Animal models of intestinal fibrosis: new tools for the understanding of pathogenesis and therapy of human disease

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Review

Fibrosis is a serious condition complicating chronic inflammatory processes affecting the intestinal tract. Advances in this field that rely on human studies have been slow and seriously restricted by practical and logistic reasons. As a consequence, well-characterized animal models of intestinal fibrosis have emerged as logical and essential systems to better define and understand the pathophysiology of fibrosis. In point of fact, animal models allow the execution of mechanistic studies as well as the implementation of clinical trials with novel, pathophysiology-based therapeutic approaches. This review provides an overview of the currently available animal models of intestinal fibrosis, taking into consideration the methods of induction, key characteristics of each model, and underlying mechanisms. Currently available models will be classified into seven categories: spontaneous, gene-targeted, chemical-, immune-, bacteria-, and radiation-induced as well as postoperative fibrosis. Each model will be discussed in regard to its potential to create research opportunities to gain insights into the mechanisms of intestinal fibrosis and stricture formation and assist in the development of effective and specific antifibrotic therapies.

Intestinal Fibrosis in Humans: The Clinical Problem

Fibrosis is the frequent result of chronic inflammation, involves components of both innate and adaptive immunity, and can cause important anatomical and functional abnormalities in any organ or system (147). Intestinal fibrosis is defined as the excessive deposition of extracellular matrix (ECM) resulting from chronic inflammatory tissue damage and impairment of intestinal wound healing (107, 112, 113). It represents a common complication of inflammatory bowel disease (IBD) with serious clinical implications for both ulcerative colitis and Crohn’s disease (10, 114). In ulcerative colitis, fibrosis is mostly restricted to the mucosal and submucosal layers and may contribute to shortening or stiffening of the colon, whereas in Crohn’s disease fibrosis is often transmural like the inflammatory process from which it derives and is eventually followed by narrowing of the lumen and stricture formation. Over time the majority of Crohn’s disease patients progress to complications, such as strictures and fistulæ, and these clinical phenotypes can occur at any time stage during the course of the disease. More than 30% of Crohn’s disease patients evolve toward a distinct fibrostenosing phenotype, with progressive narrowing of the intestinal lumen and potential obstruction. Up to 80% of all patients suffering from Crohn’s disease undergo surgery at least once during the course of their disease, stricture formation being the most common indication in a high proportion of them. Moreover, recurrence of stricture formation is common at the site of surgical anastomosis (25, 31, 102).

Despite remarkable therapeutic progress in the management of inflammation, limited progress has occurred with respect to a specific antifibrotic therapy for IBD. Often surgery is still the only practical solution for patients with complications secondary to intestinal fibrosis, and postsurgical recurrence is frequent (10, 114). Data on the effect of anti-inflammatory or immunosuppressive therapy on stricture formation are still scarce and prospective observations are lacking. Cosnes and coworkers (15) reported an unchanged incidence of stricture formation and need for surgery during the last 25 years despite the emergence of immunosuppressive medications. This conclusion has been challenged, since less than 10% of the Crohn’s disease patients undergoing surgery had received azathioprine for more than 3 mo before surgery and therefore immunosup-
pression might have been given too late and for too short periods of time. More recent observations indicate a somewhat lower risk for Crohn’s disease-related surgery with the earlier and prolonged use of anti-TNF agents and/or azathioprine (16, 24, 104, 110, 137). However, it should be noted that either the available information has been retrospective or, in the case of prospective evaluation, follow-up times were short and post hoc analyses were used. Knowledge about the effect of immunosuppressive medications on stricture formation is still evolving but, taken together, still indicates that control of inflammation alone does not completely prevent stricture formation.

A fundamental problem with studying the pathogenesis of intestinal fibrosis in humans is that by the time fibrosis (with or without stricture) is detected, early pathogenic events have already occurred and the fibrogenic process is fully established. Consequently, the early and the ensuing time-dependent steps in the pathogenesis of fibrosis can no longer be investigated. In addition, reliable predictors that can indicate the development of intestinal fibrosis are still not available. Given these circumstances, it is obvious that major obstacles exist that impede a better understanding of intestinal fibrosis and stricture formation in humans. This is especially true when aiming at mechanistic studies. Thus an alternate approach utilizing animal models seems fully justified.

**Intestinal Fibrosis in Animal Models: The Experimental Approach**

Animal models have long been adopted to reproduce, mimic, or approximate the developmental origin and basic principles of human health and disease (84, 95). They all offer advantages as well as limitations but represent indispensable tools to substitute for what cannot be studied in humans for practical and logistic reasons. Models of IBD have been used for over 40 years, and they are responsible for major advances in our understanding of disease pathogenesis in both experimental animals and human subjects (128). However, the massive amount of information generated by IBD animal models has been almost entirely restricted to the study of immunity and inflammation and the gut microbiota. Only recently these models are being utilized to study intestinal fibrosis. This has resulted in substantial advances in the characterization of a number of animal models that resemble the human disease process. In these models clues can be found to identify initiating, perpetuating, and reversing events of intestinal fibrosis, thereby allowing the creation of conceptual pathophysiological frameworks. On the other hand, differences between animal and human biological responses cannot be dismissed (86), and precaution must be taken when results of experimental systems are translated into clinical practice (87). Although animal models will never fully reproduce the complexity of human diseases, they can investigate and compare early events vs. late events, and this may lead to the development of new pathophysiology-based therapies.

Compared with the vast number of currently available experimental models for IBD (22, 106, 144), models addressing intestinal fibrosis remain limited. In this review they will be classified into seven categories: spontaneous, gene-targeted, chemical-, immune-, bacteria-, and radiation-induced as well as postoperative fibrosis. Each model provides the distinct opportunity to gain different and complementary insights into the pathogenesis of fibrosis and stricture formation. In many if not all of the models environmental and genetic factors are probably critical for fibrosis to occur, highlighting the relationship among the environment, genetic factors, and the intestinal immune system, a relationship also present in humans. This review aims at discussing some of the models of intestinal fibrogenesis, including methods of induction and key characteristics, assessing their usefulness to identify critical pathogenic events, and testing new therapeutic interventions. A synopsis of the models including advantages, disadvantages, and relevance to human disease is presented in Table 1.

**Spontaneous Intestinal Fibrosis**

Animal models that spontaneously develop intestinal fibrosis are highly attractive because of their similarity to human disease, in that inflammatory and fibrotic responses occur without any apparent exogenous manipulation. Unfortunately, these spontaneous models are quite rare, and only one has received scientific scrutiny.

The SAMP1/Yit mouse was generated by mating of a senescence-accelerated mouse line (82). Inflammation develops spontaneously at 10–20 wk of age with almost 100% penetrance after 30 wk and is most severe in the terminal ileum. The inflammation is segmental, transmural and includes granulomas in the mucosa and submucosa. Since the terminal ileum is typically the primary location of lesions in Crohn’s disease patients, this strain provides an excellent model to study the pathogenesis of intestinal stricture formation (82, 117). Disease can be adoptively transferred into severe combined immunodeficiency (SCID) mice that lack both T and B cells, and these recipient mice also develop terminal ileitis (59). SAMP1/YitFc mice do not exhibit inflammation under germ-free conditions; however, germ-free mice reconstituted by transfer of the flora from specific pathogen-free SAMP1/YitFc develop disease. A fraction of these mice spontaneously develop perianal fistulas and accumulate ECM in the small and large bowel with thickening of the muscularis mucosa, predominantly in the terminal ileum (117), a feature closely resembling stricture formation in Crohn’s disease patients. The low breeding rate of SAMP1/YitFc mice makes it difficult to use this model on a large scale, and large colonies required for routine experimentation are available only in selected laboratories. In addition, this in not an inbred mouse strain and SAMP1/YitFc mice are not commercially available, restricting the access to this model by the scientific community.

**Gene Targeting: Knockout and Transgenic Models**

The development of intestinal fibrosis by manipulations of selected inflammation-associated genes represents a second, more direct method for determining which specific immune abnormalities may lead to fibrosis.

**IL-10 deficiency.** IL-10 is an immunoregulatory cytokine with immunosuppressive and anti-inflammatory properties (88). IL-10 appears to be a major regulator of mucosal homeostasis, since IL-10-deficient mice develop a chronic enterocolitis (111). When these animals are housed under specific pathogen-free conditions they develop a mild inflammation restricted almost entirely to the colon. However, when housed under conventional conditions, IL-10-deficient mice exhibit mucosal inflammation in the upper and more so in the lower...
### Table 1. Synopsis of the major characteristics of individual animal models of intestinal fibrosis

<table>
<thead>
<tr>
<th>Model of Intestinal Fibrosis</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Site of Involvement</th>
<th>Relevance to Human Disease</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Spontaneous</strong></td>
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<tr>
<td>SAMP1/YitFc ileitis</td>
<td>• Spontaneous inflammation and fibrosis • No need for stimulus or additional manipulations • Gut histology resembles Crohn’s disease • The natural history of the disease is chronic</td>
<td>• Low breeding rate • Few colonies in existence • Long time needed to complete experiments • Not commercially available</td>
<td>• Primarily small bowel (terminal ileum) in early and late disease</td>
<td>High • Disease location, histology, presence of granulomas, fistulas, perianal disease and chronic evolution bear close resemblance to human Crohn’s disease</td>
<td>59, 82, 117</td>
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<tr>
<td><strong>Gene Knockout and Transgenic</strong></td>
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<tr>
<td>IL-10 deficiency</td>
<td>• L-10 is a cytokine with a well recognized immunoregulatory and anti-inflammatory activity • Mechanisms of inflammation resulting from its deficiency are well defined</td>
<td>• The model requires genetically manipulated animals • Appearance and severity of inflammation, and consequently of fibrosis, is variable depending on the breeding facility</td>
<td>• Primarily colon and less commonly small bowel</td>
<td>Moderate • Human infants with genetic IL-10 receptor deficiency and severe IBD have been described</td>
<td>4, 5, 61, 76, 111, 126</td>
</tr>
<tr>
<td>TGF-β1 overexpression</td>
<td>• TGF-β1 is the most potent pro-fibrogenic factor and unquestionably linked to intestinal fibrosis • Mechanisms of inflammation resulting from its deficiency are well defined</td>
<td>• The model requires transfection with an adenoviral vector producing bioactive TGF-β1 • Fibrosis is focal</td>
<td>• Colonic fibrosis with obstruction</td>
<td>Moderate • Patients with IBD have spontaneously high levels of TGF-β1 and fibrosis in the inflamed tissue</td>
<td>8, 17, 37, 62, 83, 96, 133</td>
</tr>
<tr>
<td>MCP-1 overexpression</td>
<td>• MCP-1 is chemokine involved in intestinal inflammation • Mechanisms of inflammation caused by its overproduction are well defined</td>
<td>• The model requires transfection with an adenoviral vector producing bioactive MCP-1 • Fibrosis is focal</td>
<td>• Transmural fibrosis of colon and rectum</td>
<td>Moderate • Patients with IBD have high levels of MCP-1 (and several other chemokines) and fibrosis in the inflamed tissue</td>
<td>39, 91</td>
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<tr>
<td><strong>Chemically Induced</strong></td>
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<td>TNBS-induced</td>
<td>• Colonic inflammation is clearly T cell dependent • Appearance of fibrosis follows a logical Th1 to Th2 cytokine switch</td>
<td>• The model requires repeated enemas for induction of intestinal fibrosis • There is considerable variability in the induction of colitis depending on source and amount of TNBS • Intensity of inflammation varies in different strains</td>
<td>• Colon involvement in late disease</td>
<td>Moderate • There is no evidence that haptens play a role in human IBD • T cell dependency is important in human IBD, particularly Crohn’s disease • Transmural inflammation with fibrosis and granulomas are features of Crohn’s disease</td>
<td>26, 58, 71, 81, 89, 96, 145, 148</td>
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<tr>
<td>DSS-induced</td>
<td>• The easiest and most reproducible protocol to induce colonic inflammation with associated fibrosis</td>
<td>• Initial insult is an acute chemical injury to the colon epithelium • Detailed mechanisms of colonic inflammation still not fully characterized</td>
<td>• Colon involvement in late disease</td>
<td>Low • There is no evidence that chemical damage causes IBD and fibrosis • Injury can be useful to study epithelial inflammation and subepithelial fibrosis in ulcerative colitis</td>
<td>18, 54, 76, 79, 97</td>
</tr>
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<tr>
<td>Straightforward induction of colitis by administration of peroxynitrite-containing enemas</td>
<td>• Colon involvement in subacute stage</td>
<td>Potentially high • NO is produced in large quantities in intestinal inflammation and IBD and induces tissue damage</td>
<td>Colon</td>
<td>108</td>
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<tr>
<td>The model has been described only in one report</td>
<td>• The model is laborious and artificial • The degree to which intestinal fibrosis correlates with severity or duration of inflammation is unknown</td>
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<tr>
<td>Reproducibility is unknown</td>
<td>• The model requires a surgical procedure</td>
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<tr>
<td>No direct link between NO and induction of fibrosis</td>
<td>• Colon</td>
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**Immune-Mediated**

| T cell transfer-induced | • The mechanisms of induction of intestinal inflammation are well defined • T cell abnormalities may indirectly modulate mesenchymal cell behavior | • Colon | Uncertain • Studies in this model have been heavily oriented toward immune regulation • No convincing evidence for T cell regulatory defects in IBD patients | 72, 90, 92, 105 |

| Bacteria-Induced | • The dependency of the model on bacteria-derived components is pathophysiologically relevant • The model allows a time-dependent investigation of fibrogenesis | • The model is laborious and artificial • t requires a laparotomy and intramural injection of PG-PS into the bowel wall | Small and large bowel | Moderate-high • Massive fibrosis with bowel thickening and adhesion are found in Crohn’s disease, but bacterial components derive from the lumen in human patients | 122, 125, 136, 149 |

| Gut microbiota-induced | • The dependency of the model on bacteria-derived components and activation of the TGF-β pathway are both pathophysiologically relevant events | • The model is laborious • It requires a laparotomy and intramural injection of fecal suspensions or extracts of selected anaerobic bacteria into the bowel wall | Colon | Very high • Crohn’s disease pathogenesis and complications, including fibrosis, are believed to be closely linked to an immune response to the gut microbiota | 85, 93 |

| Infection-induced | • The induction of gut inflammation and fibrosis only requires oral administration of infectious agents • Induction of increased levels of TGF-β, CTGF, and IGF-I is pathophysiologically relevant • The model allows a time-dependent evaluation of the fibrogenic response and removal of the fibrogenic stimulus with antibiotic treatment | • The intestinal fibrogenic process in this model and in humans may be pathophysiologically different | Colon | Low-moderate • No evidence that infectious agents directly cause IBD • Epidemiology studies show a correlation of acute bacterial infections and subsequent development of IBD, although not fibrosis | 38, 40, 49 |

**Radiation-Induced**

| Exteriorized bowel segment | • Choice of bowel segment to be irradiated with sparing of remaining intestine | • Colorectal fibrosis with radiation dose-dependent obstruction | Moderate • Segmental bowel radiation mimics human situation | 29, 42, 43 |
Table 1.—Continued

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<thead>
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<tr>
<td>Postoperative</td>
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<tr>
<td>Anastomotic fibrosis</td>
<td>• Results reproduce outcome of routine bowel resection</td>
<td>• The model requires a surgical procedure and bowel resection</td>
<td>Model-dependent small bowel, colon or ileocolic anastomosis</td>
<td>High</td>
<td>60, 116</td>
</tr>
<tr>
<td>Intra-abdominal adhesions</td>
<td>• Results reproduce outcome of intra-abdominal manipulation of bowel segments during surgery</td>
<td>• The model requires a surgical procedure and bowel manipulation</td>
<td>Manipulation site-dependent small or large bowel</td>
<td>High</td>
<td>13, 46, 47, 132</td>
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CTGF, connective tissue growth factor; IBD, inflammatory bowel disease; MCP-1, monocyte chemoattractant protein 1; PG-PS, peptidoglycan-polysaccharide.

intestinal tract, which can be accompanied by systemic complications (61). CD4+ T-cells are the main effector cells in this model, and their transfer into RAG-2−/− recipients, a strain lacking mature T and B cells, results in colitis, indicating that when the immunoregulatory activity of IL-10 is missing intestinal inflammation ensues. This response appears to be mediated by IFN-γ, an immunoregulatory cytokine mainly secreted by activated T cells, because the development of colitis can be abrogated by treatment with anti-IFN-γ antibodies (4). Interestingly, inhibition of prostaglandin synthesis by cyclooxygenase isoform-selective inhibitors leads to more rapid and severe intestinal inflammation in these animals (5). This model also exhibits important immunological differences with respect to the early vs. the late stages of inflammation. In fact, a typical Th1-dominant inflammatory cytokine profile (high IL-12 and IFN-γ mucosal levels) appears in the early stages, whereas in late disease a Th2 profile emerges that is characterized by high IL-4 and IL-13 levels (126). This evolutionary pattern is directly relevant to fibrosis because IL-13 is now recognized as a major profibrotic cytokine, as will be discussed later in this review. To date, this model has not been extensively used to study intestinal fibrosis. However, in the chronic phase, an accumulation of ECM is detectable in the affected intestine, and the IL-10−/− mouse has been proposed as appropriate for investigating mucosal and submucosal fibroblasts in intestinal fibrosis (76).

**TGF-β1 overexpression.** TGF-β1 is considered the most powerful driver of fibrosis in essentially all organs including the gut. Levels of TGF-β1 are elevated in the mucosa of IBD patients (3), with a differential expression of its isoforms by intestinal fibroblasts (83). Enema delivery of an adenoviral vector producing bioactive TGF-β1 leads to transfection of mouse colonic epithelium and transient TGF-β1 expression (133). This results in an inflammatory mucosal and submucosal response for 14 days with resolution of the inflammatory infiltrates thereafter. Subsequently, severe and sustained fibrosis develops from day 14 to day 28, with excessive collagen accumulation in the submucosa and in the external muscle layers. Cells with a myofibroblast phenotype emerge with the concurrent thickening of both the mucosa and muscularis propria. Preexisting inflammation accelerates the adenoviral vector-induced colonic fibrosis. The presence of fibrosis is not uniform throughout the colon but is focal, affecting regions up to 2 cm in length, and intestinal obstruction can be observed in a high proportion (50%) of the animals. Interestingly, these effects appear despite a brief transgene expression of a few days, since TGF-β1 levels peak at 2 days. The mortality in this model is low (<10%) (133).

It is known that TGF-β1-deficient mice develop severe multiorgan inflammation and expire by 5 wk of age (17, 62). This outcome occurs even under germ-free conditions (8) and is mediated by CD4+ T cells (37, 96). Therefore, neutralizing TGF-β1 in vivo as an antifibrogenesis approach in IBD may be highly problematic, as this may actually lead to disease exacerbation given the potent anti-inflammatory properties of this cytokine. On the other hand, models of TGF-β1 overexpression may still be valuable to study and dissect the distinct pathways that regulate fibrosis vs. those that regulate immunotolerance, and thereby could help in the development of highly specific antifibrotic therapies.

**MCP-1 overexpression.** Monocyte chemoattractant protein 1 (MCP-1), a member of the C-C family of chemokines, is known to play an important role in fibrosis formation in organs such as the lung, kidney, and liver (74, 80, 156). MCP-1 is a chemoattractant for monocytes and T and NK cells and is notably increased in the submucosa and muscularis propria of patients with Crohn’s disease (39). Intramural injection of an adenoviral vector for MCP-1 leads to a transmural inflammation may still be valuable to study and dissect the distinct pathways that regulate fibrosis vs. those that regulate immunotolerance, and thereby could help in the development of highly specific antifibrotic therapies.

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cient) mice fail to accumulate collagen despite stable numbers of macrophages (91). Comparable to the TGF-β1 overexpression model, fibrosis in the MCP-1 overexpression model is focal and restricted to colonic injection site of the vector. Potential mediators of MCP-1-induced fibrosis are TGF-β1, whose levels increase at days 3–7, and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), an endogenous matrix metalloproteinase (MMP) inhibitor, whose levels increase at day 7. Anti-MCP-1 therapy might serve as a novel antifibrotic therapy in the intestine, since it has been effective in attenuating fibrosis in experimental liver, lung, and kidney fibrosis (48, 131, 138).

Chemically Induced Models

Unlike the genetically manipulated animals described above, healthy wild-type animals can develop intestinal fibrosis by exposing them to exogenous agents that stimulate a local inflammatory response. Depending on the agent used and the targeted cells, these models enable studying different mechanisms of fibrogenesis. Chemical agents can damage the intestinal epithelial barrier that normally separates the mucosal immune system and the microbiota or can directly activate local immune cells. It is becoming increasingly clear that microbial components are critical to induce and perpetuate gut inflammation (121), and this response is likely to play a key role in chemically induced models and the eventual development of fibrosis. Therefore, these models are particularly useful in assessing the role of innate immunity in fibrogenesis. Some models, such as the dextran sodium sulfate (DSS)- and trinitrobenzene sulfonic acid (TNBS)-induced colitis (described below in detail), are widely used to study and test factors implicated in epithelial cell injury responses in acute and chronic inflammation. However, it has to be kept in mind that neither hapten formation (as found in the TNBS model) nor chemical epithelial injury (as found in the DSS model) have been shown to be implicated in the pathogenesis of human IBD, and therefore the true relevance of these models to intestinal inflammation and fibrosis in patients can be questioned.

TNBS-induced intestinal fibrosis. Colitis can be induced in susceptible murine strains by intrarectal instillation of the hapten TNBS diluted in ethanol (89). Ethanol breaks the epithelial barrier and TNBS is believed to modify colonic proteins and activate a delayed-type hypersensitivity reaction. This model is T cell dependent and exhibits a transmural inflammation (96). Lawrance et al. (71) induced a chronic form of colitis leading to fibrosis in this model by repeated administration of escalating doses of TNBS over 6 wk. This model is one of the most widely used to study intestinal fibrosis in vivo and reveals a distinct pattern of cytokine expression in early vs. late disease (26). In early disease typical inflammatory Th1 cytokines, including IL-12p70 and IFN-γ, mediators known to have an antifibrotic property, predominate. In the chronic stages of inflammation the concentrations of Th2 cytokines and TGF-β1 increase.

Various molecular and cellular mechanisms have been investigated in the fibrogenic process associated with TNBS colitis. Emergence of fibrosis is at least partially dependent on IL-13-induced TGF-β1 production by monocytes and macrophages, because blockade of these cytokines reduces fibrosis. This mechanism is further supported by a reduction in intestinal fibrosis in Smad3−/− mice, Smad3 being a signaling molecule essential to the biological activity of TGF-β1 (153). Early growth response gene (Egr-1) could also be identified as downstream mediators of TGF-β1 action as well as insulin-like growth factor (IGF-I) (27); the known profibrogenic action of IGF-I was recently confirmed by amelioration of collagen deposition, fibrosis, and muscle growth in IGF-I−/− mice (78). TNBS-induced fibrogenesis is also dependent on the activation of the prototypical proinflammatory transcription factor NF-κB, because blockade of NF-κB with antisense oligonucleotides abrogates intestinal fibrosis (145). This antifibrotic effect of NF-κB with antisense oligonucleotides could, however, be secondary to suppression of inflammation, since TNBS colitis also improves by blocking NF-κB (71).

There is evidence that mast cells and their products have profibrotic effects in TNBS colitis. Administration of nedocromil sodium, a mast cell stabilizer, reduces inflammation and fibrosis, and mast cell lysates increase rat intestinal fibroblast proliferation, collagen production, and contractile activity (148). Neuropeptides also appear to be involved. Treatment of TNBS colitic mice with CJ-12255, an antagonist of the neurokinin-1 receptor (NK-1R) that mediates the activity of substance P, reduces colonic inflammation, fibrosis, fibroblast accumulation, and levels of collagen, vimentin, and TGF-β1 (58). Accordingly, NK-1R−/− mice chronically exposed to TNBS have reduced colonic fibrosis and lower levels of fibrogenic factors.

The TNBS model of colitis has been widely used to investigate a variety of changes that accompany chronic colonic inflammation. Martinez-Augustin et al. (81) performed a longitudinal genomic analysis in this model and reported major disturbances in metabolic, transport, and structural genes. Among the latter, marked changes in the expression of many genes involved in matrix deposition, muscle plasticity, and angiogenesis were detected. These changes included IGF-I and IGFBP-5, genes that regulate tissue remodeling by increasing collagen synthesis and cell proliferation; several procollagen/collagen isoforms were increased, as well as genes involved in collagen processing and synthesis or in collagen and elastin fiber cross-linking. Several MMPs and multiple cytoskeletal genes were also upregulated, especially in the chronic stages of colitis.

Despite its popularity, some caveats need to be mentioned when using this model. First, colitis is not diffuse but is restricted to the site of TNBS injection and, depending on the source and the dose of this hapten, intestinal inflammation may fail to develop or be so severe that it causes unacceptably high mortality. Thus a considerable degree of experience is needed to routinely use this model. Second, TNBS colitis is strain dependent in regard to susceptibility, and, third, the presence of inflammation does not necessarily predispose to substantial fibrosis: for instance, certain strains, like C57/BL6 mice, appear to be generally more resistant to intestinal scar formation.

DSS-induced colitis. Mice drinking the sugar polymer DSS for several days develop a highly reproducible colitis with bloody diarrhea, ulcerations, and weight loss (79, 97). DSS is believed to have a toxic effect on intestinal epithelial cells leading to disruption of the epithelial barrier. In addition, activation of intestinal macrophages might contribute to intestinal inflammation. Mice deficient in T and B cells develop DSS colitis, indicating the lack of a prominent role of the
adaptive immune system in this model, at least in the early stages of inflammation (18, 97). DSS colitis in germ-free mice develops to the same extent or even more severely compared with conventionally housed mice (54). In certain strains, chronic administration of DSS for several cycles results in chronic colitis with the development of a substantial amount of fibrosis (76). In contrast to TNBS colitis where consistent dosing of the hapten is difficult to control by enema delivery, DSS administration in drinking water is easy and yields reproducible results, explaining its popularity and widespread use. The main disadvantage of this model is the questionable relevance to human IBD. DSS colitis is considered useful for studying acute events elicited by an epithelial response to injury, but it may not adequately address the events occurring during the chronic phase of inflammation. Nevertheless, some mouse strains able to resolve the initial DSS injury can provide assistance in investigating wound-healing responses developing immediately after an acute epithelial injury. This is particularly interesting because animals more prone to inflammation, such as C57BL6 mice, may progress toward chronic inflammation and thus offer the opportunity to compare acute and chronic repair mechanisms (19).

Peroxynitrite-induced colitis. Excessive production of nitric oxide (NO) is a phenomenon occurring almost universally in inflammation, and this is also true for experimental IBD. Rachmilewitz et al. (108) administered enemas containing peroxynitrite, a substance that promotes lipid peroxidation and sulfhydryl oxidation, to rats and examined their colons at 1, 3, 7 and 21 days. Damage was evident at day 1, the lumen was narrowed at day 7, and signs of stenosis were detected at day 21. Histological examination found fibrosis and transmural inflammation with thickening of the muscularis mucosae and muscularis propria. These findings were accompanied by a significant increase in NO, NO synthase, and myeloperoxidase production. Although peroxynitrite-induced colitis has been repeatedly used to study the role of reactive radicals in intestinal inflammation and cancer, the above report remains the only evidence linking NO to intestinal fibrosis in experimental IBD. For this reason, it is difficult to assess its relative value as an animal model of inflammation-driven fibrosis. However, further exploring the mechanisms of an NO-mediated intestinal fibrogenesis response makes sense and could represent a valuable addition to expand our understanding of intestinal fibrosis.

Immune-Mediated Models

Essentially all models of experimental IBD were originally developed to investigate the immune abnormalities associated with the acute inflammation response observed after the trigger stimulus, regardless of its type. Subsequently, some models were extended in time to study the changes associated with chronic intestinal inflammation, like in the case of TNBS and DSS colitis, and this approach has proved useful in paving the way to study animal models of intestinal fibrosis. Other models, however, retained a strict immunological character and continued to be used almost exclusively for the investigation of the intestinal mucosal system during gut inflammation.

T cell transfer-induced colitis. The prototypical example of these immune-mediated models is the T cell transfer model, in which naive CD45RB$^{high}$ CD4$^{+}$ T cells are injected into SCID mice that develop a wasting disease with severe transmural inflammation of the colon (90, 105). Animals given mature CD45RB$^{low}$ CD4$^{+}$ T cells or unfractionated CD4$^{+}$ T cells do not develop colitis, and the colitis induced by the naive T cells can be abrogated upon injection of CD45RB$^{low}$ CD4$^{+}$ T cells containing T regulatory cells (92). The original reports of this model described the large intestine as markedly thickened due to increased numbers of neutrophils, lymphocytes, and macrophages but also of “supporting stromal elements” resulting in a significant narrowing of the intestinal lumen. The combination of increased stromal cells and narrowing of the lumen is compatible with the notion that fibrosis is also a component of the T cell transfer model of colitis. In fact, a subsequent publication that detailed the histopathological features of the model does mention “mild fibrosis” as a component of this type of experimental transmural colitis (72). The literature is now replete of studies of this CD45RB$^{high}$ CD4$^{+}$ T cell transfer model, but all of them exclusively address immune events without paying any attention to the mesenchymal component of the colitis. Since in human IBD T cells are intimately involved in chronic intestinal inflammation and fibrosis, additional investigation of the T cell transfer model as another type of animal model of intestinal inflammation seems warranted.

Bacteria-Induced Models

As discussed in the chemically induced models, microbial components are needed to induce or perpetuate gut inflammation in most IBD animal models (121), and depending on the experimental conditions this response is likely to contribute to the development of fibrosis. Whether microbial components derive from commensal or pathogenic microbes is probably irrelevant. Nevertheless, when pathogens are used it is more difficult to dissect the fibrogenic input derived from infection virulence factors vs. physiological components of the commensal microbiota.

Peptidoglycan-polysaccharide-induced intestinal fibrosis. The intramural injection of the bacterial cell wall polymer peptidoglycan-polysaccharide (PG-PS) directly into the bowel wall of rats induces transmural enterocolitis (122). This evolves into a chronic granulomatous inflammation after 3 wk, followed by fibrosis, thickening of the intestinal wall, and adhesions (122, 125, 136). These events are associated with an increase in TGF-β1 and IGF-I production, indicating that components of nonpathogenic intestinal bacteria are sufficient to induce fibrosis once they reach the interstitial compartment of the intestinal wall. This model is well defined and allows the investigation of fibrogenesis in different stages of disease: acute inflammation, quiescence, and reactivation (136, 149). It is, however, technically demanding and is still not adapted to mice, making the study of fibrosis in genetically altered animals not feasible at the moment. A potentially unique aspect of this model is the PG-PS used in its induction. Small bowel strictures in patients with Crohn’s disease frequently occur in individuals carrying polymorphisms of the NOD2 gene, whose product is an intracellular pattern recognition receptor for muramyl dipeptide (MDP) (36). MDP is a ubiquitous component of bacterial peptidoglycan, therefore offering the theoretical possibility of using this agent and this model to study the specific innate immune mechanism leading to intestinal fibrosis in the setting of Crohn’s disease-associated gene variations.
Intestinal microbiota-induced intestinal fibrosis. Using an experimental protocol similar to the one described for PG-PS (122), a group of investigators induced colitis in rats by injecting the colonic wall with a fecal suspension of luminal contents or selected aerobic (Lactobacillus spp., Enterobacter aerogenes, Klebsiella pneumoniae, and Streptococcus viridans) and anaerobic (Clostridium ramosum, Bacteroides fragilis, and Bacteroides uniformis) strains isolated from the same animals. These were then compared with rats in TNBS-induced colitis (93). All groups developed chronic colitis with fibrosis and a high rate of bowel strictures, and colonic tissue contained increased levels of TGF-β1 and collagen. Antibiotic treatment significantly inhibited TGF-β1 and collagen production as well as stricture formation. Inoculation of bacterial suspensions into the colonic wall increased tissue TGF-β1 and collagen content, and antibodies against TGF-β1 prevented collagen accumulation. Inoculation with single anaerobic (Clostridium ramosum, Bacteroides fragilis, and Bacteroides uniformis), but not with aerobic, strains also induced collagen deposition. Therefore, components of the commensal gut microbiota have the capacity to stimulate TGF-β1 production and induce collagen deposition once they enter in direct contact with cells in the bowel wall; furthermore, on the basis of these results, decreasing bacterial load has an apparent antifibrotic effect, but this effect may be restricted to components of the anaerobic communities and not to all bacteria present populating the gut.

In a follow-up study using a similar protocol the underlying molecular mechanisms were explored by disrupting the TGF-β/ALK5 (a TGF-β type 1 receptor)/Smad signaling pathway (85). Upregulation of ALK5 and TIMP-1, phosphorylation of Smad2 and 3, and increased intestinal wall collagen deposition were found in the colitic animals, and these effects were attenuated by the ALK5 inhibitor SD-208. TGF-β1 treatment of isolated rat mucosal myofibroblasts induced phosphorylation of Smad2 and 3 and upregulation of ALK5 protein, TIMP-1, and α2 type 1 collagen gene expression, and again these effects were inhibited by SD-208, whereas ALK5, Smad2, and Smad3 RNA interference abolished the induction of TIMP-1 and α2 type 1 collagen. Therefore, the profibrotic effects of some commensal microbiota strains are apparently mediated by the same classical TGF-β-mediated pathways that also mediate fibrosis induced by exogenous chemical agents like TNBS and DSS.

Infection-induced intestinal fibrosis. Infection with live bacteria can induce intestinal fibrosis in mice. Oral delivery of Salmonella enterica serovar Typhimurium 24 h after antibiotic treatment with streptomycin leads to chronic intestinal inflammation with a severe and stable fibrotic process in the cecum and colon that is maximal at 3 wk (38). The presence of the two Salmonella virulence factors Salmonella pathogenicity islands (SPI)-1 and SPI-2 is required for the development of fibrosis (38). This model is easy to use and is reproducible. It works in several different strains, although to variable degrees, and mimics the most frequent stricture location of humans in the terminal ileum. The investigators that reported this model found an increased expression of TGF-β1, connective tissue growth factor (CTGF), and IGF-I in addition to fibroblast accumulation. However, it is unclear whether the disease mechanisms in this model are shared with human IBD, since stricture formation in IBD has not been linked to flagellated bacteria, and S. typhimurium infections in humans are not associated with clinically relevant intestinal fibrosis. On the other hand, recent evidence shows that TLR5, the specific receptor for flagellin, is expressed on human intestinal fibroblasts and its binding leads to significant fibroblast proliferation and enhanced fibronectin secretion (40) (and our own unpublished observations).

Regardless of human relevance, the Salmonella model has recently been employed to investigate the relationship between intestinal inflammation and fibrogenesis in a time-dependent fashion. Using S. typhimurium-infected mice, Johnson et al. (49) removed the inflammatory stimulus by eliminating the infectious agent with levofloxacin and then examined inflammation and fibrosis at 2, 4, 8, and 21 days postinfection. The authors found that delayed eradication of the inflammatory stimulus repressed inflammation but failed to prevent development and progress of fibrosis even in the early stages of inflammation. They concluded that, once initiated, fibrosis is self-propagating and independent, to some extent, of the initial trigger of inflammation and fibrosis. These results are particularly valuable to better understand key steps in the development of intestinal fibrosis and have potentially critical implications for the timing of implementation of antifibrotic interventions.

Radiation-Induced Intestinal Fibrosis

Exposure of the intestine to therapeutic doses of radiation frequently results in subsequent bowel inflammation followed by bowel fibrosis later on. This well-established clinical observation has led to the development of experimental models of radiation-induced intestinal fibrosis in rats, and to a lesser degree in mice, to reproduce and investigate the events responsible for the development of equivalent fibrosis developing in human disorders.

Morphological aspects and experimental approaches. From a morphological point of view, radiation-induced intestinal fibrosis recalls the appearance found in Crohn’s disease patients with a stenosing phenotype. Radiation induces a marked thickening of the bowel wall, with an enlargement of the submucosa, enhanced fibroblast and smooth muscle cell proliferation, and excessive deposition of collagen and other ECM components. Vascular sclerosis and chronic ulcers are also hallmarks of radiation-induced bowel fibrosis (43, 140). Various reports have demonstrated that in animal models the magnitude of intestinal fibrosis depends in part on the type of radiation, increases with total radiation dose, fraction size, and reduction in the interfraction interval. Additionally, the severity of bowel fibrosis has been shown to increase over time, until 26 wk after radiation (29, 65–67).

From an experimental point of view, two different experimental strategies have been utilized. In the first one, a segment of small bowel is surgically exteriorized and irradiated while the rest of the animal is shielded with a lead screen. The exteriorized bowel segment is moistened with saline buffer during the procedure and, after irradiation, returned to the abdominal cavity (42, 43). In the second strategy, a bilateral orchiectomy is performed, the inguinal ring is opened, and a segment of distal ileum is placed in the empty scrotum. The resulting “scrotal hernia” contains a fixed 4-cm segment of small bowel that is easily accessible to radiation, without the need of additional surgery and therefore minimizing manipulation artifacts (65, 67, 140–142, 157).
Pathogenesis of radiation-induced intestinal fibrosis. The postradiation events developing after the initial acute mucosal inflammation and ulceration have been called “consequential” late effects, whereas the direct radiation responses in the stromal compartment have been called “primary” late effects (140) 139. While both effects result in an increased deposition of ECM in the bowel, experiments undertaken to dissect the contribution of each component to bowel fibrosis concluded that the ulcerated lesions of “consequential” fibrosis contained three times more collagen and required a fourfold increase in peak force to rupture the intestine, compared with the nonulcerative “primary” fibrosis (30). In any case, the older notion that a single cell type could dictate the entire tissue response to radiation has been replaced by a newer concept that a more complex, coordinated, multicellular response is required to develop radiation-induced intestinal fibrosis (43).

The pathogenesis of chronic intestinal radiation fibrosis is multifactorial and includes distinct responses resulting from the impact of radiation on different cell types. Inflammation precedes and accompanies the evolution of fibrosis, microvascular insult provides an early stimulus that is followed by a secondary stimulus coming from the hypoxia due to the microvascular injury (152). These events are then followed by cytokine production, fibroblast proliferation, and activation of collagen synthesis (152). A recent report proposes that mast cells and their products (chymase, tryptase, and histamine) are essential components of radiation-induced fibrosis, on the basis of results showing that mast cell-deficient mice show less damage than control littermates (6). These results, combined with previous findings in TNBS colitis model (148), suggest that mast cell products may be more central to the overall process of intestinal fibrosis than previously anticipated.

Our knowledge of the molecular mechanisms governing the development of radiation-induced bowel fibrosis has markedly improved in recent years. Prolonged upregulation of fibrogenic cytokines, such as TGF-β1 and its main downstream effector, CTGF, in the myofibroblasts of irradiated bowel, seems to be a fundamental profibrotic steps (35, 44). In fact, irradiated NIH3T3 cells phosphorylate Smad, and treatment with a TGF-β receptor inhibitor represses this effect, whereas p38, ERK1/2, and JNK are not affected (151). In addition, the Ras homologue (Rho) and Rho-associated kinase (ROCK) signaling pathways are also involved in the development of chronic radiation enteritis (158). The deficiency of microvascular thrombomodulin and upregulation of protease-activated receptor 1 (PAR-1) observed in irradiated rats would also support the long-held notion that endothelial cell dysfunction is an early and key step for development of bowel fibrosis (142). Interestingly, the bowel response to radiation seems to be regulated in part by capsaicin-sensitive nerves. Ablation of capsaicin-sensitive enteric neurons results in an exacerbation of the early intestinal radiation toxicity but markedly attenuates the development of chronic fibroproliferative changes in rat small intestine (141).

Postoperative Fibrosis

The development of intestinal fibrosis and adhesions following intra-abdominal procedures is an essentially universal response, but one whose outcome that can range from a completely uneventful state to a serious and recurrent clinical problem. As many as 65% of Crohn’s disease patients undergo an operation over their lifetime because of uncontrollable inflammation or intestinal obstruction (25, 102). Surgery for Crohn’s disease is rarely curative and after segmental resection two distinct complications frequently occur: recurrence of inflammation with or without stenosis at the site of the anastomosis and adhesion formation.

Anastomotic postoperative fibrosis. Seventy percent of Crohn’s disease patients undergoing intestinal resection due to stricture formation have endoscopic evidence of recurrence at 1 year and up to 40% show a symptomatic recurrence after 4 years, requiring further interventions (119, 120, 143). Why some patients develop fibrosis, while others do not, is currently unknown (102), and no treatment is at hand to prevent this from happening. No animal model evaluating in detail the development of postoperative fibrosis was available until the report of Rigby et al. (116). This group of investigators showed that, after ileocecal resection in IL-10−/− mice, animals developed inflammation-driven small intestinal fibrosis at the site of the resection and in the adjacent small bowel, whereas control wild-type counterparts did not. Interestingly, IL-10−/− mice housed under germ-free conditions also failed to develop inflammation or fibrosis after ileocecal resection, suggesting the strong dependence of this response on the presence of the gut microbiota (116). This model also develops fibrosis on the proximal side of the anastomosis as seen in humans, providing an excellent model to study clinically relevant postoperative recurrence (25, 102). In addition, the dependence on the intestinal microbiota in this model offers a solid experimental base to investigate the contribution of innate immunity on fibrogenesis, an area that deserves further attention. Another report investigated the role of myofibroblasts in a model of colonic resection in normal rats (60). The authors found a progressive postoperative increase in myofibroblast growth and differentiation from days 3 to 7, a time when staining intensity for α-smooth muscle actin of colonic myofibroblasts at the anastomotic site reached the level of the muscle layer cells. This indicates that mesenchymal cell proliferation is an intrinsic response at sites of anastomosis regardless of whether inflammation was present or not in the operated bowel.

Postsurgical intra-abdominal adhesions. Adhesion formation following procedures in the abdominal cavity is a very common clinical problem with limited treatment options. In a large review of the surgical literature spanning 20 years, peritoneal adhesions caused 32% of acute intestinal obstruction and 65–75% of all small bowel obstructions. The study found that peritoneal adhesions develop after 93–100% of upper abdominal laparotomies and after 67–93% of lower abdominal laparotomies. Fortunately, only 15–18% of these adhesions required surgical reintervention (98). In addition to chronic abdominal pain and infertility, adhesions can lead to bowel obstruction and even organ failure (20, 21, 100). Complications secondary to adhesion formation may manifest at any time postoperatively, and about 20% of complications appear more than 10 years postsurgery (20). Adhesions result from the peritoneal wound healing response after surgical intervention and develop in the first 5 to 7 days after injury. Initially a fibrin gel matrix is built between apposing serosal surfaces, which can then be followed by reepithelialization and restitutio ad integrum. On the other hand, the fibrin bridge can serve as a basis for developing a fibrous band and an organized fibrotic
tissue, i.e., the abdominal adhesion (45). Postsurgical adhesions tend to recur after secondary surgical lysis, seemingly implying an underlying predisposition and an inflammatory component as drivers of the process. On the basis of the above, it is obviously desirable to study early events of fibrogenesis in adhesion formation to prevent later complications, again providing a strong rationale for the use of animal models.

Experimental intra-abdominal adhesions can be easily and reproducibly induced in mice by serosal abrasion of the cecum and abdominal wall (13, 132). The abrasions result in the development of petechiae on the serosal surface, and development of adhesions are evaluated 6 days after surgery by use of a scoring system that assesses the number, thickness, and vascularization of adhesions (13). Somewhat surprisingly, adaptive immunity is involved in the generation of adhesions, and CD4+ Th1 cells are apparently required for the development of postsurgical adhesions (13, 14). Upon abrasion of the serosal surfaces these T cells enter the peritoneal cavity, orchestrate chemokine production and leukocyte trafficking, and accumulate at the site of adhesion formation (13, 47). The Th1 cells central to adhesion development require the Th1 differentiation factor T-bet and the Th1 cell regulator Tim-3. The T cell-derived cytokines IFN-γ and IL-17 participate in this response, and adhesion formation is also dependent on the Th1 chemotactant IL-16 (13, 132). Mice deficient in IL-12, but not in IL-4, signaling show fewer adhesions compared with wild-type mice (13), underscoring the Th1-type nature of the adhesion-forming process. Microbial zwitterionic polysaccharides can prevent adhesion formation, and this protective effect can be transferred through transfer of CD4+CD45RB<low> IL-10-producing T cells (118). Injections of IL-10 alone can also prevent adhesion formation in mice (46). On the basis of these combined results it has been suggested that adhesion formation in the abdominal cavity represents a dysregulated type I immune response, at least in the experimental setting (132).

Because of its frequency and clinical significance a variety of therapeutic approaches have been attempted with the aim of decreasing intra-abdominal adhesion formation; this will be discussed later on under antifibrotic therapies.

**New Approaches to the Study of Experimental Intestinal Fibrosis**

The magnitude of the clinical problem of intestinal fibrosis in various diseases, combined with the multiple limitations of in vivo studies in humans, and the noticeably slow progress in understanding its pathophysiology should place animal models of intestinal fibrogenesis at the center of research efforts. However, it is unrealistic and to some degree futile to try to mention all potential new avenues of research that could be explored to improve our knowledge of experimental intestinal fibrosis. Therefore, we will limit the following discussion to areas of interest that are emerging as a rational follow-up to the current state of the art on this subject.

**Nontraditional sources of intestinal fibroblasts.** A new area of very high interest is the elucidation of the source of intestinal fibroblasts. Besides local proliferation, migration, and influx from the circulation and bone marrow (9, 70, 73, 113), alternative origins have been proposed to contribute to fibrogenesis in organs such as the kidney, liver, and lung. Substantial evidence now exists that epithelial as well as endothelial cells can transform into fibroblasts under inflammatory pressure (51, 146, 154, 155). Novel genetic tools in the form of double transgenic mice are available using a genetic marker of cell lineage to irreversibly tag specific cell types expressing both Cre-recombinase under the control of a cell specific promoter and a reporter gene such as LacZ or GFP that can be made functional by exposure to the Cre enzyme (75, 123, 124). Two recent reports using these constructs lend support to the notion that both epithelial- and endothelial-to-mesenchymal transition occur in animal models of IBD and contribute to intestinal fibrosis (28, 115). This approach can be used to tag potential fibroblast precursors and other cell types to determine their possible contribution to the process of intestinal fibrogenesis. Complementing this approach, cell-specific genetic knockout or knockin could be performed in vivo, such as for fibroblasts or fibroblast precursors. This would circumvent the lack of specificity of conventional knockout animals in which the gene of interest is deleted in all cells (75, 123, 124).

**ECM turnover.** The investigation of ECM production and turnover in animal models of gut inflammation is another area of valuable research. Under normal conditions excessive ECM in a fibrotic organ is degraded by MMPs, enzymes that are kept at bay by TIMPs. Reports on the expression of MMPs and TIMPs in human IBD consistently show major increases and imbalances in the affected tissues, but very few studies have been done in animals considering the plentiful MMPs and TIMPs that control ECM accumulation in the gut (11, 33, 71). This is surprising, because this area appears to be particularly promising for discovering new therapies.

**Innate immunity.** Also in need of aggressive exploration is the role of the innate immune system in fibrogenesis, specifically the involvement of Toll-like- (TLR) and NOD-like receptors (NLR), both being classes of receptors intimately involved in IBD pathogenesis (52). Both TLRs and NLRs are expressed by and known to be able to activate intestinal fibroblasts, with synergistic effects when stimulated in combination. In addition, they are able to mediate a profibrotic response by increasing the secretion of prototypical ECM molecules, such as fibronectin and collagen I (our unpublished observations).

**Novel molecular pathways.** The central importance of TGF-β in the fibrotic response is fully established, but the fine molecular mechanisms that regulate its action and related signaling pathways need to be investigated in greater depth. For example, the investigation of HSP47, a collagen-specific molecular chaperone (94), has barely started, but preliminary evidence has been published showing that gene expression of HSP47 and TGF-β1 are significantly increased in IL-10<−/−> mice and correlate with severity of inflammation (55). It would be extremely interesting to uncover the molecular steps regulating this interaction to improve our understanding of fibrotic responses in the gut and devise blocking steps aiming at therapeutic interventions. Additional molecular pathways that need investigation in regard to intestinal fibrosis are the Indian Hedgehog (Ihh) and the Wnt/β-catenin pathways. Deletion of Ihh from the intestinal epithelium of Cyp1a1-CreIhh<−/−> conditional Ihh mice triggers an influx on fibroblasts, increase in TGF-β1 production, and deposition of ECM that progressively results in intestinal fibrosis (135). The Wnt/β-catenin signaling appears important in normal wound healing, and its sustained activation also leads to fibrogenesis (64).
Epigenetic regulation. All processes are regulated at an epigenetic level, and this regulatory step of ultimate gene expression can be explored in animal models (7), including for the study of profibrotic conditions (50). Ongoing studies have begun to investigate possible epigenetic changes in the intestinal fibrosis associated with Crohn’s disease (M. Scarpa and E. Stylianou, unpublished observations), and this approach can be easily extended to animal models, like to SAMP/YitFc mouse or chronic TNBS and DSS colitis. In view of the increasingly appreciated role of microRNAs in IBD (101), this also appears to be an extremely fruitful area for new discoveries in intestinal fibrogenesis.

Relevance of Animal Models to Human Intestinal Fibrosis

The ultimate goal of developing and studying animal models of intestinal fibrosis is to improve our understanding of the pathophysiology, detection, and therapy of intestinal fibrosis in patients suffering from a variety of chronic illnesses complicated by fibrosis. We have already alluded to the fact that carrying out mechanistic studies in humans is problematic, and therefore relevant animal models become the logical as well as practical solution to learn how to approach the intestinal fibrosis dilemma. This dilemma is daunting, because the mechanisms of progressive fibrosis must take into perspective the tissue specificity of each organ and the lack of effect of anti-inflammatory therapies in suppressing the profibrotic response (34). In addition, fibrosis is traditionally viewed as the end point of a chronic response, but in reality when the key events triggering fibrosis actually commence is still a mystery, and recent studies tend to suggest that this is a very early process that goes hand in hand with inflammation, or may even precede it; in the S. typhimurium model even early eradication of the infectious agent fails to prevent development of intestinal fibrosis (49); additionally, in a sheep model of obesity the offspring intestine is already inflamed and fibrotic at the time of birth and continues for at least 22 mo (150). Thus not only do we need to consider which model to adopt and how to use it, but also when to use it, if clinically relevant information is to be obtained. It is also important to remember that current understanding of the gut immune and inflammatory response is far from complete, and the same is true for the overall response of gut tissue to insults. The fact that even the newest and most potent biologics appear to be only partially effective in stopping or reversing intestinal fibrosis in IBD (16, 24, 104, 110, 137) is the perfect example of how limited our comprehension of underlying pathogenic events is, but, at the same time, this creates a compelling reason to pursue more studies on this topic.

The various animal models discussed above can generate information valuable for human disease. However, it must first be remembered that most of these models were initially developed with the sole intent of dissecting the immune components of experimental IBD and only later some of them were modified and extended in time to look at late inflammatory events, such as intestinal fibrosis. Thus it is possible that other models not addressed in this review have the potential of yielding supplementary information relevant to the intestinal fibrosis. Second, the limitations intrinsic to each model also need to be taken into account. For instance, whereas investigators can exploit the unique features of each model to ask very specific questions, no animal model recapitulates all of the pathogenic and clinical features of human intestinal fibrosis. Another important consideration is that, unlike what happens in the actual human situation, in most murine models intestinal fibrosis spontaneously reverses as soon as the inducing stimulus is removed. Moreover, although mice offer the distinct advantage of being susceptible to genetic engineering for detailed cellular and molecular studies, many of these manipulated mice are available on a C57/BL6 background, a strain that is not particularly susceptible to fibrogenesis. Despite these limitations, the contribution of animal models has been immense and will likely be even greater as we rationally and methodologically improve the models and their utilization. One example of such improvement is the recently reported use of a new reporter mouse, which expresses fluorescently labeled proteins under the control of promoters for collagen I and mesenchymal cell markers (α-smooth muscle actin) in the DSS and TNBS models of intestinal fibrosis (19). Using this approach investigators will be able to investigate the type, number, and relative contribution of specific mesenchymal cell subpopulations to ECM deposition. In addition, tools such as this could set the foundation for future cell-targeted antifibrotic therapies. Additional examples in regard to detection of fibrosis in the gut and its experimental therapy are described below.

Detection. An unresolved issue of major clinical relevance and with serious therapeutic implications is whether symptoms referred by the patient are due to the presence of a significant accumulation of scar tissue in the bowel, especially in the absence of clear-cut signs of intestinal obstruction. This is a very common situation, and routine radiological examinations may at best suggest but not prove the presence of substantial fibrosis. Endoscopic mucosal biopsies are too superficial and not informative of the histological changes in the deeper layers of the bowel. To help this state of affairs new imaging techniques are being developed and tested in animal models to detect intestinal fibrosis and assess their potential translation for human use.

Ultrasound elasticity imaging (UEI), a noninvasive method that allows characterization of intestinal tissue with high spatial resolution, can provide a simple and accurate assessment of severity of fibrosis in the colon of rats with TNBS colitis (53). When applied to resected bowel segments from these animals to look for evidence of inflammation and fibrosis, UEI is able to differentiate acutely inflamed vs. chronic fibrotic changes, and between unaffected and fibrotic intestine in a pilot study of Crohn’s disease patients (127). Magnetization transfer (MT) is another new imaging modality. When applied to rats with PG-PS-induced fibrosis, the mean MT ratio in rats with late-phase fibrosis was higher than that of animals with early inflammation and of animals without late-phase fibrosis; furthermore, the MT ratio showed a correlation with the amount of tissue fibrosis (2). Additional experimental imaging techniques are being constantly tested, and the availability of animal models of fibrosis makes their ex vivo and in vivo application a practical possibility, opening the door to far better ways of detecting intestinal fibrosis in humans.

Therapy. The vast majority of patients with significant intestinal fibrosis have had or still has a substantial degree of associated inflammation. Because of this, it has been particularly frustrating to realize, while recognizing that available data are still scarce, that even the most effective currently available
anti-inflammatory agents seem unable to fully stop or prevent further progress of the fibrotic process (16, 24, 104, 110, 137). This is true even in face of experimental evidence that blocking certain proinflammatory mediators does ameliorate fibrotic processes. For instance, tumor necrosis factor (TNF-α) has been unequivocally shown to induce proliferation of murine intestinal myofibroblasts and induce collagen accumulation in vitro; TNR2 is the primary mediator of TNF-α activity through ERK1/2 to induce proliferation and through STAT3 to stimulate TIMP-1 and inhibit collagen degradation (129). On the basis of these results one might expect that anti-TNF-α agents should have the ability to inhibit intestinal fibrosis. However, considering the still-evolving evidence mentioned above, this effect may be only partial. Thus it seems appropriate to consider alternative approaches that can be tested in properly selected animal models, and with pharmacological strategies that most closely mimic the real clinical situation (57). A wide variety of disparate compounds have been tested in the models listed in this review and are briefly discussed below.

Because of its well-established and potent fibrogenic effect, by far the most coveted target of antifibrotic therapy has been TGF-β. A large number of neutralizing antibodies, kinase and peptide inhibitors, ligand traps, anti-inflammatory drugs, anti-allergic drugs, and mimetics have been developed for several human fibrotic conditions, including Crohn’s disease (reviewed in Ref. 37) (41). Most of them have not been evaluated in animal models of intestinal fibrosis, a challenging enterprise that should yield relevant information on both mechanisms and efficacy. A different approach to anti-TGF-β therapy has been the use of a TGF-β1 peptide-based virus-like particle vaccine (77). When tested in mice with TNBS-induced chronic colitis, inflammation and collagen deposition improved together with decreased Smad3 phosphorylation and other inflammatory markers.

Statins have also been tried in various models of intestinal fibrosis. Pravastatin has been reported not only to prevent but also to ameliorate established radiation-induced fibrosis in rats (42, 43). Simvastatin improved TNBS-induced colitis in mice, a response accompanied by antifibrotic effects demonstrated by a decrease in CTGF levels (1). Statin-containing cellulose film appears to help preventing postoperative adhesions in rats submitted to the cecal abrasion protocol (63).

PEG 15–20, a high-molecular-weight polyethylene glycol-based copolymer, prevents radiation-induced intestinal injury in rats and also displays an antifibrotic effect (134), and treatment of TNBS murine colitis with the pentoxifilline metabolite M-1 has been reported to significantly reduce colon weight, thickness, and total collagen content (103). Biological films or gels containing hyaluronan, modified chitosan-dextran, or carboxymethyl chitosan have been used with some success in preventing or decreasing intra-abdominal adhesions abrasion and anastomosis models in rats (23, 69, 99, 139).

A series of natural plant- and animal-derived products have been tested for possible antifibrotic actions. Daikenchuto, a traditional Japanese medicine product, significantly prevented formation of intestinal adhesion in rats, an effect possibly mediated through the transient receptor potential vanilloid type 1, since capsaicin also displayed antifibrotic effects (130). Extracts of anti-inflammatory Boswellia and antiinflammatory Scutellaria plants reduced inflammation and fibrosis in rats with TNBS colitis, together with a significant reduction of α-smooth muscle actin, collagen I-III, CTGF, TGF-β1, and Smad 3 and 7 (68). Even honey and pollen have been reported to be effective in preventing postoperative intra-abdominal adhesion in the cecal abrasion model (12). Few studies have addressed potential mechanisms underlying the antifibrotic effects of natural substances. One such study found that resveratrol, a phytoalexin found in berries, peanuts, grapes, and red wine, induced cycle arrest and decreased collagen synthesis and apoptosis of rat intestinal smooth muscle cells in vitro (32). These effects are compatible with a potential antifibrotic effect in vivo, an outcome corroborated by a subsequent report showing that oral administration of resveratrol in the PG-PS rat model lowered levels IL-1β, IL-6, TNF-α, and TGF-β1 mRNA as well as procollagen I and III and IGFI-F mRNA (109).

Finally, as a complement to a potential “natural approach” to managing intestinal fibrosis, there is some evidence that dietary manipulations may reduce intestinal fibrosis in animals. Knoch et al. (56) performed transcriptome analysis using whole genome microarray analysis of RNA isolated from colonic tissue of colitic IL-10−/− mice fed with an oleic acid- or arachidonic acid-based diets. They found that several genes implicated in intestinal fibrosis were expressed at a lower level in the colon of mice fed with the arachidonic acid-based diet, and these animals exhibited reduced colonic shortening and deformation. Accordingly, they found a downregulation of Colla2 and IGFBP-5 genes, both of which are typically involved in collagen deposition and tissue remodeling. Although it is difficult to separate the putative antifibrotic effects from the anti-inflammatory ones, this study opens a new window of opportunity for the potential management or prevention of intestinal fibrosis with selected dietary components.

**Summary and Conclusions**

To date, intestinal fibrosis is still viewed as an inevitable consequence of chronic bowel tissue injury. No specific antifibrotic therapy is available in humans, and scientific advances in this field have been hampered by ethical, practical, and logistic reasons. The development of animal models of intestinal fibrosis not only allows the investigation of pathogenetic mechanism of fibrosis but also opens the door to preclinical therapeutic studies. Such studies are badly needed to extend therapeutic options for patients with fibrostenotic Crohn’s disease beyond the option of surgical interventions. Which animal model is the most relevant and appropriate will fundamentally depend on the context of the research question asked and at present no single recommendation can be correctly given. This review will hopefully provide researchers with a specific interest in the study of intestinal fibrosis some guidance for the selection process. Thoughtful use of currently available models and creation of additional experimental tools should lead to the development of pathophysiologically based therapies in intestinal fibrosis.

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ANIMAL MODELS OF INTESTINAL FIBROSIS

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