Can we influence fibrosis in Crohn's disease ?

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Abstract

Despite recent advances in the therapy of active Crohn's disease (CD) fibrostenosis remains a challenging complication of the disease. Transmural inflammation of CD is associated with phenotypic switch of the mesenchymal cells resulting in proliferation and collagen deposition. Both resident myofibroblasts and smooth muscle are candidate progenitor cells of the fibrogenic cells in CD stenoses. The principal growth factors involved in intestinal fibrosis have not been identified although TGF-β1 and 2, PDGF and IL-1 may be involved. Research aimed at elucidating the basic mechanisms underlying fibrosis in the gut has been hindered by the lack of an adequate animal model. Recently, however, new rodent models of chronic inflammation with distinct fibrosis have been described. Cell culture research has provided more information about possible pathways that may limit uncontrolled mesenchymal proliferation in the inflamed intestinal wall. The modulator role of neurotransmitters such as VIP, nitric oxide and prostaglandines is an important target for therapeutic intervention. Interfering with the phenotypic switch of mesenchymal cells may offer new therapeutic perspectives in the prevention of fibrostenosis. Further in vitro and animal studies as well as restenosis prevention studies are needed to develop pharmacological tools in the prevention of Crohn's disease fibrostenosis. (Acta gastroenterol. belg., **2001**, 64, **193-196**).

Introduction

In the last decade several powerful therapeutic tools have been developed aimed at modifying mucosal inflammation in inflammatory bowel disease (IBD). This has resulted in improved outcome and quality of life for patients with both Crohn's disease and ulcerative colitis. However, in the case of Crohn's disease which affects the entire bowel wall, the proliferation of mesenchymal cells in the lamina propria and the muscalaris propria resulting in intestinal strictures and obstruction still represents a challenging clinical problem. Mucosal healing does not always prevent fibrosis nor does it exclude thickening of the deeper layers. Moreover, as in other organs the very process of healing may be fibrogenic per se and may affect the functional integrity of the bowel. Inflammatory cytokines may exert opposite effects in the mucosa and in the mesenchymal layers. Transforming growth factor beta (TGFβ), for instance, inhibits mucosal inflammation but at the same time this cytokine induces mesenchymal cell proliferation in the intestinal wall.

Wound healing in the intestine induces phenotypic changes of mésenchymal cells resulting in proliferation of fibroblasts and myocytes and in the deposition of collagen. (for a review see refer. 1). In contrast to the body of knowledge that has been recently developed concerning mediators of mucosal inflammation in IBD much less is known about growth and differentiation factors controlling mesenchymal cell homeostasis in the deeper layers of the gut wall. However, evidence is emerging to support a role for transforming-growth factor-beta (TGFβ), insulin like growth factor-1 (IGF-1), platelet derived growth factor (PDGF), tumor necrosis factor (TNF) and interleukin-1 (IL-1) as growth factors for intestinal mesenchymal cells (2-4). Nevertheless, the reason why a subgroup of CD patients have a "fibrostenotic" disease phenotype is not known. The fact that these patients maintain their tendency to develop stenoses in the absence of frank mucosal inflammation in post-operative recurrences only extends the enigma.

Because of the importance of intestinal fibrosis in the clinical presentation of Crohn's disease, therapeutic strategies aimed at limiting mesenchymal cell proliferation in intestinal tissue repair may prevent strictures and thus the need for repeated surgical interventions and the progression to short bowel syndrome.

Pathogenesis of intestinal strictures in Crohn's disease

1. *Cellular mechanisms of healing and fibrogenesis*

The cellular mechanisms of fibrosis in the intestinal wall have not been investigated to the same extent as in other organs such as the liver or the vascular system and many questions remain unanswered. As in other organs, acute injury to the intestine triggers healing mechanisms that restore the original architecture of the bowel wall. This self-limiting process requires equilibrium between pro- and anti-inflammatory signaling. An uncontrolled proliferation of mesenchymal cells and excessive production of extra-cellular matrix (ECM) proteins will inevitably lead to fibrostenosis as occurs in the chronic inflammation of CD. The cellular origin of inflammatory mediators that provoke fibrosis has not been elucidated. Since fibrostenosis is observed in transmural inflammatory disorders it is tempting to hypothesize that infiltrating immune cells such as lymphocytes, neutrophiles and macrophages secrete cytokines and growth factors in the mesenchymal layers. Nevertheless, it is known

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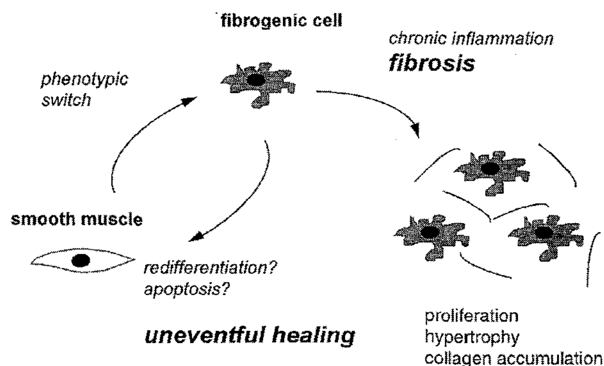

Fig. 1. — Hypothetical model of fibrogenesis in chronic intestinal inflammation.

that smooth muscle cells are a cellular source of cytokines such as IL-1, IL-6 and TNF that might act in an autocrine manner to promote fibrosis. Studies investigating wound healing in skin regeneration have shown that TGF-β is the key mediator of myofibroblast-induced wound contraction. Of the TGF-β isoforms TGF-β1 and to a lesser extent TGF-β2 have been implicated in pathogenic fibrosis in the skin, lungs and liver. TGF-β3, on the contrary is considered to inhibit fibrosis by down-regulating the two other isoforms. Therefore, a disturbed balance of the TGF-β isoforms may contribute to a fibrogenic condition in CD. Both mesenchymal cell hyperplasia and hypertrophy and collagen deposition are involved in fibrostenosis. It is unclear whether inflammatory cytokines promote both mitogenesis and ECM deposition. In vitro experiments, for instance, have shown that IL-1 is mitogenic for intestinal smooth muscle cells, but at the same time inhibits collagen secretion by these cells (4). Recently, a role for mast cells was suggested in the regulation of mesenchymal cell growth in fibrotic strictures of Crohn's disease (5).

2. *Phenotype of mesenchymal cells in fibrotic segments*

The main mesenchymal cell types in the intestinal wall are smooth muscle cells (myocytes), interstitial cells of Cajal and fibroblasts. All these cell types are found in substantial density and have been implicated in intestinal fibrogenesis. In contrast, in the vascular wall there are few if any fibroblasts. Stellate cells in the liver and fibroblasts in the skin are the only cell type with fibrogenic potential. This unique complexity of the intestinal mesenchymal layers hinders the clear phenotypic characterization of the fibrogenic cell in the gut. The different mesenchymal cell types can be discriminated based on the relative presence of filaments such as actin, vimetin and desmin. Furthermore, myocytes and fibroblasts secrete different collagen types, which may help to characterize them. Fully differentiated smooth muscle cells are typically a-smooth muscle actin (A) and desmin (D) positive (A+/D+). Fibroblasts are invariably vimentin positive (V+), while myofibroblasts are V+/A+. In fibrotic strictures are generally a-smooth muscle actin positive, but may loose desmin expression and vimentin filaments may appear (1). Therefore, there is an on-going controversy whether the fibrogenic cell type originates from the smooth muscle or the fibroblast lineage. The identification of collagen isoform secretion in fibrotic strictures has been used in an attempt to settle the argument. In normal intestine there is a preponderance of collagen type I (70%). Graham first reported an abundance of type III collagen in fibrotic structures, which would imply a smooth muscle source of the extracellular matrix. However, recent work (6) has shown that V+/A+ and V+/A- fibroblasts secreting type I are also clearly present in strictures. Whether these contradictory findings can be attributed to differences in time course of stricture formation at which the tissue samples have been obtained is still open to debate.

3. *Phenotypic switch : the plasticity of mesenchymal layers*

Injury to most mesenchymal layers in the human body results in phenotypic alterations in the resident cell population. This phenomenon has been best characterized in atherosclerosis. Mature vascular smooth muscle with contractile properties de-differentiate into a proliferative and secretory cell type with capabilities of producing cytokines and extracellular matrix (7). The same switch may occur in the bowel wall transforming smooth muscle in myoblasts or fibroblasts in myofibroblasts. Discrimination based on structural characteristics such as cytoplasmatic filaments (actin, vimentin...) may prove virtually impossible. Alternatively, a dormant mesenchymal stem cell might be forced to proliferate in inflammatory conditions, and overpopulate the resident smooth muscle and fibroblasts. However, such stem cells have not be found yet. Figure 1 represents a hypothetical model of fibrogenesis based on phenotypical switch and proliferation of a fibrogenic cell. In most instances phenotypic switches of mesenchymal cells in pathological conditions are reversible and inducing re-differentiation of fibrogenic cells represents a key therapeutic target in the prevention of intestinal fibrosis. Interestingly smooth muscle cells primary culture also transform into secretory and proliferating cells and to a certain extent re-differentiation is observed in myocyte cultures. Therefore, they provide a potential model to study the molecular mechanisms driving phenotypic plasticity.

4. *Extracellular matrix deposition*

The relative importance of collagen deposition versus mesenchymal cell proliferation in intestinal strictures has not been established, but extracellular matrix expansion is essential to fibrogenesis since it provides anchoring for contractile cells. In fibrostenotic segments mRNA for collagen types I, III, IV and V are found (1). Of these collagen III and V may be of particular importance and specificity although the cellular source of the

collagen overproduction has not been found. As in other diseases involving extracellular matrix deposition the role of collagen degrading enzymes has been evaluated for Crohn's disease strictures. Increased expression of metalloproteinases was found in both CD and UC and therefore a deficiency of these enzymes can not explain the fibrotic nature of CD (8). It has to be said that these observations were made in mucosal biopsies and further studies in resection specimens are warranted.

Experimental models of fibrogenesis

1. *Animal models*

Most experimental models of human IBD are acute models aimed at studying mucosal repair that may not be a true reflection of slowly developing fibrostenosis. In acute TNBS colitis for instance a substantial thickening of mesenchymal layers is observed, but this coincides with major architectural damage to the muscularis and extensive cellular infiltrates throughout the colonic wall. Data on fibrogenesis during chronic inflammation are much more limited. Recently, two rodent models have been introduced that have promising features for the study of fibrotic phenomena. In a first model submaximal doses of TNBS/ethanol were administered by enema to CD-1 mice at regular intervals for several weeks. A marked submucosal thickening was observed in these animals with phenotypic changes in the fibroblasts and collagen deposition (9). In another rodent model bacterial cell wall polymers, peptidoglycan-polysacharides (PG-PS) were injected intramurally in the ileum of rats. The injection resulted in chronic granulomatous enterocolitis 17 to 26 days after treatment with an increase in collagen type I and TGF-β1 expression in myofibroblasts at the periphery of granulomas (10). These two models may provide interesting working tools to unravel the early stages of fibrogenesis.

2. *Cell culture systems*

a. Smooth muscle and fibroblast cultures

Primary intestinal smooth muscle cultures obstained from human or animal muscularis segments have been extensively characterized. Phenotypic switch is generally observed in proliferating cells in culture and some plasticity towards re-differentiation can be found when cells become confluent and start to grow in layers. These cells proliferate when stimulated by fetal serum, TNF or IL-1 and can express major histocompatibility complex-II (MHC-II) and inflammatory cytokines. Myofibroblasts obtained from human and animal intestinal submucosa and serosa have also been successfully cultured using conventional media (11-14). Fibroblasts from CD serosa were found to have an enhanced capacity to contract in a fibroblast-populated collagen lattice culture system as compared to cells from non-IBD bowel. Earlier work has also shown that fibroblasts cultured from CD strictures display an increased synthesis of collagen type III. The increased type III collagen deposition in these strictures may therefore originate from fibroblasts as well as from smooth muscle. Although observations in cell culture have to be carefully interpreted with regard to the situation in the native bowel wall, the mesenchymal cells in culture may be the ideal model to study phenotypic plasticity and offer the advantage of accessibility for molecular studies.

b. Neuron-smooth muscle co-cultures

Neuron-smooth muscle or neuron fibroblast interactions may have a role in the control of mesenchymal cell role. Structural alterations in enteric nerve plexus is a distinctive feature of Crohn's disease (15) and local inflammation of the myenteric plexus in macroscopically normal resection margins of CD affected ileum is associated with early recurrence (D'Haens et al. preliminary data). In skin regeneration it has been suggested that neuronal outgrowth fosters the repair of mesenchymal layers through the secretion of neuropeptides (16). Recent work in nerve-smooth muscle co-cultures has shown that intrinsic nerves may inhibit proliferation and induce differentiation of smooth muscle (17).

Therapeutic perspectives

Obviously controlling disease activity and preventing post-operative recurrence will have a major role in the prevention of stricture formation. Nevertheless, rapid mucosal repair by powerful drugs such as anti-TNF agents may not be beneficial for the fibrosis in deeper mesenchymal layers and therefore unraveling the very mechanisms of fibrogenesis may open new therapeutic perspectives for the future. Alternatively, endoscopic dilation offers an effective treatment for short-segment strictures but the technique is hindered by a high relapse rate. Attempts to prevent re-stenosis with intramural or topical corticosteroids have been performed in clinical practice but no controlled studies are available at present. Since stenosis in postoperative recurrence of CD is a common feature even in the absence of clinical signs of active disease, selective targeting of the mesenchymal layers is an important topic for further research.

Most of the preliminary information on possible targets for therapy comes from cell culture work and from data in cardiovascular research. In intestinal smooth muscle cells it has been shown that blocking Ca2+-entry with verapamil inhibits cytokine-induced proliferation (18). We have shown that an increase in cyclic-AMP induced by neuropeptides or by phosphodiesterase inhibitors significantly inhibits colon smooth muscle proliferation (19) and these data have been confirmed in cultured intestinal myofibroblasts (13) and in bronchial smooth muscle (20). Similar observations of a growth-modulatory role for neurotransmitters have been made in the vascular wall. In atherosclerosis research nitric oxide gene transfer and prostaglandin treatment are currently being investigated in the prevention of intima and

smooth muscle hyperplasia (21,22). It may be time to export these observations to animal models and test their relevance in experimental enterocolitis and eventually from the bench to the clinic.

References

1. GRAHAM M.F. Pathogenesis of intestinal strictures in Crohn's disease. *Inflamm. Bowel Dis.*, 1995, **1** : 220-227.
2. LUND P.K. The alpha-smooth muscle promoter : a useful tool to analyse autocrine and paracrine roles of mesenchymal cells in normal and diseased bowel. *Gut*, 1998, **42** : 320-322.
3. PERR H.A., GRAHAM M.F., DIEGELMANN R.F., DOWNS R.W. Cyclic nucleotides regulate collagen production by human smooth muscle cells. *Gastroenterology*, 1989, **96** : 1521-1528.
4. GRAHAM M.F., WILLEY A., ADAMS J., YAGER D., DIEGELMANN R.F. IL-1 down-regulates collagen and augments collagenase expression in intestinal smooth muscle cells. *Gastroenterology*, 1996, **110** : 344-350.
5. GELBMANN C.M., MESTERMANN S., GROSS V., KOELLINGER M., SCHOELMERICH J., FALK W. Strictures in Crohn's disease are characterised by an accumulation of mast cells colocalised with laminin but not with fibronectin or vitronectin. *Gut*, 1999, **45** : 210-217.
6. PUCILOWSKA J.B., WILLIAMS K.L., LUND P.K. Fibrosis in inflammatory bowel disease : cellular mediators and animal models. *Am. J. Physiol.*, 2000, **279** : G653-659.
7. ROSS R. Atherosclerosis – An inflammatory disease. *N. Engl. J. Med.*, 1999, **340** : 115-126.
8. SCHUPPAN D., HAHN E.G. MMPs in the gut : inflammation hits the matrix. *Gut*, 2000, **47** : 12-14.
9. LAWRANCE I.C., WEST G., WILLIS J., FIOCCI C., CHAKRAVARTI S. A novel murine method of chronic inflammation induced intestinal fibrosis. *Gastroenterology*, 2000, **118** : A575.
10. VAN TOL E.A., HOLT L., FENG L.F. *et al.* Bacterial cell wall polymers promote intestinal fibrosis by direct stimulation of myofibroblasts. *Am. J. Physiol.*, 1999, **277** : G245-G255.
11. STALLMACH A., SCHUPPAN D., RIESE H.H., MATTHES H., RIEKEN E.O. Increased collagen type III synthesis by fibroblasts isolated from strictures of patients with Crohn's disease. *Gastroenterology*, 1992, **102** : 1920-1929.
12. PLATEROTI M., RUBIN D.C., DULUC I. *et al.* Subepithelial fibroblast cell lines from different levels of the gut axis display regional characteristics. *Am. J. Physiol.*, 1998, **274** : G945-G954.
13. JOBSON T.M., BILLINTON C.K., HALL I.P. Regulation of proliferation of human colonic subepithelial myofibroblasts by mediators important in intestinal inflammation. *J. Clin. Invest.*, 1998, **101** : 2650-2657.
14. REGAN M.C., FLAVIN B.M., FITZPATRICK J.M., O'CONNELL P.R. Stricture formation in Crohn's disease. The role of intestinal fibroblasts. *Ann. Surg.*, 2000, **231** : 46-50.
15. DVORAK A.M., SILEN W.S. Differentiation between Crohn's disease and other inflammatory conditions by electron microscopy. *Ann. Surg.*, 1985, **201** : 53-56.
16. MARTIN P. Wound healing – Aiming for perfect skin regeneration. *Science*, 1997, **276** : 75-81.
17. BLENNERHASSETT M.G., LOURENSSEN S. Neural regulation of intestinal smooth muscle growth in vitro. *Am. J. Physiol.*, 2000, **279** : G511-519.
18. SCHEINFELD B.A., COLLINS S.M., BLENNERHASSET M.G. Verapamil inhibits interleukin-1b mediated hyperplasia of human intestinal smooth muscle cells. *Gastroenterology*, 1994, **106** : A768.
19. VAN ASSCHE G., DEPOORTERE I., DE VOS R. *et al.* Region specific anti-proliferative effect of VIP and PACAP38 on rabbit enteric smooth muscle. *Am. J. Physiol.*, 1999, **276** : G303-310.
20. BILLINGTON C.K., JOSEPH S.K., SWAN C., SCOTT M.G., JOBSON T.M., HALL I.P. Modulation of human airway smooth muscle proliferation by type 3 phosphodiesterase inhibition. *Am. J. Physiol.*, 1999, **276** : L412-419.
21. WU K.K. Prostacyclin and nitric oxide-related gene transfer in preventing arterial thrombosis and restenosis. *Agents Actions*, 1997, Suppl. 48 : 107-123.
22. OWENS G.K., WISE G. Regulation of differentiation/maturation in vascular smooth muscle cells by hormones and growth factors. *Agents Actions*, 1997, Suppl. 48 : 3-24.