Fibrogenesis
IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models

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Pucilowska, Jolanta B., Kristen L. Williams, and P. Kay Lund. Fibrogenesis. IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models. Am J Physiol Gastrointest Liver Physiol 279: G653–G659, 2000.—The cellular mediators of intestinal fibrosis and the relationship between fibrosis and normal repair are not understood. Identification of the types of intestinal mesenchymal cells that produce collagen during normal healing and fibrosis is vital for elucidating the answers to these questions. Acute injury may cause normal mesenchymal cells to convert to a fibrogenic phenotype that is not maintained during normal healing but may lead to fibrosis when inappropriately sustained. Proliferation of normal or fibrogenic mesenchymal cells may lead to muscularis overgrowth associated with fibrosis. The presence of increased numbers of vimentin-positive cells within fibrotic, hypertrophied muscularis in Crohn’s disease suggests that changes in mesenchymal cell phenotype and number may indeed be associated with fibrosis. Fibrosis is induced in rats by peptidoglycan polysaccharides or trinitrobenzene sulfonic acid-ethanol administration, but inducing fibrosis in mice has been technically challenging. The development of current mouse models of colitis, such as dextran sodium sulfate or trinitrobenzene sulfonic acid-ethanol administration, into models of fibrosis will allow us to use genetic manipulation to study molecular mediators of fibrosis.

A WORKING MODEL OF THE CELLULAR BASIS OF FIBROSIS

The model in Fig. 1 borrows from available data in the literature on intestinal inflammation (9, 18) and on fibrosis in other organs, especially the liver (12). In the liver, activated stellate cells are the mediators of fibrosis. An activated stellate cell expresses &alpha;-smooth muscle actin (SMA) and has increased capacity for synthesis of extracellular matrix (ECM), including collagen type I. Acute and chronic injury of the liver causes stellate cell activation, but only chronic damage results in expansion of the activated stellate cell population and fibrosis (12). In the intestine, acute or chronic injury may cause a similar activation of a corresponding fibrogenic mesenchymal cell type.

Fibrosis in CD can be viewed as an overzealous healing response to injury (Fig. 1). This model predicts that injury causes initial activation of normal intestinal mesenchymal cells to a “fibrogenic” phenotype that we will define here as having an increased capability for ECM synthesis. Following acute injury, however, normal intestinal architecture is restored because posttranscriptional and posttranslational mechanisms prevent the net accumulation of ECM while fibrogenic cells are eliminated. In contrast, in fibrosis mechanisms to degrade ECM are not operative at appropriate levels and fibrogenic cells are not only maintained but are expanded in number. Mechanisms regulating these effects are unknown but may include factors associated with the presence of vimentin-positive cells.
with CD, such as Th1 cytokines or transmural inflammation (7). Unraveling the mechanisms that cause fibrosis requires the identification of these target fibrogenic cells.

Some important points should be made about fibrosis in CD. First, aside from their ability to manufacture large amounts of ECM, we do not yet have a useful definition of an “activated” or fibrogenic phenotype of intestinal mesenchymal cells. In the liver, the induction of α-SMA has proved very useful in identifying and functionally characterizing fibrogenic mesenchymal cells. The situation is more complicated in the intestine than in the liver because the normal intestine has a large, heterogeneous population of mesenchymal cells, some of which synthesize significant amounts of collagen. These cells could be considered to have a constitutive fibrogenic phenotype. This article will therefore review what is known about mesenchymal cell phenotypes in normal and inflamed intestine and offer suggestions as to how we might better define and study the activated or fibrogenic phenotype of intestinal mesenchymal cells during inflammation. A second point is that we need a better understanding of the transcriptional, posttranscriptional, and posttranslational mechanisms that regulate both ECM synthesis and net ECM accumulation in the normal vs. diseased intestine. New observations and animal models that may prove useful in studying these mechanisms will be described in this review. A third point is that hyperproliferation or inappropriate survival of normal or fibrogenic mesenchymal cells may be either of equal or greater importance than ECM synthesis in the development of fibrosis, obstruction, and stricture. We will therefore consider what is known about the relationship between overgrowth of muscularis layers and fibrosis. Finally, even though comparisons between UC and CD are useful to study mesenchymal cell responses during mucosal vs. transmural inflammation, interpretation of these results is complicated by the fact that the initiating factors for each disease are probably different (7). Animal models provide the opportunity to induce mucosal injury or transmural inflammation with the same initiating factor to study whether fibrosis is an excessive variant of normal tissue healing or whether it is specifically associated with transmural inflammation. These studies are feasible only in animal models, and potentially useful animal models will be described.

**Model of normal tissue repair and fibrosis**

![Model of normal tissue repair and fibrosis](image)

Fig. 1. Hypothetical model of normal tissue repair and fibrosis. Acute injury causes normal mesenchymal cells to be activated to a fibrogenic phenotype but may be followed by normal healing or fibrosis. During normal healing, excess extracellular matrix deposition is prevented by posttranscriptional or posttranslation regulation of collagen, reversal of the fibrogenic phenotype, or selective death of fibrogenic cells. If these events do not occur, are not sufficiently active, or if the fibrogenic cell population expands, fibrosis results.

**MESENCHYMAL CELLS IN NORMAL AND FIBROTIC INTESTINE: IS THERE AN ACTIVATED, FIBROGENIC PHENOTYPE?**

Mesenchymal cells in the intestine can be broadly classified as fibroblasts, smooth muscle cells, or myofibroblasts on the basis of immunostaining properties with antibodies to vimentin (V) and α-SMA (A) (18, 19). Typically, fibroblasts are V^+/A^- and are present in the submucosa and serosa of normal intestine. V^-/A^- smooth muscle cells predominate in the normal muscularis mucosa and muscularis propria. Subepithelial myofibroblasts (SEMF) with V^+/A^+ phenotype are found adjacent to epithelial cells (18, 19). Although α-SMA and vimentin are useful phenotypic markers, it is increasingly evident that intestinal mesenchymal cells are more heterogeneous than previously suspected. Enteric smooth muscle cells with mature or immature phenotypes differ in levels of α-SMA and γ-SMA expression (4). Some cells that share common features with V^+/A^- myofibroblasts do not express...
In normal intestine, SEMF and fibroblasts found in submucosa, serosa, and intermuscular connective tissue are the primary sites of expression of collagen mRNA and protein. These cells could therefore be considered a resident population of constitutively activated intestinal mesenchymal cells. In UC, collagen mRNA expression is upregulated in SEMF, suggesting that chronic inflammation further increases the activity of fibrogenic cells. Graham (9) was the first to propose that smooth muscle cells may be the cellular mediators of fibrosis in CD. He and others (9, 29) have demonstrated that cells that retain phenotypic characteristics of smooth muscle cells in culture, including α-SMA and tropomyosin expression, can synthesize collagen. However, whether or not cells with a normal smooth muscle phenotype are the same cells that synthesize excessive collagen in CD in vivo is still open to debate. Our recent studies in fibrotic intestine from CD patients indicate that V1/A+ or V2/A+ fibroblasts and myofibroblasts are the major sites of increased type I collagen mRNA expression and collagen deposition in muscularis. These collagen-producing cells also do not express desmin (19). It is possible that these cells are modified smooth muscle cells activated to express collagen (5). The cells could also derive from the activation or expansion of local fibroblasts or myofibroblasts that reside in connective tissue between smooth muscle bundles or from the migration of activated fibroblasts and myofibroblasts from the mucosa or submucosa to the muscularis layers.

Another possibility is that ICC could transform into a collagen-expressing fibroblast or myofibroblast phenotype. Our studies in involved intestine from CD patients failed to reveal c-kit positive ICC, even though such cells were evident in uninvolved bowel from the same CD patients and in intestine from noninflammatory controls (19). Because we did detect V1/A+ cells and collagen mRNA and protein in these regions, it seems possible that modified ICC could indeed be cellular sources of collagen. Another possibility is that ICC are destroyed during fibrosis and replaced by cells with a fibroblast phenotype. A role for ICC in fibrosis was suggested recently (18), and in vitro studies with isolated strips of muscularis propria indicate that ICC are altered by inflammatory stimuli (13). Defining the lineage of the collagen-expressing V1/A+ or V2/A+ cells at sites of fibrosis in CD requires additional studies. Analyses at the onset of fibrosis would be especially useful. Because resection of fibrotic intestine in CD generally occurs in late-stage disease, such studies are not feasible on human tissue samples. Animal models of inflammation-induced fibrosis represent an obvious alternate approach, as discussed in ANIMAL MODELS OF INFLAMMATORY BOWEL DISEASE.

Cultured intestinal fibroblasts, myofibroblasts, and smooth muscle cells are under investigation by a number of groups and have already provided insight into differences in cytokine expression, mesenchymal cell phenotype, and collagen synthesis in normal and diseased intestine (9, 22–24, 29). More detailed studies of these cultured cells following exposure to cytokines, immune cells, or ECM are required to provide a useful definition of an activated, fibrogenic phenotype of intestinal mesenchymal cells. It will also be useful to examine how the phenotype of cultured cells relates to the phenotype of cells in the intestine in vivo during onset and progression of fibrosis.

Inflammatory cells that infiltrate the gut in UC and CD include macrophages, lymphocytes, and plasma cells. These may have important interactions with mesenchymal cells and thereby impact fibrosis. Mast cells have recently been shown to be increased in number in the submucosa and muscularis propria of patients with CD (8). Mast cells express the c-kit receptor, facilitate chemotaxis via stem-cell factor/c-kit interaction, and can stimulate mesenchymal cell proliferation (25). Recent studies indicate that mast cell-deficient rats show reduced inflammation induced by dextran sodium sulfate (DSS) (1) and reduced fibrosis of the intestine after radiation-induced injury (28). The role of mast cells in fibrosis of the intestine thus warrants further investigation.

COLLAGEN SYNTHESIS AND DEGRADATION IN INFLAMMATORY BOWEL DISEASE

Increased levels of collagen type I, III, IV, and V mRNA and protein occur in strictured intestine from patients with CD (9, 14). Of these, collagen V may be preferentially increased (9) and may therefore be diagnostic or functionally relevant to fibrosis in CD. More detailed studies of the cell phenotypes expressing collagen V will help to define fibrogenic intestinal mesenchymal cells. Studies by Matthes et al. (14) indicate that both UC and CD are associated with increased levels of mRNAs encoding collagens I, III, and V. Elevated collagen mRNA was, however, limited to the mucosa in UC and was not associated with increased collagen protein (14). Together, these observations support a model whereby there is activation of intestinal mesenchymal cells in both UC and CD, but the two diseases differ in terms of posttranslational regulation of collagen. Recent studies have evaluated levels of matrix metalloproteinases (MMPs) in biopsies from patients with inflammatory bowel disease (discussed in Ref. 21). These studies indicate that both UC and CD show elevated mRNA and protein levels of mesenchymal cell-derived MMPs, including MMP-1, -2, -3, and -14, without increases in the expression of inhibitors of these proteases. Thus it does not appear that development of fibrosis in CD can be easily explained by a
lower expression of matrix-degrading enzymes. So far the biochemical analyses have not measured or localized the actual activities of the MMPs and have been limited to mucosal biopsies, and so differences in other intestinal layers cannot be excluded (21). Expression of other MMPs derived from epithelial cells or immune cell or MMP expression at earlier stages in disease than those examined may differ in UC and CD. Mice with targeted deletion of specific MMPs (15) provide new models to address the role of MMPs in normal mucosal repair and fibrosis.

**TRANSCRIPTIONAL ACTIVATION OF COLLAGEN SYNTHESIS AS A POSSIBLE MARKER OF FIBROGENIC PHENOTYPE**

Detection of transcriptional activation of collagen genes could provide an early biochemical or histological marker of conversion of normal cells to a fibrogenic phenotype. Although steady-state levels of mRNAs encoding a number of collagen subtypes are elevated in inflammatory bowel disease, little is known about transcriptional activity of collagen genes. Our model (Fig. 1) predicts that intestinal mesenchymal cells have activated collagen genes during normal tissue healing and fibrosis, but this has not been examined directly. A novel transgenic model was developed recently that will facilitate direct analyses of collagen gene activation in vivo and in vitro. The green fluorescent protein (GFP) reporter gene linked to a procollagen α1(I) promoter (10) provides a simple, highly sensitive, and readily quantifiable measure of transcriptional activation of the procollagen α1(I) gene and serves as a histological marker of cell types that exhibit type I collagen gene activation. We have recently begun studies using these procollagen α1(I) promoter-GFP mice and cultured intestinal mesenchymal cells derived from them. Preliminary data indicate that the procollagen α1(I)-GFP reporter gene is induced in vivo by DSS and in vitro by fibrogenic cytokines such as transforming growth factor-β (P. K. Lund, unpublished observations). These data indicate that the procollagen α1(I) promoter-GFP mice should provide useful models for defining the phenotype of fibrogenic intestinal mesenchymal cells and elucidating the transcriptional and posttranscriptional mechanisms that regulate collagen synthesis in cultured cells or in vivo during intestinal inflammation. The mice serve as a prototype for similar approaches linking GFP to promoters that regulate expression of other collagen types or other ECM components. This system could prove useful for testing interventions aimed at controlling collagen synthesis.

**MUSCULARIS OVERGROWTH DURING INTESTINAL INFLAMMATION AND FIBROSIS**

Overgrowth of the muscularis mucosa and muscularis propria occurs in CD but not UC, and this contributes to development of stenosis, stricture, and obstruction (2). Muscularis overgrowth also occurs in some animal models of chronic intestinal inflammation (3, 6, 27, 29). There have been few, if any, detailed studies of cells within hypertrophied muscularis layers. Our recent studies in CD indicate that thickening of the muscularis layers is associated with an increase in the number of vimentin-positive cells in these areas. In severely fibrotic samples, entire layers of histologically normal muscularis are populated primarily by V−/A− and V+/A+ fibroblasts and myofibroblasts rather than normal V+/A+ smooth muscle cells (19). These data support the concept that muscularis overgrowth in CD involves a change in enteric smooth muscle cells toward a fibroblast or myofibroblast phenotype.

We do not yet have a clear understanding of the contributions of increased cell size, increased cell proliferation, or reduced cell death to muscularis overgrowth during CD. Figure 2 illustrates two hypothetical models describing the development of muscularis overgrowth and fibrosis. In one model, acute injury...
induces activation of fibrogenic cells, followed by expansion of this fibrogenic cell population during chronic inflammation. In the second model, proliferation precedes activation. Either model would result in expanded cell number and increased ECM synthesis. The possibility that normal intestinal smooth muscle cells might transiently expand in number during injury has not, to our knowledge, been addressed. Quantitative analyses of muscularis overgrowth over the course of inflammation and fibrosis in animal models using either morphometric measurements or quantitative assays of cytoskeletal antigens (3) in conjunction with assays of cell phenotype and levels of collagen synthesis will determine which of the models in Fig. 2 is correct. Direct evaluation of mesenchymal cell proliferation and apoptosis would also be valuable.

ANIMAL MODELS OF INTESTINAL INFLAMMATION, MUCOSAL HEALING, AND FIBROSIS

A number of animal models of intestinal inflammation have been the topic of detailed reviews (6, 16, 20). Several models of acute injury are currently being used to study characteristics of normal mucosal repair. Rectal administration of acetic acid has probably been most extensively used, but colitis caused by indomethacin, trinitrobenzene sulfonic acid-ethanol (TNBS), peptidoglycan polysaccharides (PG-PS), and DSS all have useful features for analyses of mucosal repair following acute inflammation (6). It is noteworthy, however, that studies of mucosal repair in these models have focused more on epithelial than mesenchymal cells. Analyses of collagen gene activation, mesenchymal cell phenotypes present, and the levels of ECM synthesis and degradation in these models would be worthwhile.

Fibrosis during chronic inflammation has been less extensively studied in animal models. Excessive collagen deposition in the rat PG-PS and TNBS models is probably best characterized. These models show transmural inflammation, fibrosis, and muscularis overgrowth as in CD (27, 29). Additionally, the rat PG-PS model has some unique features that make it useful for comparing normal mucosal repair and fibrosis in response to the same stimulus. Intramural injection of PG-PS into the ileum and cecum causes acute inflammation that peaks at 1–2 days, followed by remission and mucosal repair. Spontaneous reactivation occurs in genetically susceptible rat strains at 12–17 days after injection. Reactivation is accompanied by progressive worsening of transmural granulomatous enterocolitis and development of severe transmural fibrosis in ileum and colon (20). A model of radiation-induced fibrosis of the intestine, although not well characterized with respect to its relevance to inflammatory bowel disease, may also be useful and has the advantage that small intestine and colon may be treated (28). As with the models of mucosal healing, we currently have little detailed information about mesenchymal cell phenotypes, collagen gene activation, ECM synthesis, and degradation in these models. More detailed analyses of fibrosis and muscularis overgrowth in small intestine and colon are warranted.

To date, the PG-PS, TNBS, and radiation models have been most commonly applied to rats. A mouse model of inflammation-induced fibrosis of the intestine is highly desirable for studies using genetic manipulation to study the cellular and molecular mediators of fibrosis. Of the models of inflammation listed above, the DSS model has been most extensively applied to mice. DSS is ingested in drinking water, and this model is technically very easy to develop. A single treatment with DSS results in focal but widespread mucosal damage from direct toxicity of DSS to epithelial cells that does not require T or B cells (6). Acute colitis is followed by restoration of mucosal architecture (6), making this an ideal model to study normal mucosal healing. In a number of studies, acute DSS colitis has been used in genetically manipulated mice to define the role of specific molecular and cellular mediators in mucosal damage and repair (Refs. 17 and 26). We believe that this model will also prove useful to study mesenchymal cell phenotypes, transcriptional and posttranscriptional regulation of collagen synthesis, and the possibility that there is transient overgrowth of enteric smooth muscle during or following acute inflammation. We note that doses of 5–10% DSS for 5–7 days that have been commonly used in several mouse strains led to unacceptably high morbidity or...
need for euthanasia during recovery in mice on C57BL/6, C57BL/6/SJL, and FVB-N genetic backgrounds. Studies aimed at mucosal repair should therefore begin with lower doses of DSS. We have found that 3% or 3.5% DSS for 5 days permits high survival rates and normal mucosal repair within 7–21 days following DSS.

A single treatment with 3–5% DSS does not lead to significant fibrosis at the end of DSS or during recovery (P. K. Lund and K. L. Williams, unpublished observations). We have begun to examine collagen synthesis and histological evidence of fibrosis in mice treated with multiple cycles of DSS to determine whether the DSS model may be used for studies of fibrosis as well as mucosal repair. Previous studies have reported that chronic colitis occurs in some mouse strains after multiple cycles of DSS (6). Chronic DSS colitis shares some common features with CD, including activation of T and B cells, induction of T cell-derived cytokines, prominent lymphoid aggregates, and fissuring ulcerations (6). Preliminary data indicate that two cycles of DSS in C57BL/6 and C57BL/6/SJL mice cause transmural inflammation, increased collagen mRNA abundance, and increased collagen deposition in lamina propria, muscularis mucosa, submucosa, and muscularis propria (Fig. 3). Muscularis overgrowth also occurs after two DSS cycles and may be as severe as in the rat PG-PS model (Fig. 3). We have not yet observed other features of CD such as stricture and obstruction in mice given two cycles of DSS. Ongoing studies are testing whether an increase in the number of DSS cycles causes more severe fibrosis.

Another potentially useful mouse model of fibrosis and muscularis overgrowth is the TNBS model (11). Recent studies in CD-1 mice indicate that repeated treatments with progressively increasing doses of TNBS over a 6- to 8-wk period cause increased ECM deposition and a high occurrence of fibrosis (11). We believe that these mouse models offer great promise to permit the use of genetic manipulation in unravelling the molecular and cellular mechanisms that lead to inflammation-induced intestinal fibrosis and for testing of therapies aimed at controlling fibrosis. Two new mouse models of spontaneous ileitis have been reported recently that share features in common with CD and may prove useful to study mesenchymal cell responses to inflammation of the small intestine (16).

In closing, there is growing consensus in the fibrosis field that mice are more resistant to fibrosis than other species. This has certainly been our experience in liver as well as in the intestine (unpublished observations). From a practical standpoint, this means that studies of fibrosis in mice are more time-consuming than anticipated. However, the slow development of fibrosis in mouse models may ultimately prove to be an advantage for tracking changes in phenotype and number of mesenchymal cells during acute and chronic injury from onset to progression to fibrosis. Defining which of the two models proposed in Fig. 2 is operative or whether muscularis overgrowth and increased collagen synthesis occur concurrently will help tailor preventive therapies for fibrosis in CD toward controlling conversion to a fibrogenic phenotype, expansion of the normal or fibrogenic mesenchymal cell populations, or both of these processes.

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