Lymphology 34 (2001) 22-29

NEWLY-FORMED LYMPH NODES IN THE SUBMUCOSA IN CHRONIC INFLAMMATORY BOWEL DISEASE*

E. Kaiserling

Institute of Pathology, University of Tübingen, Tübingen, Germany

ABSTRACT

Background and Aims: Routine diagnostic work revealed cell aggregates reminiscent of lymph nodes in the bowel submucosa in occasional cases of chronic inflammatory bowel disease. We therefore investigated whether they fulfill criteria for classification as lymph nodes.

Methods: Colon with terminal ileum from a patient with florid Crohn's disease and a colectomy specimen from a patient with ulcerative colitis were investigated. Sections were immunostained with antibodies that recognize endothelial and sinus-lining cells, immune-accessory cells, and lymphoid cells.

Results: Circumscribed collections of cells that fulfill all the major criteria for classification as lymph nodes were found in the large and small bowel. They had marginal and intermediate sinuses (positive for BMA 120, CD34, CD31, X-11, and von Willebrand's factor), afferent lymph vessels, T- and B-regions, and a capsule. Small collections composed predominantly of B cells that had only a marginal sinus were also occasionally observed.

Conclusion: Secondary mucosa-associated lymphoid tissue, typically seen as follicular lymphoid hyperplasia, also appears to occur as secondary submucosal lymph nodes. This phenomenon seems inconsistent with the notion that lymph nodes do not develop after birth. We have also noted secondary development of lymph nodes in lymphangioma and lymphangioleiomyomatosis. It is possible that local lymph vessel proliferation, possibly with chronic lymphedema of the tissue involved, is an important prerequisite for lymph node neogenesis.

The anatomy of mucosa-associated lymphoid tissue (MALT) of the gastrointestinal tract is well established (1-3). A distinction is made between isolated lymph follicles (folliculi lymphatici solitarii), which are also known as lymphoglandular complexes if there is a close topographic relationship to the colonic mucosa, and closely-packed collections of lymph follicles (folliculi lymphatici aggregrati), which typically are found in the terminal ileum, where they are known as Peyer's patches.

In chronic inflammatory bowel disease, especially Crohn's disease and ulcerative colitis, there is hyperplasia of the local lymphoid tissue, and secondary MALT develops by the formation of other lymph follicles, especially in the submucosa, although they occur in all the layers of the bowel wall in Crohn's disease.

Detailed investigation of this secondary MALT reveals various differences compared to primary MALT, which have not previously been described.

MATERIALS AND METHODS

Histologic and immunohistochemical

*Dedicated to Professor D. Harms, Kiel, Germany, on the occasion of his 65th birthday.

TABLE 1
Antibodies Used in the Study

Antibody	CD	Main Reactivity	Source
L26	CD20	B cells	Dakopatts, Hamburg, Germany
CD3	CD3	T cells	Dakopatts
KiM4P		B-immune-accessory cells	Prof.Wacker, Kiel, Germany
Anti-S100 protein		T-immune-accessory cells	Dakopatts
CD1a	CD1a	T-immune-accessory cells	Immunotech, Hamburg, Germany
X-11		B-immune-accessory cells	Immunotech
IC/70A	CD31	endothelial cells	Dakopatts
QBEND10	CD34	endothelial cells	Dianova, Hamburg, Germany
BMA120		endothelial cells	Behringwerke, Marburg, Germany
Anti-vWF		endothelial cells	Dakopatts
sm-actin		smooth muscle actin	Dakopatts

Anti-vWF= anti-von Willebrand's factor; sm-actin= anti-human smooth muscle actin

investigations were performed on tissue from two patients with chronic inflammatory bowel disease: Case 1, resected colon with terminal ileum from a 25-year-old man with a 3-year history of Crohn's disease and involvement of the colon and ileum; and Case 2, colon and terminal ileum from a 37-year old man with a 10-year history of ulcerative colitis and florid disease at the time of operation.

The surgical specimens were fixed in formalin and sections were stained with H&E, the periodic acid-Schiff reaction (PAS), Giemsa, and Gömöri's silver impregnation. Immunohistochemical investigations were also carried out by the peroxidase-antiperoxidase method (4) using the antibodies listed in *Table 1.*

RESULTS

Histologic changes typical of Crohn's disease were seen in the specimen from Case 1, an acute relapse of Crohn's disease involving the large and small intestine. The mucosa exhibited edema, which was marked in places, multiple fissures, and filiform polyposis, which is sometimes found in association with Crohn's disease (5). There was marked fibrosis of the submucosa at several sites. As far as the lymphoid tissue was concerned, there was an increase in lymph follicles in the form of lymphoglandular complexes with and without germinal centers, a large number of lymph follicles in the submucosa, muscularis propria and subserosa (also with and without germinal centers), and markedly enlarged Peyer's patches with numerous germinal centers. The regional lymph nodes were also enlarged and exhibited pronounced follicular lymphatic hyperplasia. No granulomas were detected.

One of the lymph follicles in the submucosa of the small bowel was particularly noteworthy *(Fig. 1).* It exhibited at its periphery a loose, cell-depleted meshwork, which had the immunohistochemical characteristics of the marginal sinus

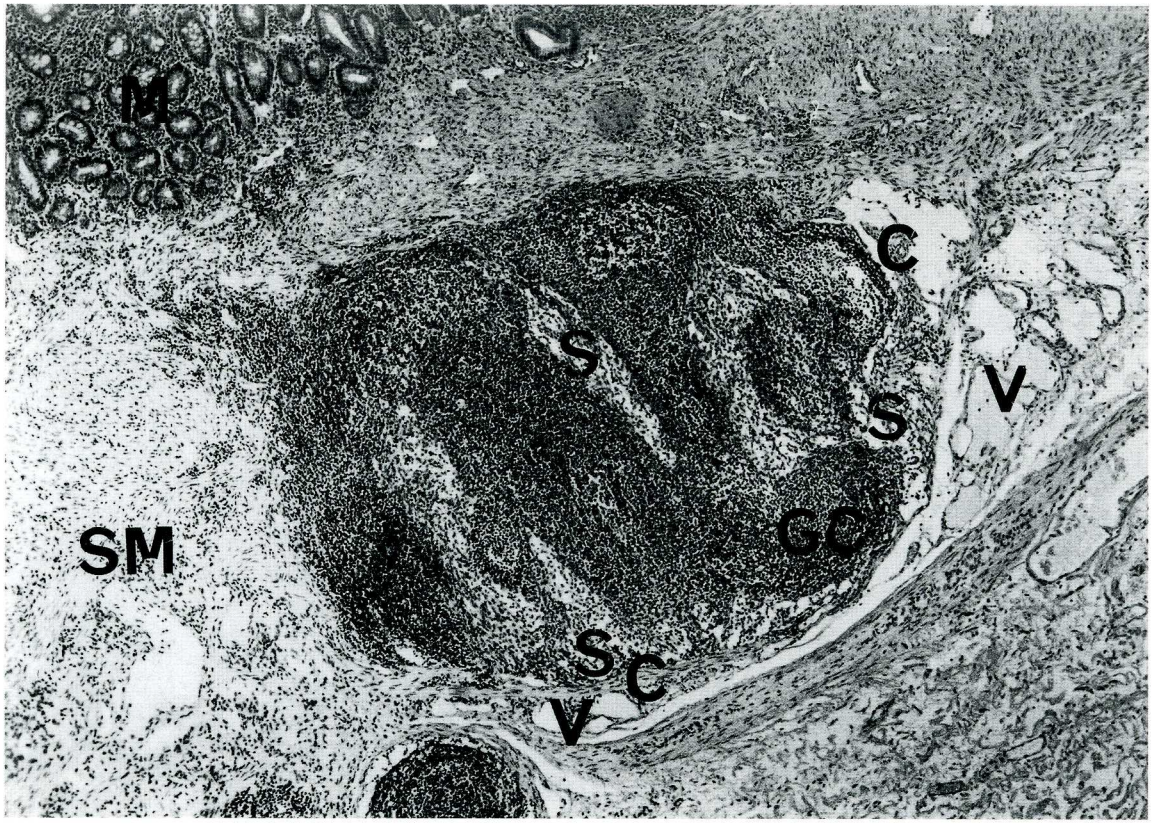

Figure 1. Lymph node in the submucosa (SM) of the small bowel in Crohn's disease. The lymph node capsule (C), surrounding ectatic blood and lymph vessels (V) and a germinal center (GC) are clearly visible. S=marginal and intermediate sinuses. M=mucosa. Giemsa, 35x

of a lymph node. Both parietal and visceral wall cells expressed CD31 and the former also expressed CD34. Both also expressed BMA 120, X11, and von Willebrand's factor. Organization into T- and B-cell areas was demonstrated by staining for these cells. No S100 protein-positive or CD1a-positive immune accessory cells were detected. The lymph follicle had the general appearance of a lymph node, and was separated from the surrounding tissue by a capsule that was rich in collagen fibers and contained sm-actin-positive smooth muscle cells. Staining for the latter also delineated lymph node trabeculae, which ran from the capsule to the center of the lymphoid tissue. Ectatic lymph vessels, which, as expected, were negative for CD34, were found in the vicinity of this submucosal lymph node, and were sometimes seen to communicate with the marginal sinus.

A similar structure was also noted in the submucosa of the large intestine. Like the above, this structure also had a diameter of 0.8 mm. Again, several secondary follicles, which were lined up along the periphery of the node, and both marginal and intermediate sinuses were seen *(Fig. 2)*. It was surrounded by a fibrous capsule containing a few sm-actin-positive smooth muscle fibers. There was some connection between the marginal sinus and the lymphatics in the surrounding connective tissue. The lymphoid tissue was divided into T- and B-cell areas. Typical epithelioid venules were seen in the T-cell

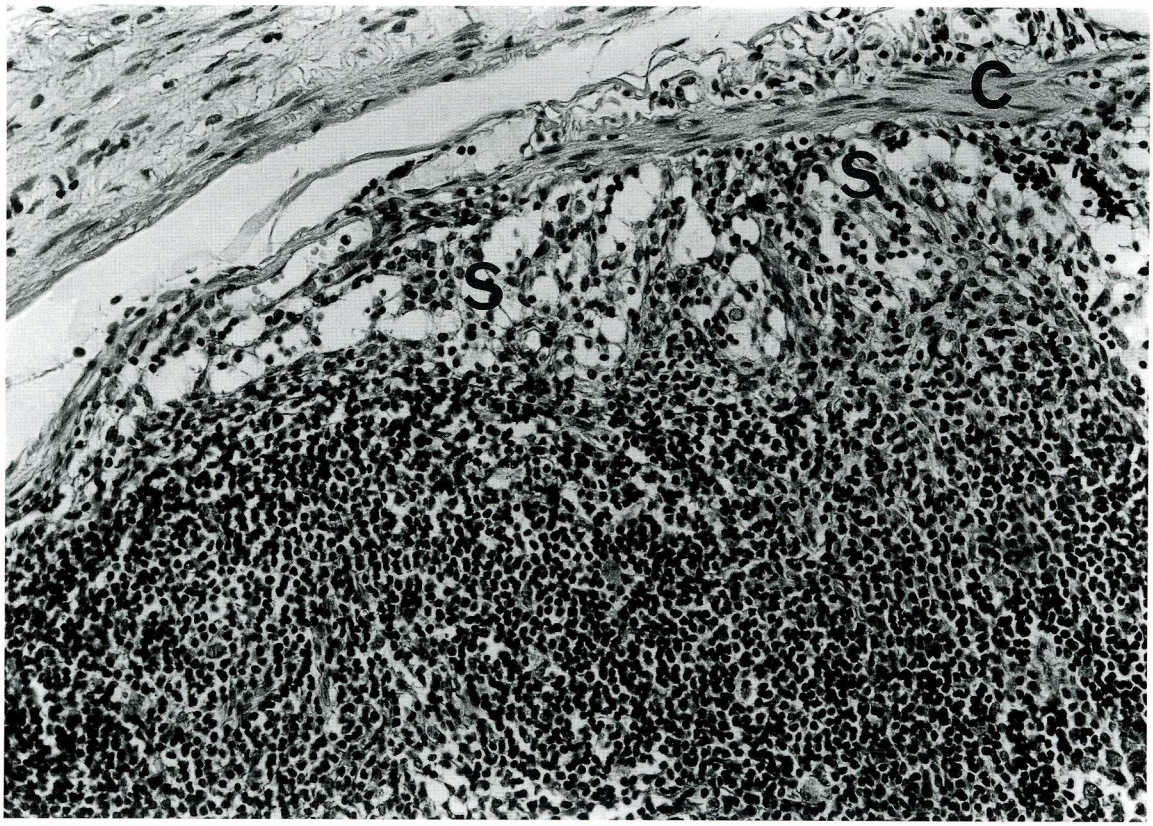

Figure 2. Part of a submucosal lymph node in the ascending colon. The ectatic marginal sinus (S) and capsule (C) are seen. Giemsa, 350x

areas. The germinal centers were composed of B lymphocytes and also contained KiM4P-positive follicular dendritic reticulum cells. A few S100-positive immune accessory cells were found in the T-cell areas. No reactivity for CD1a was seen. The cells of the sinus walls expressed CD31, X11, BMA 120, and, on the parietal side, CD34 *(Fig. 3)*. Small lymphocytes were seen in the lumen. A second follicle of the same size exhibited no sinuses but was also divided into T- and B-cell areas.

For the sake of completeness it is necessary to mention the solitary lymph follicles of the large intestine, in the submucosa or in the form of lymphoglandular complexes, which showed the same morphology as under normal conditions but were present in particularly large numbers. Some contained typical epithelioid venules, and some were found in close association with thin-walled, ectatic blood vessels and lymphatics (CD34-, BMA 120+, von Willebrand's factor+). Semicircular fissures were also often seen in the follicles, but these were considered mainly to be fixation artifacts. A pericolic lymph follicle that consisted mainly of a germinal center, marginal sinus, and capsule was also found *(Fig. 4)*.

In Case 2, in which the clinical diagnosis was ulcerative colitis, an almost identical collection of lymphocytes was seen, again with marginal and intermediate sinuses. It

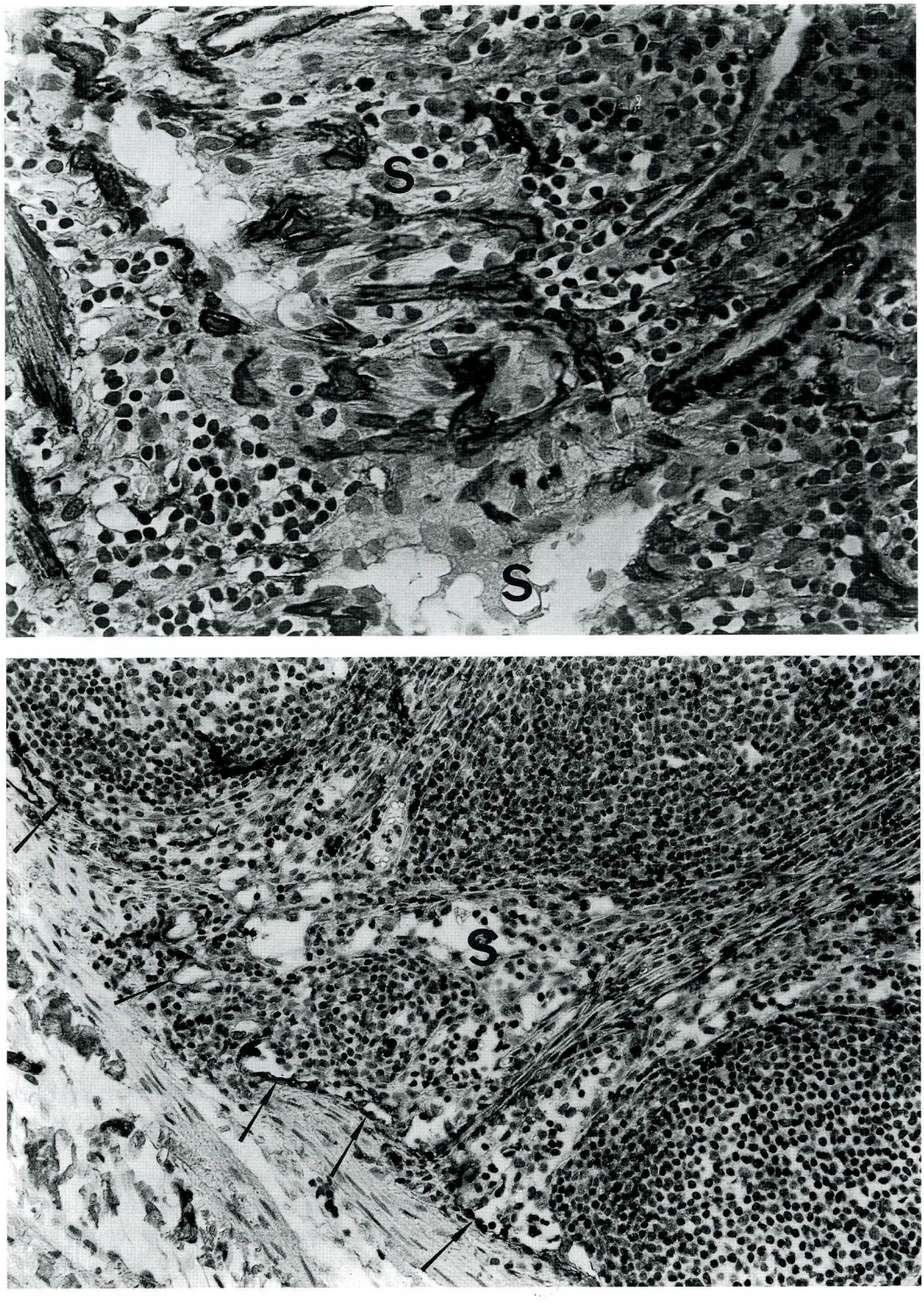

Figure 3. (A) Note intense staining of the sinus wall cells by the antibody BMA 120. (B) The parietal sinus wall cells are weakly positive for CD34 (QBEND10; arrows). S = sinus lumen. A 350x; B 560x

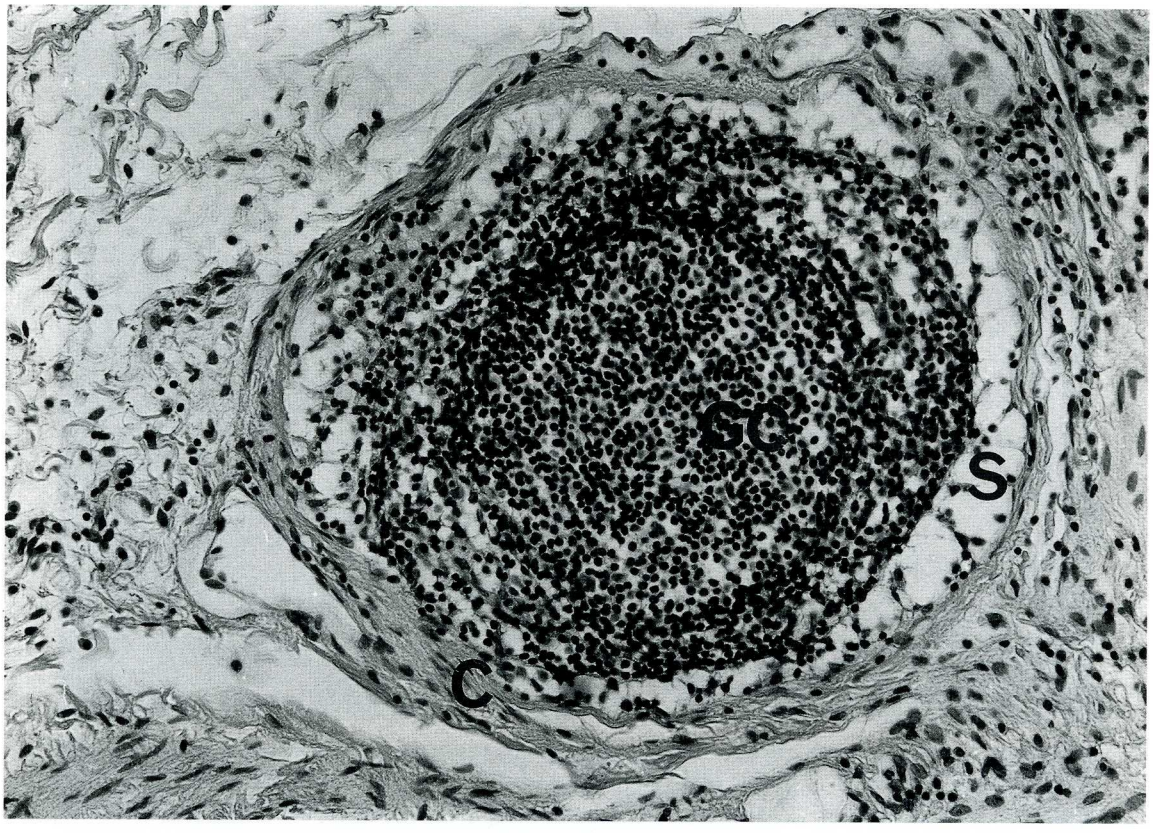

Figure 4. A small collection of lymphocytes with a germinal center (GC), a sinus-like circular lumen (S), and a capsule (C) is seen in the pericolic connective tissue. Giemsa, 140x

was divided into T- and B-cell areas and was found in the upper part of the submucosa of the small intestine. Ectatic lymphatics were seen in the surrounding connective tissue, some of which communicated with the marginal sinus. Further immunohistochemical investigation was not possible.

DISCUSSION

We found structures in the submucosa in two patients with chronic inflammatory bowel disease that exhibited the basic architecture of lymph nodes: marginal and intermediate sinuses, epithelioid venules, a capsule of collagen fibers, afferent lymphatics, and lymphoid tissue divided into T- and B-cell areas. Such structures are not found in this part of the large and small intestine under physiological conditions. Our hypothesis is that these lymphoid aggregates represent newly formed lymph nodes or their equivalent, which have developed from pre-existing lymph follicles (folliculi lymphatici solitarii) or inflammation-induced lymph follicles in the submucosa.

Normally, lymph nodes develop from lymph sacs, which are formed during the second month of embryonic life and are fully developed in 30-mm embryos (6-8). The lymph nodes themselves are fully developed at birth. There have been no reports of lymph node neogenesis after birth, although our investigations have revealed various findings that are suggestive of this phenomenon.

The first findings that prompted us to pursue the possibility of lymph node neogenesis after birth were noted during investigations concerning lymphangioleiomyomatosis and lymphangioma (9,10). The case of lymphangioleiomyomatosis studied exhibited lymphangial lymph follicles that were partly or completely surrounded by lymph vessel endothelial cells, and lymph follicles that lay against the inside of the vessel wall and exhibited sinus-like structures in the area of contact with it. These apparently early lymph nodes exhibited T- and B-cell areas, with the corresponding T- and B-immune accessory cells. The morphological diversity of these intravascular and parietal lymphocyte aggregates, similar structures to which were also noted in lymphangioma, suggested that these had not formed during prenatal life, but had formed subsequently in the lumen of the neoplastic vessels. Pre- or perinatal lymph node neogenesis was particularly unlikely in the patient with lymphangioleiomyomatosis, which had been diagnosed in a 46-year-old woman.

The findings we describe in chronic inflammatory bowel disease support the notion developed in the study of lymphangioma and lymphangioleiomyomatosis that new lymph nodes can develop after birth.

Under physiological conditions, solitary lymphatic follicles of the large bowel (in the submucosa and in the form of lymphoglandular complexes) may exhibit associated blood vessels (e.g., epithelioid venules) or immunohistochemically detectable von Willebrand's factor+ and CD34- (11) lymph capillaries. These follicles are found in greater numbers in chronic inflammatory bowel disease, especially Crohn´s disease. Lymph follicles that exhibit semicircular fissures, probably mainly fixation artifacts, are also typical.

It is generally accepted that lymphatic follicles do not exhibit sinuses under physiological conditions. Our own (unpublished) findings suggest that lymph follicles with sinuses are sometimes also seen in association with tumor-related inflammation, and in various other chronic inflammatory bowel diseases.

In the patients described in this report, the sinuses were lined by endothelial sinus wall cells arranged into parietal and visceral layers. A network of collagen fibers covered by endothelial cells was seen in the lumen. This was true of both the typical lymph nodes and the submucosal lymph nodes described. The same immunophenotype was seen in both: reactivity for BMA 120, X-11, CD31, CD34, and von Willebrand's factor (12,13). We do not know whether lymph node generation in postnatal life is also seen in other disorders or in other areas.

The finding that submucosal lymph nodes develop in chronic inflammatory bowel disease but not in other disorders in which there is lymphoid hyperplasia may have something to do with the fact that lymphangiectasia is often seen in Crohn's disease and ulcerative colitis. It cannot be said whether this in itself leads to more lymph follicles containing a sinus. The removal of substances that are carried in the lymph would definitely be optimized by the presence of sinus-containing lymphoid organs, providing efferent vessels are also present, which can, however, be assumed.

The mechanism of organogenesis of the submucosal lymphoid tissue in chronic inflammatory bowel disease can only be speculated upon. It is interesting to note, however, that Futterer et al (14) showed in experiments on genetically altered mice that lymphotoxin beta receptor-negative mice do not develop colon-associated lymphoid tissue, Peyer's patches, or lymph nodes. This finding suggests that this receptor plays a key role in ontogenesis of secondary lymphoid tissue and could form the basis of an experimental approach to the problem of lymph node neogenesis in inflammatory diseases of the bowel.

REFERENCES

1. Bienenstock, J, MMcDermott, D Befus, et al: A common mucosal immunologic system involving the bronchus, breast and bowel. Adv Exp Med Biol 107 (1978), 53-59.
2. Isaakson, PG, AJ Norton: Mucosa-associated lymphoid tissue (MALT) and the MALT lymphoma concept. In: *Extranodal Lymphomas*. Edinburgh, London, Madrid, Melbourne, New York, Tokyo: Churchill Livingstone, 1994, pp 5-14
3. Gebert, A: The role of M cells in the protection of mucosal membranes. Histochem Cell Biol 108 (1997), 455-470.
4. Hsu, SM, L Raine, H Fanger: Use of avidin-biotin-peroxidase complex (ABC) inimmuno-peroxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29 (1981), 577-580.
5. Rozenbajgier, C, P Ruck, H Jenss, et al: Filiform polyposis: A case report describing clinical, morphological and immunohisto-chemical findings. Clin Invest 70 (1992), 520-528
6. van der Putte, SCJ: The development of the lymphatic system in man. Adv Anat Embryol Cell Biol 51 (1975), 1-60.
7. Töndury, G, S Kubik: Zur Ontogenese des lymphatischen Systems. In: *Handbuch der Allgemeinen Pathologie*, Vol. III/VI, Lymph Vessel System, p., Berlin, Heidelberg, New York: Springer, 1972, 1-38.
8. Wilting J, H Neef, B Christ: Embryonic lymphangiogenesis. Cell Tissue Res. 297 (1999), 1-11.
9. Földi, E, E Kaiserling: Ein Fall von Lymphangiom kombiniert mit einer Lymphangioleiomyomatose. Vasomed 8 (1996), 204-208.
10. Kaiserling, E: Morphologie der Lymphan-giome. Lymph Forsch 2 (1997), 59-67.
11. Kaiserling, E, S Kröber: Lymphatic amyloidosis, a previously unrecognized form of amyloid deposition in generalized amyloidosis. Histopath 24 (1994), 215-221.
12. Wacker, HH, HJ Radzun, V Mielke, et al: Selective recognition of rat follicular dendritic cells (dendritic reticulum cells) by a new monoclonal antibody Ki-M4R in vitro and in vivo. J Leukocyte Biol 41 (1987), 70-77.
13. Kaiserling, E, J-C Xiao, A Adam, et al: Der Lymphknotensinus als Leitungssystem. Morphologische und immunhistochemische Befunde. In: *Lymphologica 1997 Year Book*. Kaiserling E, SM Kröber, P Ruck (Eds.), Bonn: Kagerer Kommunikation, 1998, pp. 37-43.
14. Futterer, A, K Mink, A Luz, et al: The lymphotoxin beta receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity 9 (1998), 59-70.

Professor E. Kaiserling
Institute of Pathology
University of Tübingen
Liebermeisterstrasse 8
72076 Tübingen
Germany
Tel: ++49 (0)7071 2982991
Fax: ++49 (0)7071 292991
e-mail: enkaiser@med.uni-tuebingen.de