Contribution of morphology for the comprehension of mechanisms of fibrosis in inflammatory enterocolitis

K. P. Geboes[1], L. Cabooter[2], K. Geboes[1]

(1) Internal medicine and (2) GI Pathology, KULeuven.

Abstract

Strictures are a common complication of Crohn's disease and an indication for surgery in approximately 50% of patients. Morphologic studies have shown that fibrosis of the submucosa and muscularis propria are common in Crohn's disease, especially in strictures. Immunohistochemical and in situ hybridization studies have demonstrated a marked increase of various subtypes of collagens in Crohn's disease. Collagens type I and III are present in ulcerated areas where they appear around capillaries and in a linear deposition at the junction between the granulation tissue and the necrotic debris. Collagens type IV and V show a prominent perivascular expression, increased deposition in the muscularis propria and increased expression around ganglia. Initiation and maintenance of the connective tissue changes are related with the inflammatory infiltrate. Inflammatory cells can further alter smooth muscle proliferation and migration and promote the formation of myofibroblasts. These alterations together with increased collagen deposition are involved in the complex process of strictures and bowel wall alterations in Crohn's disease. (Acta gastroenterol. belg., **2000**, 63, **371-376**).

Key words : Crohn's disease, collagen, tenascin, fibrosis.

Introduction

In the Vienna Classification of Crohn's disease (CD), a distinction is made between Inflammatory, Stricturing and Perforating disease. Strictures are a classic complication of CD and an indication for surgery in approximately 50% of CD patients. Morphologic studies have described edema and fibrosis in the submucosa and thickening of the muscularis mucosae and propria in CD, especially in strictures. In addition the muscularis can be highly irregular and show segments with complete loss of smooth muscle cells, the presence of an additional muscle layer or layers in the submucosa and areas where there is fusion of the muscularis mucosae and propria. A substantial increase of smooth muscle cells in the submucosa is also shown with staining using antibodies directed against smooth muscle actin (19.3% vs. 7.2% in controls, $p < 0.0001$). Ultrastructural studies confirmed a marked increase in number and size of smooth muscle cells of both muscularis mucosae and propria. Electron microscopy furthermore demonstrated the presence of irregular collagen deposits in the lamina propria underneath the basement membrane, in the submucosa and even in the ganglia of the myenteric plexus. Biochemical studies have confirmed increased collagen deposition in CD compared with ulcerative colitis. Fibrosis and strictures are furthermore associated with alterations of the extracellular matrix (ECM). It has been suggested that the significant increase of Ki67 + (proliferating cells) CD3+ (T cells) in the muscle layers and subserosa compared with the lamina propria in CD may contribute to fibrosis and muscle hyperplasia, both phenomena which are involved in stricture formation (1). This review of literature and experimental data will concentrate on the morphologic features of fibrosis and associated phenomena such as abnormalities of the extracellular matrix.

Collagen in the normal intestine

The predominant connective tissue protein in the intestine is "collagen". It is produced by mesenchymal cells, especially fibroblasts. Fibroblasts, even from a single tissue, are a heterogeneous population. Phenotypic changes can be induced during disease. Several genetically determined types of collagen have been identified, each having a specific distribution and architectural function. Types I, II and III are the ubiquitous interstitial collagens. They are fibrillar collagens and, together with elastin, they form the fibrous backbone of the extracellular matrix. They are linked to the non-fibrillar collagens (type IV), laminin and entactin. Type I collagen is the most abundant collagen in the body. Its function is to give tissue tensile strength. Type III collagen is associated with tissues and organs that require a motile structure. Type IV is a major component of epithelial basement membranes and type V is a pericellular collagen that can be produced by smooth muscle cells. In the normal intestinal wall type I collagen (68%), type III collagen (20%), type V collagen (12%), type IV and type VII collagen are present (2). Type IV collagen is present in basement membranes underneath the epithelium, in the lamina propria stroma and encapsulating smooth muscle cells (Fig. 1). Type V collagen type is found underneath the epithelium, around blood vessels in the lamina propria and submucosa, in the smooth muscle tissue and around submucosal and myenteric ganglia. Due to its pericellular localization it may interact with cell surfaces

Address for correspondence : K. Geboes, Dept. of Pathology, Univ. Hospital K.U. Leuven, Minderbroedersstraat 12, 3000 Leuven.

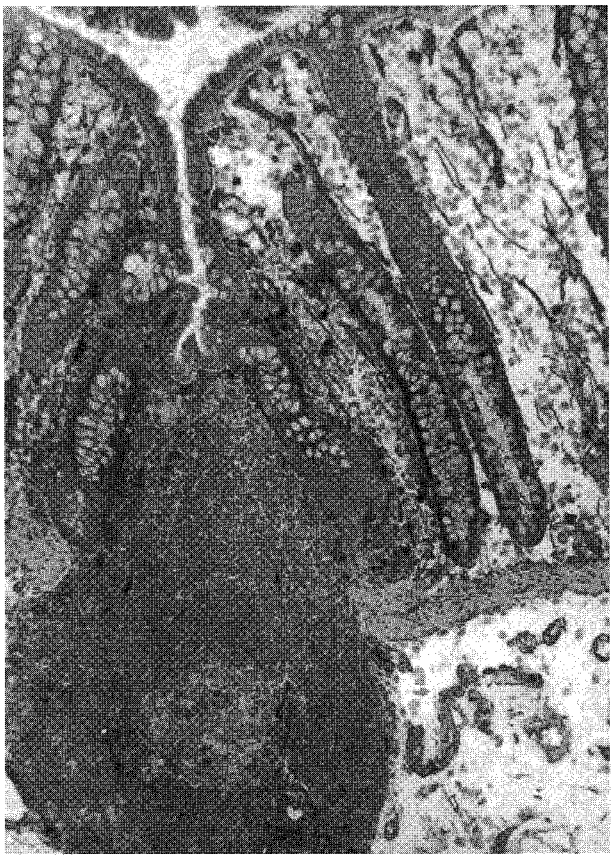

Fig. 1. — Immunohistochemical staining using antibodies directed against collagen type IV, showing a positive staining of the subepithelial basement membrane (magnification × 125).

and modulate cellular activities such as adhesion, differentiation, migration and / or synthesis. In the colon, type VII collagen is confined to the basement membrane of intercryptal surface epithelium in a punctate manner (3). Immunohistochemical studies furthermore show the presence of laminin (alpha 1 chain) in basement membranes of epithelia, muscularis mucosae and blood vessels (3). The lamina propria is a loose connective tissue with collagen types I, III, IV and V. Analysis of the collagen types in human intestinal muscle reveals a predominance of types I and III. Hypertrophy of intestinal muscle secondary to experimentally induced obstruction is associated with an increase in collagen content, particularly in the muscle layer (originating from and maintained by the muscle itself) (4).

Extracellular matrix in the normal intestine

The extracellular matrix (ECM) has an influence upon cell growth, differentiation and migration in normal and pathological conditions. The skeletal network of ECM is a complex structure essentially composed of proteins. Collagens form the fibrillar backbone. The other proteins consist of glycoproteins and complex carbohydrates known as glycosaminoglycans, usually covalently linked to core proteins to form proteoglycans. They are structurally and functionally very heterogeneous and vary from one tissue to another. Fibronectin, collagens, elastin, laminin, thrombospondin and tenascin are among the most important ECM glycoproteins. Proteoglycans like perlecan and fibromodulin are much more abundant. They form the non-fibrillar component of ECM. Tenascin (Tn) is a large glycoprotein of the ECM produced by mesenchymal cells. Three major oligomeric forms of intestinal Tn (320 kDa, 220 kDa and 200 kDa) have been identified in both the human fetus and adult. Tn is involved in cell proliferation, differentiation and migration during embryogenesis and in proliferative processes such as wound healing. In the normal colon mucosa, immunoreactivity for tenascin is confined to the basement membrane of the intercryptal surface epithelium and the muscularis mucosae (while it is absent from the fetal colon) (3) (Fig. 2). It is not normally present around the crypts except for an occasional punctate staining around the upper part of some crypts.

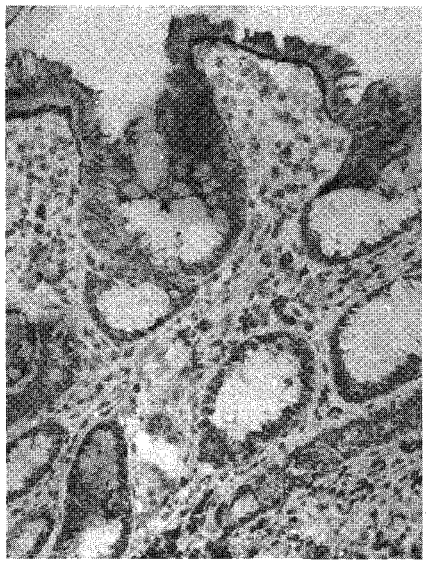

Fig. 2. — Immunohistochemical staining for tenascin in the normal colon showing a positive expression of the basement membrane, in the intercryptal area (magnification × 125).

In the small intestinal mucosa it is present in the basement membrane underneath the cells lining the villi and absent from the basement membrane lining the crypts. A subepithelial distribution has also been described in human fetal tissue by 13 weeks of gestation (5). Tn is further present in the muscularis mucosae, with a more intense immunoreactivity towards the crypts and around capillaries. In the submucosa Tn is found around the small ganglia and some larger capillaries. Tn immunoreactivity is intense also in the muscularis propria and in the basement membrane around the ganglia of the

myenteric plexus. A relation between Tn and smooth muscle is already observed in human fetal small intestine. By 9 weeks, villus rudiments are formed but TN and alpha-smooth muscle expression remain restricted to the muscularis propria. It has been suggested that Tn could have a role in the differentiation of intestinal contractile cells (5). The ECM proteins play a key role in cell movement through the various tissues. They can serve as the functional storage of cytokines, but can also restrict or modulate cytokine activities at target sites. The ECM is also involved in interactions with T cells through the presence of adhesion mediating receptors such as the very late antigen integrin VLA-4 (6).

Inflammation and connective tissue

Inflammatory cells orchestrate "connective tissue degradation" either directly by secretion of elevated levels of "matrix metalloproteinases (MMPs)", or indirectly by proinflammatory cytokines. These cytokines have been shown to induce MMP gene expression in the resident cell population. MMPs are a family of zinc containing enzymes with distinct specificities for the individual components of the extracellular matrix. The collagenases are the most specific of the MMPs, cleaving the triple helix of types I, II, and III collagens at a single site. This action causes the molecule to unwind and become susceptible to further proteolytic attack by gelatinases. The gelatinases are a group of enzymes which cleave denatured collagens (gelatins), collagen types IV, V, VII, X, elastin and fibronectin. Gelatinase A can be constitutively present. It is produced by macrophages. Gelatinase B is produced by a variety of cells including neutrophils, eosinophils and macrophages. The stromelysins (MMPs 3 and 10) have a much broader substrate specificity and degrade a range of extracellular matrix components including proteoglycans, the non-helical regions of type IV collagen, laminin, and fibronectin.

Lymphocytes and other cells interact with ECM proteins. In UC patients, peripheral blood T cells show greater T cell adhesion to fibronectin and collagen IV. In general lamina propria lymphocytes can adhere and be costimulated by ECM proteins. Generally the changes are stronger in acutely ill patients. Remodelling of ECM components is also present in CD.

Collagen in Crohn's disease (and UC)

Immunohistologic and in situ hybridization studies have demonstrated a marked increase of types I, III and V collagens and RNA transcripts in muscularis mucosae and propria in CD. The expression of mRNA for types I, III and V correlates with the intensity of the inflammatory reaction. Large quantities of type V collagen have especially been detected in stenotic lesions from patients with CD (7,8). We have studied the presence of collagen in tissue samples from the ileum and colon obtained from 5 patients with established CD (3 male, 2 female patients, age range : 25-48 years), one patient with UC (35 yrs), one patient with drug related colitis (46 yrs) and two patients operated for multiple polyps (59 yrs) using immunohistochemistry and a panel of monoclonal antibodies directed against various types of collagen. CD patients were or had been treated with 5- ASA, corticosteroids, azathioprine (n = 1) and infliximab (n = 1). Indications for operation were strictures (n = 3) and persistent inflammation (n = 2). This study confirmed the presence of an increased deposition of different collagen subtypes in the different layers of the bowel wall in CD when compared with normal controls. A similar increase was not observed in NSAIDS related colitis. In CD, a band-like deposit of collagens I and III was observed in ulcerated areas, at the junction between the necrotic material and underlying granulation tissue at the luminal border (Fig. 3). Type IV and V collagens showed a mainly perivascular expression (Fig. 4). In ulcerated and non-ulcerated areas, type I and type V collagen are clearly increased in the muscularis. Type I is mainly found in the septa between smooth muscle cell bundles and type V mainly around individual smooth muscle cells. In the samples from the patient treated with infliximab and azathioprine, the deposition of collagen V in the muscularis propria was less pronounced however. *Type V collagen* (produced by smooth muscle cells) is further also increased in the subepithelial collagen table, in the perivascular zone especially in the submucosa and around the ganglia of the submucosal and myenteric plexuses. Collagen type V deposits can even be found within the ganglia of the myenteric plexus (Fig. 5).

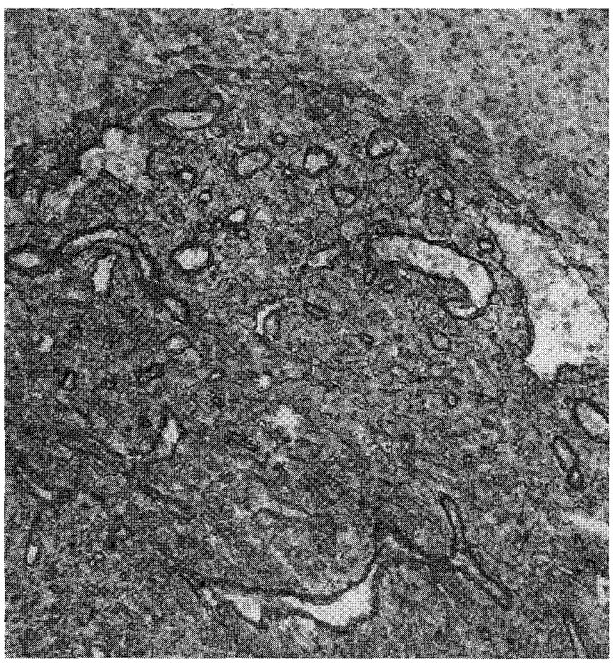

Fig. 3. — Immunohistochemical staining for collagen III showing deposition around the capillaries of the granulation tissue and in a linear band just underneath the necrotic debris of the ulcer base (magnification × 125).

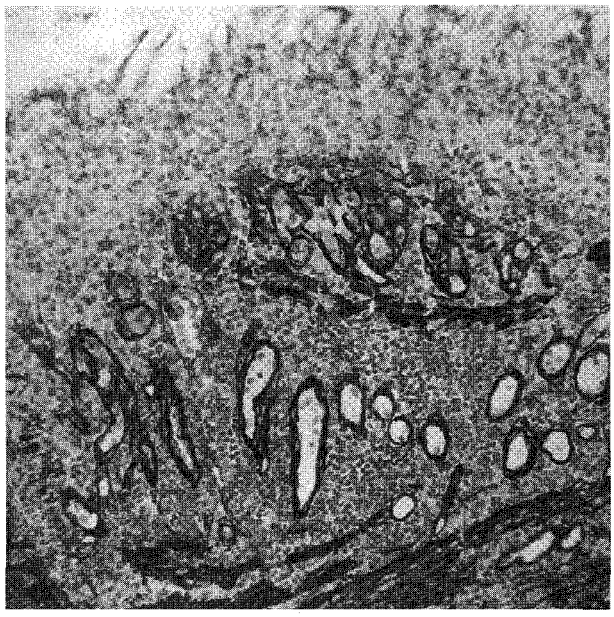

Fig. 4. — Serial section of figure 3 : immunohistochemical staining for collagen IV showing deposition around the capillaries of the granulation tissue and in the smooth muscle tissue in the depth of the ulcer (magnification × 125).

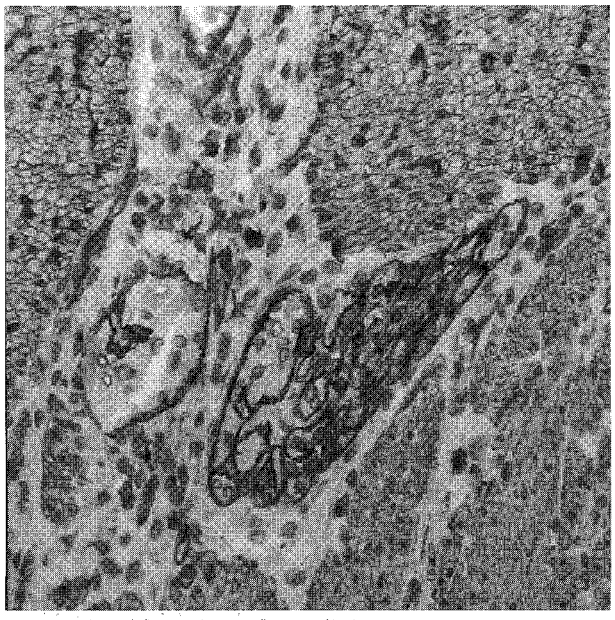

Fig. 5. — Immunohistochemical staining for collagen V showing depostion around individual smooth muscle cells of the muscularis propria and in and around ganglia of the myenteric plexus (magnification × 325).

Type III collagen (fibroblasts) is abundantly present in CD in both ulcerative and nonulcerative lesions ; it is increased in granulation tissue where it lines newly formed vessels and forms a discontinuous, almost band-like structure at the luminal front of the granulation tissue and the junction with the necrotic debris, pro viding together with collagen type I, a surface for epithelial cell migration and recovery of the ulcerated surface, while collagen V is found more deeper in the granulation tissue as irregular short bundles.

The expression of type III within the muscle layer is less significant. Fibroblasts isolated from stenotic Crohn's lesions and stimulated with transforming growth factor beta produce increased quantities of type III collagen.

In a series of 20 consecutive ileal resections for proven Crohn's disease and 10 control specimens, no difference was found in submucosal type III collagen distribution compared to control values (14.5% vs. 14.2%). There was however a significant difference between the disease phenotypes :, 19.8% stenosed vs. 12.7% perforated and 11.7% ulcerated ($p < 0.005$) (9). In the "perforating disease" phenotype a substantial increase of smooth muscle cells in the submucosa has been described (22.9% vs 17.9% stenosed and 15.8% ulcerated). In the muscularis propria the expression of pericellular type IV collagen is also increased.

Myofibroblasts might be involved in the formation of pseudopyloric gland metaplasia because they have been shown capable of inducing "intestinalization of undifferentiated gastric cells" in the duodenal mucosa (10).

Tenascin in Crohn's disease

In samples from diseased tissue obtained from surgical specimens from patients with CD immunoreactivity for Tn is increased in the colon and small intestine in active and inactive disease, and occasionally in normal appearing tissue. The increase is observed in the subepithelial area which appears thickened and in the mucosal stroma where Tn appears as coarse fibers (Fig. 6). Tn immunoreactivity appears also around some crypts and capillaries. An increase of Tn immunoreactivity is also observed around blood vessels, in the muscularis mucosae and in the upper part of the submucosa where it appears again as coarse fibers lying in an irregular fashion.

MMPs in Crohn's disease (and UC)

No increased positive staining for collagenase has been reported for CD, neither in inflamed nor in normal appearing tissue. In contrast, in sections from "inflamed" tissue from patients with CD ($n = 6$), large numbers of polymorphonuclear cells and monocytes staining positive for gelatinase B can be observed, throughout the intestinal wall. The greatest number is observed in the larnina propria. Stromelysin was detected on the extracellular matrix in areas of smooth muscle proliferation in CD and in the lamina propria directly below the basement membrane in regions of mucosal damage in both CD and UC (9).

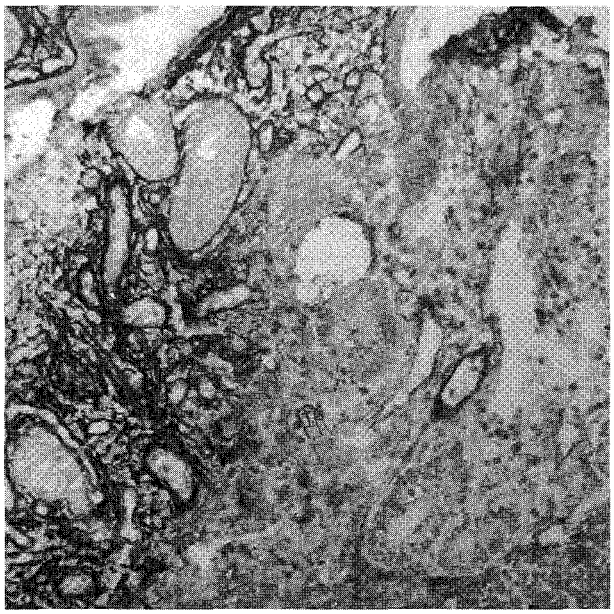

Fig. 6. — Immunohistochemical staining for Tenascin showing increased deposition in the ulcer area and in a subepithelial position at the edge of the ulcer (magnification × 125).

The inflammatory reaction and fibrosis

The inflammatory infiltrate of active CD is responsible for initiating and maintaining a series of connective tissue changes : the intensity of the infiltrate correlates with the expression of mRNAs for several collagen types ; the levels of pro-inflammatory cytokines such as TNFα and IL-10 are increased. In CD, the inflammatory reaction is classically transmural. Hence, connective tissue changes will not be limited to the mucosa but appear also in the deeper layers. The main source of TNFα in the mucosa are lymphocytes and monocytes. Similar cells can also be found in the submucosa and muscularis propria. TNFα levels are much more increased in diseased tissue from patients with CD compared with UC. TNFα stimulates fibroblast proliferation and collagen synthesis. Macrophages produce also other fibrogenic cytokines including IL- lalfa and beta, PDGF and TGFβ.

PDGF is a key mitogen for many fibroblasts and other mesenchymal cell populations. It is released by platelets involved in coagulation and vascular alterations are common in Crohn's disease either as a primary event according to Wakefield's hypothesis or as a secondary phenomenon. Collagen V deposition is however not particularly increased in the immediate vicinity of granulomas or isolated giant cells although collagen I deposition may be increased. PDGF is a potent chemoattractant and mitogen for fibroblasts and smooth muscle cells and a stimulator of collagen synthesis by fibroblasts. TGFβ (also produced by T lymphocytes) promotes collagen production by fibroblasts and smooth muscle cells in the small intestine. TGFβ-3 unlike TGF,β-1 & 2 promotes wound healing without fibrosis. In the normal intestine it is the predominant bioactive isoform released by myofibroblasts (11). The exact mechanisms responsible for the disease specific effects of cytokines and other factors on collagen synthesis are not understood. One level of control may be achieved through differential expression of TGFβ-receptor isoforms (12). In CD there is a marked overexpression of TGFβ-1, TGFβ-3 and TGFβ-receptors II (present in 94% of tissue samples). TGFβ-receptors are expressed in epithelial cells in the upper crypts of the normal small intestine but in CD a disordered expression pattern of receptors I and II is seen (11). There is a relative reduction in the release of TGFβ-3 bioactivity by CD myofibroblasts which may be important for the formation of fibrosis (13). Inflammatory cells can further alter smooth muscle cell proliferation and migration and the activity of the matrix degrading enzymes. Neutrophils (gelatinase B positive) can affect type IV collagen in subepithelial and perivascular basement membranes. By doing so they can increase permeability.

Conclusion

Stricture formation is a common complication of CD. Morphologic studies show that this is due to a complex process which involves increased collagen production and fibrosis but also alterations of extracellular matrix components and of smooth muscle cells. The different abnormalities are related to connective tissue degradation, induced by the inflammation and subsequent healing and remodelling of tissue. The extent of the fibrosis which can involve all layers of the bowel wall, is explained by the transmural nature of the inflammation in CD.

References

1. FELL J.M., WALKER-SMITH J.A., SPENCER J., MACDONALD T.T. The distribution of dividing T cells throughout the intestinal wall in inflammatory bowel disease (IBD). *Clin. Exp. Immunol.*, 1996, **104** : 280-285.
2. SEKI T., NAITO I., OOHASHI T., SADO Y., NIONOMIYA Y. Differential expression of type IV collagen isoforms, alpha5(IV) and alpha6(IV) chains in basement membranes surrounding smooth muscle cells. *Histochem. Cell. Biol.*, 1998, **110** : 359-366.
2. GABELLA G., YAMEY A. Synthesis of collagen by smooth muscle in the hypertrophic intestine. *Quarterly Journal of Experimental Physiology*, 1977, **62** : 257-264.
3. LOHI J., LEIVO L., TANI T., KIVILUOTO T., KIVILAAKSO E., BURGESON R.E., VIRTANEN I. Laminins, tenascin and type VII collagen in colorectal mucosa. *Histochem. J.*, 1996, **28** : 431-440.
4. SCHREIBER S., NIKOLAUS S., RAEDLER A. Stenoses de la maladie de Crohn : mecanisme et traitement. *In* : LEMANN M., MODIGLIANI R. (eds). *Maladies inflammatoires de l'intestin.* Doin, Paris, 1998, 191-195.
5. BEAULIEU J.F., JUTRAS S., DURAND J., VACHON P.H., PERREAULT N. Relationship between tenascin and alpha- smooth muscle actin expression in the developing human small intestinal mucosa. *Anat. Embryol. Berl.*, 1993, **188** : 149-58.
6. POZAROWSKI P, GORSKI A. The role of extracellular matrix proteins in IBD. *In* : EMMRICH J., LIEBE S., STANGE E. F. (eds). *Innovative concepts in Inflammatory bowel disease.* Dordrecht, Kluwer Academic Publishers, 1999, 129-135.

7. GRAHAM M.F. Stricture formation in Crohn's disease : Wound healing in the intestine. *In* : TARGAN S., SHANAHAN F. (eds). Inflammatory Bowel Disease : from bench to bedside. Williams & Wilkins, Baltimore, 1994, 174-179.
8. BORLEY N.R., MORTENSEN N.J., JEWELL D.P., WARREN B.F. Connective tissue changes in Crohn's disease : relationship to disease phenotypes and ulcer associated cell lineage. *Gastroenterology*, 1999, **116** : G1664.
9. BAILEY C.J., HEMBRY R.M., ALEXANDER A., IRVING M.H., GRANT M.E., SHUTTLEWORTH C.A. Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collagenase in Crohn's disease and normal intestine. *J. Clin. Pathol.*, 1994, **47** : 113-116.
10. STALLMACH A. Role of fibroblasts in inflammatory bowel disease. *In* : EMMRICH J., LIEBE S., STANGE E.F. (eds). *Innovative concepts in Inflammatory bowel disease*. Dordrecht, Kluwer Academic Publishers, 1999, 120-128.
11. MC KAIG B.C., HAWKEY J.C., MAHIDA Y.R. Differential expression of transforming growth factor β (TGFβ) isoforms by normal, ulcerative colitis (UC) & Crohn's disease (CD) intestinal myofibroblasts. *Gastroenterology*, 1999, **116** : G3330, A768.
12. FRIESS H., DIMOLA F.F., EGGER B., SCHEUREN A., KLEEFF J., ZIMMERMANN A., BUCHLER M.W. Transforming growth factor-betas steuern die Pathogenese des Morbus Crohn. *Langenbecks Arch. Chir. Suppl. Kongressbd.*, 1998, **115** : 994- 997.
13. McKaig B.C., Hawkey J.C., Mahida Y.R. Differential expression of transforming growth factor β (TGFβ) isoforms by normal, ulcerative colitis (UC) & Crohn's disease (CD) intestinal myofibroblasts. *Gastroenterology*, 1999, **116**, G3330, A768.