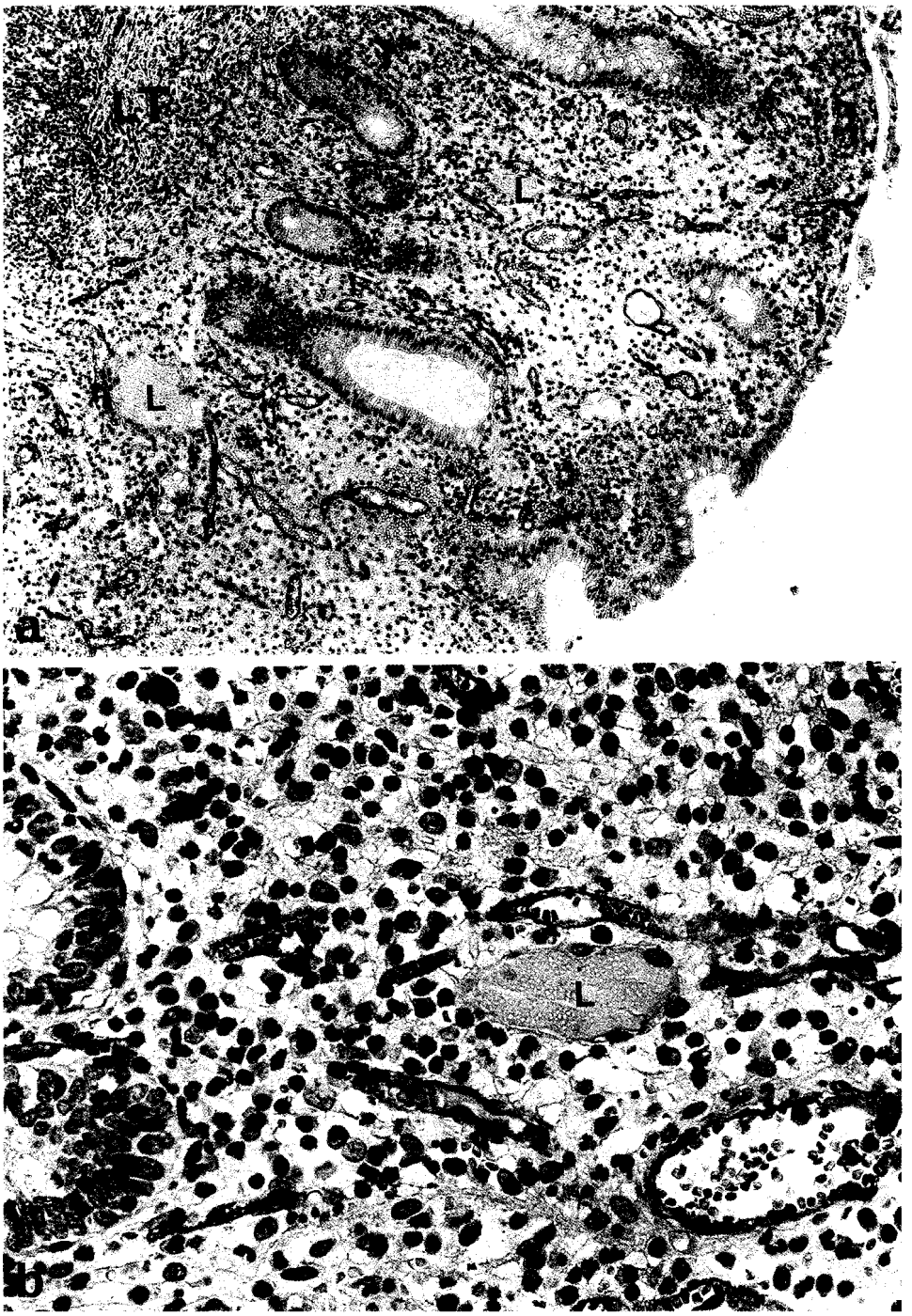

Fig. 2. Ulcerative colitis. The crypts of the colonic mucosa (a) are dilated and shortened and the mucosa-associated lymphoid tissue (LT) is prominent. The arrow marks CD34-negative lymphatics are seen in an area in which lymphatics are not found in the presence of non-altered mucosa. (b) shows one of the lymphatics (L) at higher magnification and the abundant CD34-positive blood vessels. (a) 140x, (b) 580x

from the muscularis mucosae into the middle layer of the mucosa, a few podoplanin-positive vessels (i.e., lymphatics) were seen in association with these fibers *(Fig. 1)*. When there was hyperplasia of the MALT with diffuse and follicular lymphoid infiltration of all layers of the mucosa, several CD34- or weakly positive lymphatics were also seen, even in the uppermost layer of the mucosa *(Fig. 2)*.

In specimens in which there was partial atrophy of the crypts and in those with ulceration or erosions, a few ectatic, CD34- lymphatics were seen in the middle or upper layer of the mucosa *(Fig. 2)*. In specimens with fibrosis of the submucosa, including those with polypoid or filiform thickening of the mucosa, increased numbers of lymphatics *(Fig. 3)*, which were also ectatic, were seen in both the mucosa and the submucosa. Blood vessels (CD34+) were markedly increased in number in both the mucosa and submucosa.

In the submucosa, circumscribed areas with dilated lymphatics that were sometimes associated with lymph follicles were common.

In 2 of 5 specimens with dysplasia or carcinoma, there was a close topographical relationship between CD34- or weakly + lymphatics and the areas of dysplasia *(Fig. 4)*. In these two specimens, the dysplastic epithelial cells at the bases of the crypts were in close proximity to the lymphatics, which were typically dilated and exhibited a topographical relationship to the muscularis mucosae. The middle and upper layers of the mucosa contained no lymphatics. In the other three specimens, no relationship between lymphatics and dysplastic epithelium was identified.

With carcinoma, no lymphatics were found in the vicinity of the tumor. One specimen exhibited massive lymphangitic carcinomatosis, and the lymphatics involved were those associated with both mucosal and submucosal aspects of the muscularis mucosae *(Fig. 5)*. Unlike other lymphatic vessels, those containing tumor cells strongly expressed CD34+ and podoplanin.

DISCUSSION

Colonic mucosa does not normally exhibit lymphatic vessels between the crypts of Lieberkühn. Lymphatics begin as a capillary plexus that appears to be wrapped around the muscularis mucosae. This plexus sends small branches into the mucosa, but these do not reach further than the base of the crypts (6). This anatomical situation has important consequences for oncology. Invasive growth into the colonic mucosa with glands that exhibit a cribriform pattern or lie "dos á dos" is classified as severe dysplasia or carcinoma in situ (pTis) at this site, whereas in the stomach it would be labeled invasive carcinoma (pT1) as lymphatics reach into the upper layers of the gastric mucosa, which presumably facilitates lymphatic spread of the cancer.

The question as to whether lymphatics are found in the mucosa of the large intestine and, if so, under what circumstances, has not been investigated before. As we have shown, intramucosal lymphatics may be found in specimens of ulcerative colitis of many years duration. However, chronic inflammation alone does not appear to lead to intramucosal lymphatic proliferation. The prerequisite is a structural change in the mucosa, in which there is either widening of the muscularis mucosa or projection of smooth muscle fibers into the middle or upper layers of the mucosa. Hyperplasia of the MALT may also be accompanied by increased intramucosal lymphatics, as may polypoid or filiform mucosal hyperplasia, in which structural changes in the submucosa with partial or complete atrophy are typically seen. The number of lymphatics in the submucosa may also be increased, usually accompanied by hyperplasia of the lymphoid tissue. This morphology is common in Crohn´s disease, in which lymphangioma-like ectasia and hyperplasia of the lymphoid tissue are characteristic, but is less common in ulcerative colitis.

The significance of the presence of

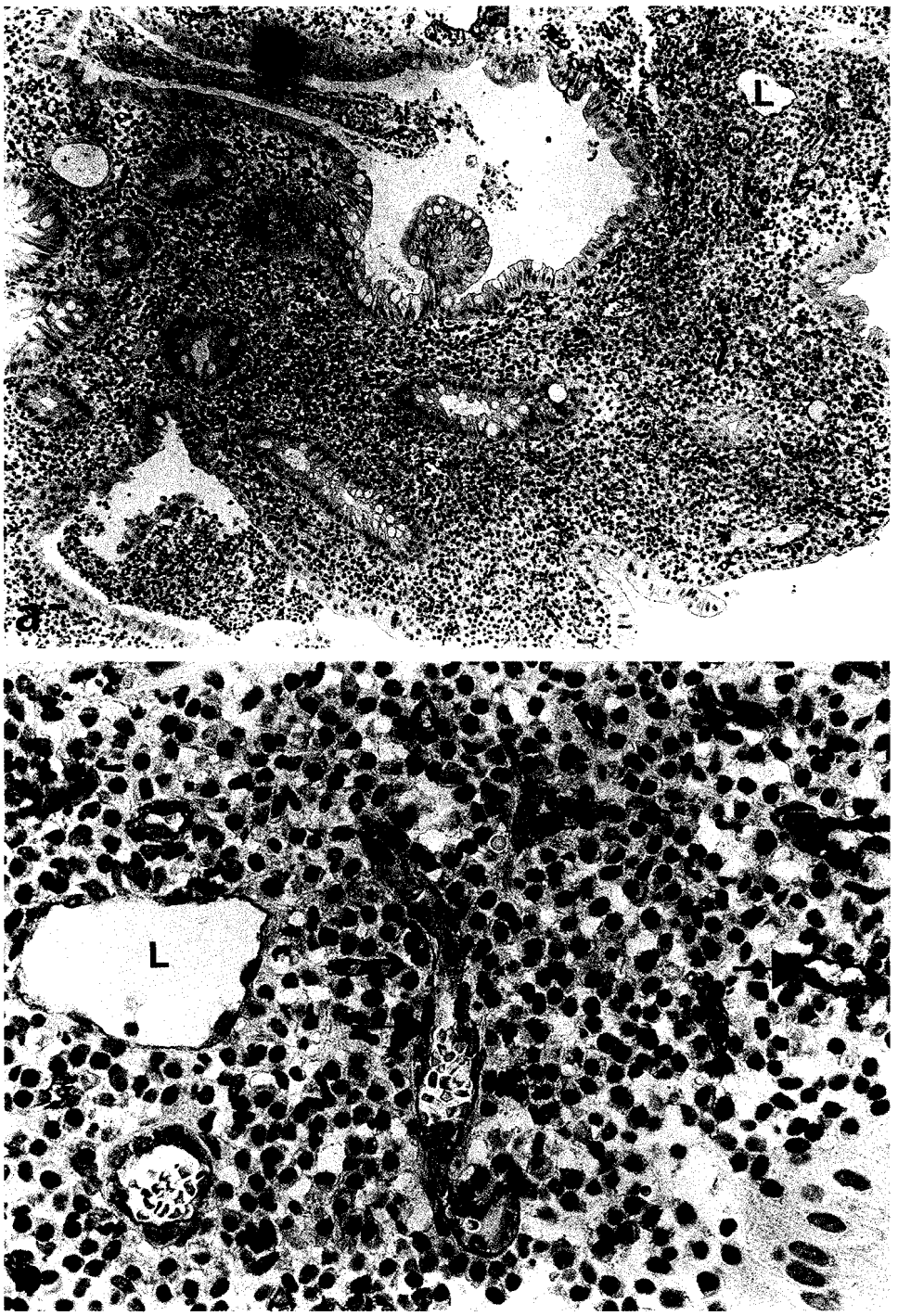

Fig. 3. (a) Colonic mucosa from a case of ulcerative colitis with filiform polyposis. There is a CD34-negative lymphatic (L) in the upper part of the mucosa (i.e. adjacent to the lumen). (b) The lymphatic vessel (L) is shown at higher magnification. The blood vessels are lined by a band of intense immunoreactivity for CD34 (arrow). (a) 140x, (b) 580x

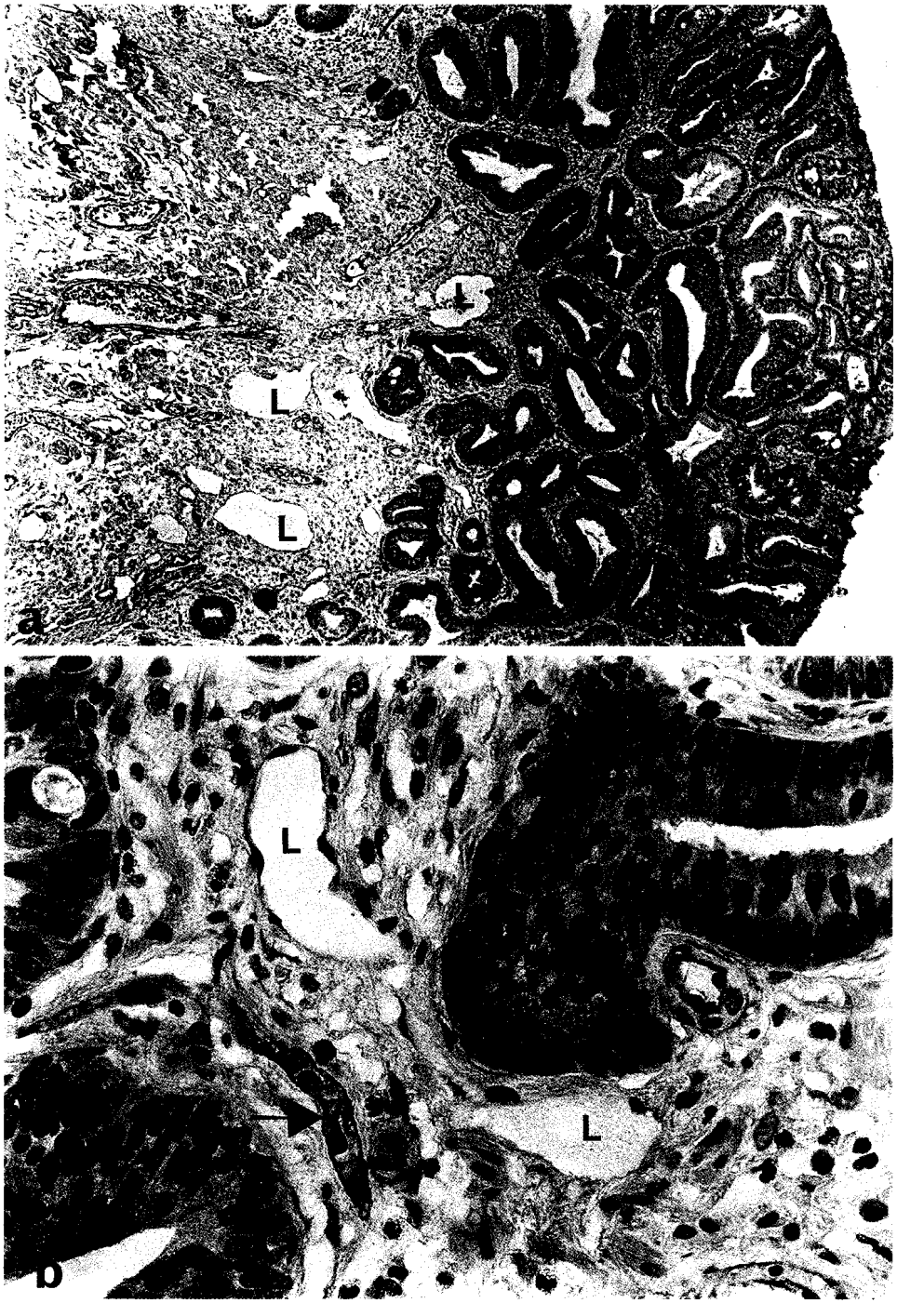

Fig. 4. (a,b) Mucosa with low-grade dysplasia of the glandular epithelium in a case of long-standing ulcerative colitis. There are numerous CD34-negative lymphatics (L) in the vicinity of the dysplastic epithelial cells in the lower part of the crypts. No lymphatics are found in the middle and upper parts of the dysplastic mucosa. (a) 140x, (b) 580x

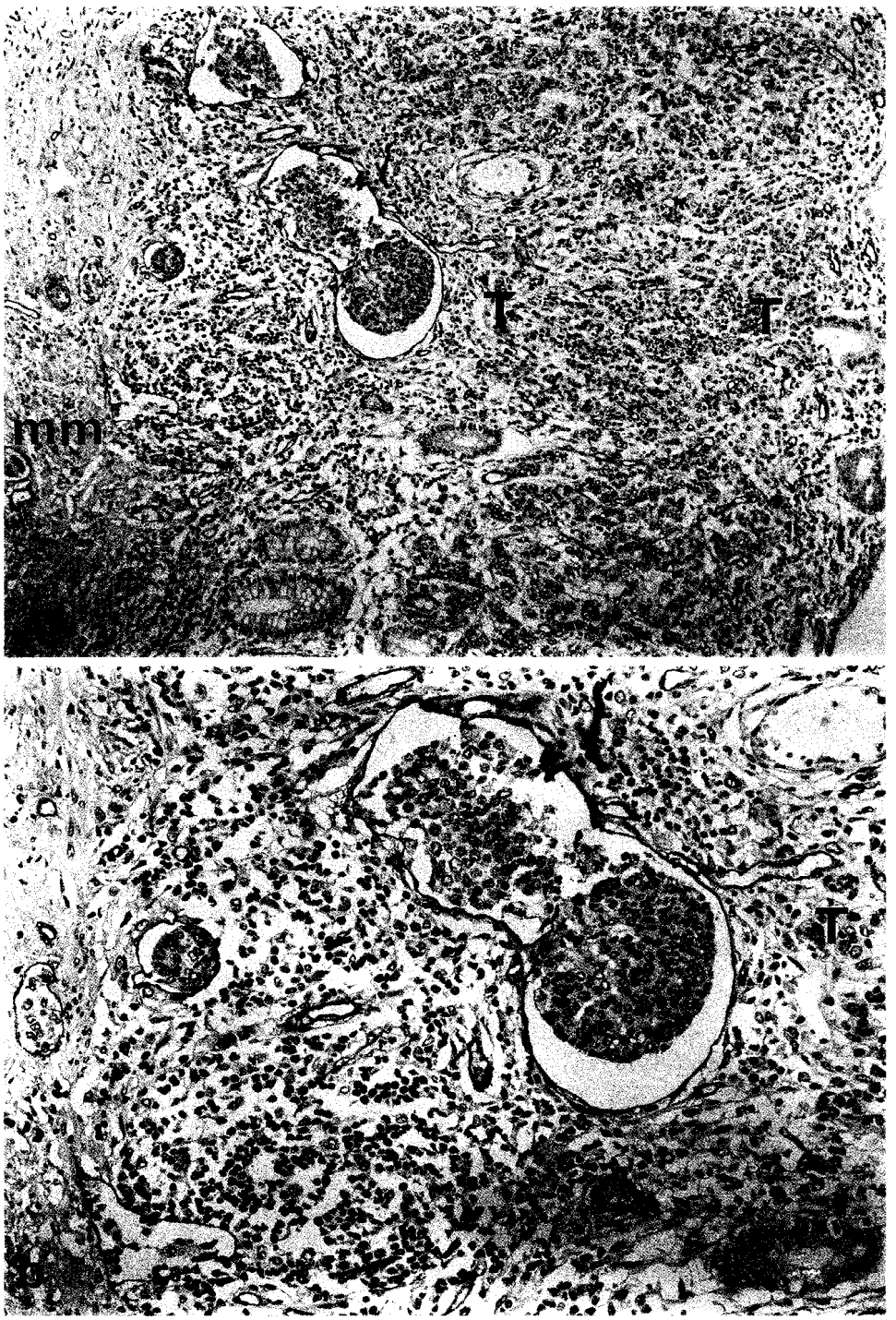

Fig. 5. (a,b) Lymphangitis carcinomatosa in a long-standing case of ulcerative colitis. The tumor itself (T) contains no lymphatics. Tumor cells are seen in the lymphatics of the lower part of the mucosa and the muscularis mucosae (mm). (a) 140x, (b) 280x

intramucosal colonic lymphatics has not been established, although one can postulate their presence would facilitate metastatic spread in the event of a malignant tumor. Indeed, increased numbers of lymphatics in the vicinity of the muscularis mucosae in ulcerative colitis are involved in the lymphatic transport of atypical epithelial cells, but it cannot be ascertained whether this finding is also valid for lymphatics in the middle and upper layers of the mucosa. It is also possible that increased numbers of lymphatics in the mucosa have a completely different function, that is, removal of increased amounts of substances that are carried in the lymph, derived, for example, from interstitial edema or cell detritus. Yet inflammation itself does not stimulate proliferation of the lymphatics (see above).

The question as to whether and to what extent preexisting lymphatics or newly formed intra- and peritumorous lymphatics are involved in the process of metastatic spread is complex and the answer probably relates to the tumor type. In a study of breast carcinoma (7), we very rarely found peritumoral lymphatics, and never intratumoral lymphatics, although tumor metastasized via lymphatics. This paucity of lymphatics finding is in sharp contrast to the marked blood vessel vascularity of this tumor. In ulcerative colitis the massive lymphangiectasia leaves little doubt that the muscularis mucosa-associated lymphatics are involved in tumor dissemination, but it remains unclear whether this phenomenon also applies to the lymphatics of the middle and upper layers of the mucosa.

It is not clear whether precancerous or hamartomatous polyps of the mucosa of the large intestine are supplied with lymphatics. In an earlier publication we suggested this to be the case (8), having observed lymphatic-like structures in the area of the stalk. However, because of the lack of specific endothelial markers at that time, we were unable to undertake the necessary immunohistochemistry to confirm their identity.

Few immunohistochemical markers are available for the identification of lymphatic vessels (9,10). However, an antibody against podoplanin (a glycoprotein expressed on the surface of podocytes), which is not yet commercially available, appears to be particularly useful for this purpose. This antibody labels the endothelium of all lymphatic vessels [in the same distribution as vascular endothelial growth factor receptor (VEGFR-3)], but not that of blood vessels (11,12). It should be noted that reactivity is also seen in osteoclasts and pulmonary alveolar cells (13) and, according to our findings, various other cells (mesothelial cells, follicular dendritic reticulum cells, cells of the walls of lymph node sinuses, certain, not yet identified lymphocytes, activated macrophages, and nerve fibers) (7).

Anti-CD34 (QBEND10) is also used for the identification of lymphatics, but is less reliable than podoplanin. Lymphatics are negative (or at most weakly positive) for this antigen, whereas blood vessel endothelium is strongly positive (14,15). Lymphatics are sometimes weakly positive with inflammation, and positive intramucosal lymphatics have been observed in cases of ulcerative colitis with lymphangitic carcinomatosis. Weakly positive lymphatics cannot be distinguished with certainty from blood vessels, but a negative reaction confirms a vessel to be a lymphatic.

A reliable histochemical marker for lymphatics would be a very useful. In the gastrointestinal tract, it would be of relevance to ascertain whether and to what extent adenomas and carcinomas are supplied with lymphatics, because these malignancies are particularly prone to lymphatic spread. The large intestine is also a useful model to study lymphatic angiogenesis and its inhibition, because this is an area that is easily accessible for biopsy and contains zones with no lymphatics (middle and upper layers of the mucosa) in close association with zones containing abundant lymphatics (the muscularis mucosae).

ACKNOWLEDGMENTS

The authors wish to thank Ms. A. Adam, Ms. A. Mall, and Ms. F. Müller for skillful technical assistance and Dr. M. Ruck for help in preparation of the manuscript.

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