Chapter 5
Cytokine and Anti-Cytokine Agents as Future Therapeutics for Fibrostenosing IBD

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Abstract The pathogenesis of stricture formation in inflammatory bowel disease is a complex process with a wide variety of clinical, genetic, epigenetic, and environmental risk factors. Originally thought to be a consequence of chronic inflammation, new evidence arises for non-inflammatory contributors to stricture formation, suggesting an intricate interplay of cellular, molecular, and additional host/environmental factors. Although no specific medical treatments for fibrostenotic intestinal strictures currently exist, understanding the molecular pathways involved in stricture formation will undoubtedly guide therapeutic developments. As mediators of inflammation *and* immunoregulation, cytokines are key effectors in the fibrotic process. Accordingly, targeting inflammation, in part via cytokine blockade, has been the mainstay of therapy in IBD. In many cases, inflammatory disease is associated with significant fibrotic change, as increased inflammation perpetuates the cascade of mucosal repair. Thus, inflammatory cytokine-targeted therapy may serve as one potential avenue for treating fibrostenosis. As regulatory and repair mechanisms have been implicated in fibrosis as well, either as sequelae of inflammation or via de novo pathways, a parallel route for treating intestinal fibrosis may be the targeting of "regulatory" cytokines. This chapter will highlight the relevant contributions and potential therapeutic targeting of cytokines involved in inflammatory and regulatory pathways leading to fibrosis.

Keywords Inflammatory bowel disease · Strictures · Crohn's disease · Ulcerative colitis · Fibrostenosis

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5.1 Introduction

Approximately 40% of CD patients with ileal disease will develop clinically apparent strictures throughout their lifetime [1]. The frequency of fibrostenosing complications has still remained significant despite immunosuppressive therapy in CD patients in the form of steroids or immunomodulators [2, 3]. Since a myriad of genetic and epigenetic variables are thought to contribute to fibrostenosing disease, including those that affect cytokine biology, the investigation of specific therapeutics targeting those pathways has become prevalent. The potential adverse effects of inhibiting pathways involved in tissue repair and mucosal healing, as well as the relatively slow evolution of fibrosis in CD has made precise targeting of fibrosis difficult. Despite these potential deterrents, cytokine-targeted therapy has become the pillar of treatment for many inflammatory conditions and is being evaluated for fibrotic disorders. The question of whether anti-cytokine therapy will prove useful for intestinal fibrosis still remains, however. This chapter will review current cytokines involved in fibrosis and their potential targeting for treatment.

5.2 "Inflammatory" Cytokines

Targeting inflammation has been the mainstay of therapy in IBD. As such, anti-inflammatory cytokine therapeutics have provided significant advances in treating IBD patients. In many cases, inflammatory disease is associated with significant fibrotic change, as increased inflammation perpetuates the cascade of mucosal repair. Thus, since fibrogenesis may be a consequence of increasing inflammation, the hope of treating resulting fibrosis by preventing and suppressing inflammatory insults has emerged.

5.2.1 TNFα

TNFα is a multifunctional cytokine, often considered proinflammatory (but with important immunomodulatory properties, as well). A variety of cell types can secrete TNFα, including activated macrophages, B cells, T cells, keratinocytes, and fibroblasts. Depending upon the conditions, TNFα can trigger either pro-inflammatory or anti-inflammatory pathways by engaging one or both of two distinct transmembrane receptors: TNF-Receptor 1, and TNF-Receptor 2. In addition to its pro-inflammatory effects, TNFα may potentiate fibrosis via induction of tissue inhibitor of metalloproteinase-1 (TIMP-1) and reduce MMP-2 activity and collagen degradation [4]. Treatments targeting TNFα are some of the most widely used anti-cytokine therapies for inflammatory disorders, but mixed evidence has surfaced for using these agents in pro-fibrotic diseases. In some animal models of liver and renal

fibrosis, TNF blockade reduced organ inflammation and fibrogenesis [5, 6], but a recent clinical study investigating adalimumab for fibrotic kidney disease (FSGS) failed to meet its primary outcome [7]. An open-label pilot study in 16 systemic sclerosis patients demonstrated improvement in skin scores with reduction in collagen secretion noted from cultured lesional fibroblasts (Table 5.1) [8–10].

In contrast, there is evidence suggesting that TNFα is an antifibrogenic cytokine and its blockade might therefore promote fibrosis. In some studies, TNFα can exhibit antifibrotic properties by reducing the expression of collagen and connective tissue growth factor in dermal fibroblasts [11], and via suppression of TGFβ signaling through NFKB induction of Smad 7 in other cell types [12]. The differing results may separate at the level of the individual TNF receptors on specific cell-types. Diminished TNFR1 signaling accelerates wound-healing, increases collagen deposition, and angiogenesis at wound sites in TNFR1-deficient mice [13]; whereas

Table 5.1 Cytokine and drug targets in fibrosis

Cytokine	Effect on fibrosis	Cellular/molecular mechanism	Drug (mechanism of action)
"Inflammatory"			
TNFα	↑/↓	Induction of TIMP, ↓ MMP ↓ Fibroblast collagen, CTGF, TGFβ	Infliximab, adalimumab (anti-TNF Ab)
IL-4 IL-13	↑ ↑	Fibroblast activation, ↑ collagen ↓ MMP, ↑ TGFβ	Lebrikizumab, tralokinumab (anti-IL-13 Ab)
IFNγ	↓	↓ Fibroblast proliferation, migration ↓ Collagen production	Recombinant human IFNγ, HSc025 (upregulates YB-1)
IL1β	↑/↓	↑ TNF-α, IL-6; transcription of TGF-β ↑ Collagenase, ↓ collagen production	Canakinumab (anti-IL1β Ab)
IL-17	↑	Activation of fibroblasts, ↑ collagen Promotion of EMT, ↑ TGFβ	Secukinumab (anti-IL-17 Ab)
TL1A	↑	Activation of fibroblasts, ↑ collagen ↑ TIMP	In development
"Regulatory"			
TGFβ	↑	↑ Fibroblast activation, proliferation ↑ Collagen, fibronectin, TIMP ↓ MMP Promotion of EMT and EndoMT	Metelimumab, fresolimumab (anti-TGFβ Ab) SD-208, EW-7197, IN-1130, SM16 (TGFβR inhibitor) Pirfenidone, ACEi/ARB, statin (↓ TGF syn/signaling) Cilenglitide (integrin inhibitor, ↓ TGFβ activation)
IL-10	↓	T-reg associated suppression of cell activation	Recombinant human IL-10

TNFR2-deficient intestinal myofibroblasts demonstrate reduced cell proliferation and decreased collagen synthesis [4].

Pertaining to intestinal fibrosis per se, the evidence for utilizing TNF antagonists as *anti-fibrotic* agents has remained questionable. Initial studies of TNF blockade reported concerns due to obstructive complications in some patients that accompanied mucosal healing. In vitro studies with myofibroblasts from CD patients treated with infliximab, however, showed that TNF blockade decreased collagen production [14]. Later multivariable analyses from the observational TREAT registry and the ACCENT I multicenter trial determined that disease duration, severity, location, and new corticosteroid use are factors associated with stricture formation, rather than TNF-antagonist use [15]. Some efficacy has now been seen in a few patients with inflammatory or mixed stenoses [16, 17], as well as small case series reporting intralesional injection of infliximab [18]. Cohort studies suggest that these agents may reduce the need for surgery, as rates of surgery ranged between 27 and 61% within the first 5 years after diagnosis before the use of TNF antagonists, and between 25 and 33% after the introduction of these agents [19, 20]. Indeed, anti-TNF agents are recommended to reduce the risk of post-operative recurrence after surgery. Discerning between unique antifibrotic effects in these cases and modification of the fibrotic program due to reduction in inflammation may be difficult.

5.2.2 Th1 Cytokines

Despites its proinflammatory potential, IFNγ may also have anti-fibrotic effects. IFNγ can inhibit fibroblast proliferation and migration [21]. Treatment with IFNγ reduces collagen deposition associated with chronic granuloma formation in schistosomiasis-induced fibrosis [22]. Similar results were obtained in models of pulmonary and kidney fibrosis [23, 24]. IFNγ may exert some if its anti-fibrotic activity by suppressing profibrotic cytokines such as TGFβ through the action of Y box-binding protein YB-1 (YB-1). An orally administered agent that promotes nuclear translocation of YB-1 resulted in the improvement of murine liver fibrosis and TNBS-induced murine chronic colitis [25–27]. These outcomes were not replicated in human studies, however. A randomized trial of subcutaneously injected recombinant IFNγ did not demonstrate improvement in survival of patients with idiopathic pulmonary fibrosis (Table 5.1) [28].

5.2.3 IL-1 Cytokines

There are 11 members of the IL-1 family of ligands; IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-18, and recently, IL-33, have been studied in vitro, in animal models of disease, and in humans. In humans, IL-1β blockade has been utilized clinically. IL-1β is a cytokine with major roles in inflammation and innate immune

response. Activated monocytes, macrophages and dendritic cells produce IL-1β, which can then induce the production of additional pro-inflamamtory cytokines such as TNF-α and IL-6, or chemokines, as well as proteases associated with proliferation of resident fibroblasts [29]. Cell assembly of the NLRP3 inflammasome containing caspase 1 is required to cleave pro-IL-1 into active IL-1 [30]. Studies using KO mice for several components of inflammasome pathway including NLRP3, showed a reduction of IL-1β and consequent reduction of experimental pulmonary fibrosis induced by bleomycin [31]. In an alveolar basal epithelial cell line, IL-1β stimulates transcription of TGF-β [32]. Notably, collagen deposition is reduced in interleukin-1 (IL-1) receptor deficient mice [29]. IL-1β and inflammasome pathway have been reported to play an important role in chronic liver inflammation leading to fibrosis and cirrhosis [33]. In rats, IL-1Ra administration attenuated dimethylnitrosamin-induced liver cirrhosis [34]. In contrast to these data, however, in the gut, IL-1β has been shown to *downregulate* collagen production [35]. Moreover, it has been shown that corticosteroids repress the IL-1β-induced secretion of collagenase in human intestinal cells [36]. Thus, with regards to fibrosis, IL-1β may have tissue-specific, differing effects regarding fibrosis. Canakinumab, a human anti-IL-1β monoclonal antibody that neutralizes IL-1β signaling, has been developed for suppression of inflammation in patients with disorders of autoimmune origin. In 2009, the drug was approved by the FDA for the treatment of familial cold auto-inflammatory syndrome and Muckle-wells syndrome, which are inflammatory diseases associated with elevated IL-1β levels. It is currently undergoing clinical trials for a variety of inflammatory disorders, but not for yet fibrotic diseases [37].

IL-33 is a member of the IL-1 family, which behaves as both an extracellular cytokine and nuclear transcription factor [38], signaling through a unique receptor: suppression of tumorigenicity 2 (ST2) [39]. IL-33 was initially considered a potent activator of type 2 immune responses integral to adaptive immunity. However, IL-33 is now known play a role in both innate and adaptive immunity. IL-33 is released by epithelial and endothelial cells in response to cell injury and necrosis, thereby acting as an 'alarmin' to initiate the innate immune response. Recent studies have demonstrated nuclear IL-33 is important in synovial fibroblasts, skin keratinocytes, and bone-marrow-derived mast cells [39]. A recent study demonstrated that intestinal IL-33 expression is localized to the pericryptal fibroblasts during homeostasis and is increased during infection [40]. ST2 is expressed by various immune cells, most notably T cells, including Th1 cells, Th2 cells, group 2 innate lymphoid cells (ILC2s), regulatory T (Treg) cells, and CD8+ T cells.

Elevated expression of both IL-33 and ST2 has been reported in inflamed mucosa from IBD patients. Intestinal epithelial cells (IEC) and sub-epithelial myofibroblasts (SEMFs) have been identified as the principal source of IL-33 in UC, along with smooth muscle cells, endothelial cells and adipocytes [41, 42]. Studies in colitis mouse models have suggested a mixed role for IL-33/ST2 in disease, with IL-33 administration attenuating chronic colitis, but neutralization of ST2 resulting in amelioration of disease [43, 44]. Interestingly, although fibrosis is usually associated with CD, it has been reported that IL-33 is expressed in activated SEMFs situated

below ulcerative lesions predominantly in UC, as opposed to in CD [42, 45]. Recently, however, IL-33 has been associated with pediatric fibrostenosing CD patients [46].

As in experimental colitis, there have been mixed data regarding the effects of IL-33/ST2 on various fibrotic disaeases. Inhibition of IL-33 in mice suppressed bone marrow-derived fibroblast accumulation and myofibroblast formation in the kidneys after ischemia-reperfusion stress injury, which was associated with less expression of extracellular matrix proteins [47]. Increased hepatic IL-33 expression was noted in the murine bile-duct ligation (BDL) model of fibrosis and in surgical samples obtained from patients with liver fibrosis. Liver injury, inflammatory cell infiltration and fibrosis were reduced in the absence of ST2, and the activation of hepatic stellate cells (HSCs) was decreased in ST2-deficient mice. Interestingly, however, while administration of recombinant IL-33 significantly increased hepatic inflammation in sham-operated mice, it did not enhance BDL-induced hepatic fibrosis [48]. Similarly, endogenous IL-33 had no effect on the progression of fibrosis during experimental steatohepatitis [49]. Thus, mixed data and partially disparate roles for ST2 and IL-33 with regards to liver fibrosis have been demonstrated recently. Further studies are warranted to evaluate the impact of IL-33/ST2 on intestinal fibrosis.

5.2.4 Th2 Cytokines

Th2 cytokines, IL-4 and IL-13, promote fibroblast activation, proliferation, and collagen synthesis [50, 51]. IL-4 is increased in the bronchoalviolar lavage of patients with idiopathic pulmonary fibrosis [52]. IL-4 also increases the expression of collagen in cultured hepatic fibroblasts [53]. IL-13, which shares overlapping functions with IL-4 due to a common receptor subunit (IL-4-Receptor alpha), is involved in many Th2-mediated diseases and has a role in fibrosis as well. IL-13 signals through a complex receptor system comprised of IL-4Ralpha and two IL-13 binding proteins, IL-13Rα1 and IL-13Rα2. Many cell types express IL-13 receptors, including human hematopoietic cells, endothelial cells, fibroblasts, multiple epithelial cell types, and smooth muscle cells [54]. Intestinal samples from fibrotic CD patients expressed increased IL-13 mRNA. Fibroblasts from these samples expressed elevated levels of IL13Rα1 and subsequently down-regulated MMP in response to IL-13 [55]. Interestingly, in another study, elevated IL-13 production was not detected in UC or strictured CD [56]. These associations led to experimental IL-13 pathway targeting. In vivo inhibition of IL-13Rα2 decreased collagen deposition in bleomycin-induced lung fibrosis and reduced production of TGFβ1 in oxazolone-induced colitis [57]. In TNBS-induced colitis, similar inhibition of IL-13 signaling by targeting the IL-13Rα2 with small interfering RNA, reduces fibrosis and expression of TGFβ [58]. In another animal study, IL-13 blockade reduced experimental hepatic fibrosis [59]. With the experimental benefits of IL-13 antagonism, clinical trials with anti-IL-13 antibodies lebrikizumab and tralokinumab have been launched

for pulmonary fibrosis (NCT01872689, NCT01629667). The study with lebrikizumab is ongoing, but the trial with tralokinumab was terminated early due to lack of efficacy. Clinical studies targeting IL-13 or IL-13 receptor may be anticipated for fibrosis in CD.

5.2.5 Th17 Cytokines

IL-17A-F act through the IL-17 receptor and make up the IL-17 family of cytokines. IL-17 is a significant cytokine involved in chemokine production for granulocyte activation and increasing inflammation [60]. IL-17 has demonstrated pro-fibrotic function by enhancing activation pathways in human colonic myofibroblasts [61]. It also sustains fibrotic activity in a number of cells such as stellate cells [62] and lung epithelial cells [63]. Anti-IL-17A monoclonal antibody administered after the onset of myocarditis in mice mitigates cardiac fibrosis and maintains ventricular function [64]. As IL-17A supports the synthesis and secretion of collagen via epithelial-mesenchymal transition in alveolar epithelial cells, its blockade resolves bleomycin-induced acute inflammation, attenuates pulmonary fibrosis, and increases survival [63]. IL-17's contribution to CD is complicated, however, as both clinical and experimental data suggest divergent inflammatory and regulatory functions. IL-17's effects on clinical disease activity in animal models of IBD has resulted in contrasting findings depending on the model used [65]. In vitro experiments on human samples showed that IL17-stimulated myofibroblasts from CD strictures generate more collagen and TIMP-1 than controls, and intestinal tissues expressed elevated levels of IL-17A [66]. In a clinical trial of patients with inflammatory CD, blockade of IL-17A by administration of the anti-IL-17A antibody, secukinumab, failed to meet its primary endpoint [67]. A subgroup of patients who demonstrated clinical benefit from anti-IL-17 carried a *TNFSF15* (rs4263839) SNP in post hoc analysis, however. The potential functional consequences of this allele include elevated production of TL1A protein. Under TL1A-upregulated conditions in adoptive transfer-induced colitis, IL-17A deficiency ameliorated colonic inflammation via reducing Th1 and Th9 effector responses while enhancing regulatory responses [68]. Thus, there exists a subset of patients (those that overexpress TL1A due to e.g. a *TNFSF15* variant) who could potentially benefit from IL-17 blockade. As TL1A overexpression in this subset of patients may promote their fibrotic disease, IL-17 blockade may have a positive impact both on inflammation and fibrosis.

5.2.6 TL1A

TL1A (a protein encoded by *TNFSF15*) is a member of the TNF superfamily, modulates numerous cellular functions by binding to death domain receptor 3 (DR3, also known as TNFRSF25), which is expressed on a broad array of cells [69–71].

TL1A is produced by endothelial cells induced by IL-1β and TNFα, macrophages and dendritic cells in response to Toll-like receptor stimulation, as well as in some lymphoid lineage cells [72–75].

Developmental, immunoregulatory and pro-inflammatory effects have been described for DR3, which shares homology to TNFR1. Early work on DR3-deficient mice demonstrated that it is required for negative selection in the thymus and in embryonic cells, it can induce FADD- and caspase-8-dependent apoptosis [76, 77]. Conversely, however, DR3 activation of NF-KB in human cell lines upregulates c-IAP2, an NF-KB-dependent anti-apoptotic protein, which protects against apoptosis [78]. DR3 can also be upregulated on Th17 cells, promote T cell expansion, and cytokine production during immune responses [79–81]. The pro-inflammatory effects of TL1A-DR3 likely contribute to this pathway's effect on fibrosis, but more direct evidence has shown that DR3 is an important receptor for fibroblast development, maturation and function. Owing to the fact that DR3 is expressed on intestinal fibroblasts, DR3-deficient mice display reduced number of colonic fibroblasts, reduced fibroblast activation (as evidenced by decreased expression of alpha smooth muscle actin) and expression of collagen induced by TL1A stimulation [82].

Human IBD studies found that a TNFSF15 haplotype is associated with higher TL1A expression, increased risk of CD, intestinal fibrostenosis, and greater need for surgery [83–85]. Consistent with these findings, TL1A overexpression in mice causes spontaneous ileitis with increased collagen deposition [86, 87]. Under induced colitogenic conditions by chronic DSS treatment or adoptive T cell transfer, increased inflammation, fibrosis, and fibrostenotic lesions in the gut are seen [88]. These results support the role of TL1A in induction of intestinal inflammation and suggest its contribution to fibrogenesis in the gut. The potential for TL1A as a therapeutic target in intestinal fibrosis was demonstrated in a study evaluating the effect of anti-TL1A Ab in chronic DSS and adoptive T-cell transfer models of IBD. Treatment with neutralizing TL1A Ab attenuated disease and reversed colonic fibrosis. Additionally, TL1A blockade reduced the number of fibroblasts and myofibroblasts in colonic cell isolates and lowered expression of CTGF, TGFβ1 and IGF-1 [82]. The promising data with TL1A blockade in experimental IBD and increasing evidence as to its relevance in human disease makes TL1A a potential novel target for fibrosis.

5.3 "Regulatory" Cytokines

As mentioned previously, the frequency of fibrostenosing complications has still remained significant despite immunosuppressive therapy in CD patients in the form of steroids or immunomodulators. This may be due to some fibrotic pathways being separate from inflammatory pathways, or alternatively, the ability of some cytokines to promote inflammatory and anti-fibrotic effects simultaneously. Consequently, significant attention has been devoted to targeting those cytokines that might be involved in the "aftermath" of the inflammatory assault, regulating immune function and stimulating tissue repair.

5.3.1 TGFβ

TGFβ is a pleiotropic cytokine inducing proliferation, differentiation, inflammation, immunoregulation, wound healing and fibrosis [89]. TGFβ is perhaps the most widely studied cytokine relevant to fibrosis. Elevated levels of TGFβ and its receptors have been described with numerous fibrotic including heart, lungs, liver, kidney, skin, and intestines. Likewise, genetic over-expression or exogenous administration of TGFβ in animals promotes wide-spread fibrotic disease [90]. TGFβ supports activation and differentiation of fibroblasts and production of collagen and fibronectin, expression of adhesive receptors and contractile elements, and inhibition of matrix metalloproteinases [89, 91, 92]. TGFβ can also induce fibrogenesis via additional mechanisms of fibrosis including epithelial to mesenchymal transition and endothelial to mesenchymal transition [93]. Role of TGFβ and therapeutic targets in fibrosis is further described below and summarized in Table 5.1.

Three main isoforms of TGFβ exist: TGFβ1, TGFβ2, and TGFβ3. These isoforms are secreted as latent precursor molecules containing a latency associated peptide region (LAP), and complexed with latent TGFβ binding proteins (LTBP). The cytokine is active when LTBP is removed extracellularly via proteolytic cleavage by proteases such as plasmin or thrombin; or by interactions of LAP with other proteins such as thrombospondin-1 or integrins [89]. TGFβ signals through two receptors, TGFβR1 and TGFβR2. These receptors form transmembrane serine/threonine kinase, hetero- or homo-dimeric complexes that induce phosphorylation of Smad 2 and Smad 3 proteins. Once phosphorylated, Smad 2 and 3 complex with Smad 4, translocate to the nucleus, and activate transcription. Smad 7 regulates Smad 2/3, by inhibiting binding of Smad 2/3 to the receptor complex. TGFβ can also signal through ERK1/2, c-Jun N terminal kinase, p38 kinases and members of the JAK/STAT family [89, 94].

TGFβ, is also a potent immune modulator central to immune tolerance and development of innate and adaptive immunoregulatory cells. Systemic blockade of TGFβ might therefore upset vital immune homeostasis resulting in troublesome effects. Alternatively, complete antagonism of TGFβ might be ineffective due simultaneous blockade of fibrogenic and regulatory functions. Thus, several direct TGFβ antagonists were found to be ineffective or led to possible drug associated mortality [95, 96]. In an attempt to inhibit TGFβ-driven fibrosis while sparing its immunomodulatory effects, alternative strategies have focused on specific pathways in TGFβ signaling, synthesis, activation, or other downstream mediators. Accordingly, blockade of TGFβR1 signaling by an injectable inhibitor (SD-208) was evaluated in two experimental animal models of intestinal fibrosis: anaerobic bacteria- and trinitrobenzensulphonic acid-induced colitis (TNBS). SD-208 reduced fibroblast activation, phosphorylation of Smad 2 and Smad 3 proteins, and intestinal wall collagen deposition in both models [97]. Similarly, more recent studies on blockade of TGFβR1 with oral inhibitors have demonstrated efficacy in animal models of renal fibrosis, carbon tetrachloride- or bile duct ligation-induced cirrhosis

[98, 99], pressure-overload-induced cardiac fibrosis [100], and bleomycin-induced pulmonary fibrosis [101]. These agents are being investigated in oncologic trials, with testing ongoing for fibrotic disorders.

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone) is another orally-administered molecule that has demonstrated anti-fibrotic effects partly by inhibiting synthesis of TGFβ. This agent has been efficacious in patients and experimental models of pulmonary and renal fibrosis [102, 103]. Pirfenidone has been evaluated in randomized, double-blind, placebo-controlled clinical trials where it reduced the rate of decline in lung function as well as improved mortality [104, 105]. Consequently, it has been approved in Europe and by the FDA for treatment of IPF. Pirfenidone, however, has not been consistently efficacious in all trials. No clinical or histologic benefits were observed in myelofibrosis [106], or primary sclerosing cholangitis, while being associated with increased adverse events [107].

Downregulation of TGFβ without known adverse immunological effects has been demonstrated by two classes of medications currently in widespread use in primary care: HMG-CoA reductase inhibitors (statins) and antagonists of Renin-Angiotensin system (RAS). Statins may reduce fibrosis, in part, through decreasing expression of TGFβ. Simvastatin reduces TGFβ1 expression in human fibroblasts by inhibition of Smad 3 phosphorylation [108]. In TNBS-induced colitis, it had anti-fibrotic effects by decreasing the level of connective tissue growth factor (CTGF) and inducing apoptosis in fibroblasts [109]. As the primary mediator of the RAS, Angiotensin may contribute to fibrogenesis via induction of TGFβ expression and promotion of collagen production [110]. With regards to intestinal fibrosis, early studies have reported that Angiotensin is increased in the mucosa of CD patients [111]. In TNBS-induced colitis, administration of the ACE inhibitor, captopril, or the angiotensin receptor blocker, losartan, reduced colonic inflammation and fibrosis via reduction in TGFβ [112, 113]. Given the safety and ubiquity of statins and RAS antagonists, future investigations will be feasible and determine if they are capable of favorably impacting fibrogenesis.

An important regulatory step in TGFβ signaling, which might be targeted therapeutically, is the activation of TGFβ from its latent precursor state. AlphaV (αV)-type integrins can bind LAP and activate TGFβ. Integrin-blocking therapeutics such as vedolizumab, have proven effective with regards to inflammation in IBD. These agents may reduce fibrosis via their effects on TGFβ activation. For example, αVβ6 integrin is upregulated in various fibrotic diseases and its blockade has been effective in models of pulmonary fibrosis and liver fibrosis [114]. Similarly, αVβ3 integrin contributes to excess smooth muscle cell proliferation and hyperplasia in intestinal strictures of CD [115]. Cilengitide, an αVβ3 inhibitor, reduces the development of fibrosis in chronic TNBS-induced colitis [116]. Future studies will determine if integrin inhibitors will be effective at treating fibrosis in IBD.

Targeting specific mediators in the TGFβ signaling cascade represents another possibility. This option may provide more specificity by focusing on individual mediators of TGFβ signaling, rather than TGFβ itself. Two such potential strategies are Smad 3 antagonism and Smad 7 agonism. Increased Smad 3 and decreased

Smad 7 expression have been observed in intestinal strictures in CD [117]. Furthermore, in multiple animal models, loss of Smad 3 or increase in Smad 7 confers resistance to fibrosis in several organs [118–120]. There has been focus on inhibition of Smad 7 in IBD via antisense oligonucleotides (and subsequent increase in Smad 3 transduction with potential TGFβ-mediated shift towards immune-regulation). This strategy may be problematic with regards to fibrogenesis, however. An ideal solution might be to clearly identify those patients that would be more prone to develop fibrotic/stricturing disease vs predominantly inflammatory pathology through functional, genetic and epigenetic studies.

5.3.2 IL-10

As a product of regulatory T cells, IL-10 has an established role with regards to immune regulation [121]. In contrast to TGFβ, however, IL-10 has been shown to inhibit fibrosis. Mice treated with IL-10 develop less liver and lung fibrosis when administered carbon tetrachloride or bleomycin [122, 123]. Similarly, IL-10 deficiency aggravates kidney inflammation and fibrosis in the unilateral ureteral obstruction mouse model [124]. With regards to human IBD, however, although polymorphisms in the IL-10 locus have been associated with IBD [125], treatment of CD patients with recombinant IL-10 has not been significantly effective (Table 5.1) [126].

5.4 Concluding Remarks

Cytokine targeting has proven to be effective in treating inflammation in IBD. Cytokine blockade for intestinal fibrosis has been challenging, however, given the multiple diverging and converging pathways of many cytokines. The genetic heterogeneity present across patient populations may also cause diverse pathogenesis of disease; broad cytokine targeting may then result in contrasting rates of response. Indeed, this has been observed with anti-TNF agents in terms of inflammation, and may be one source of failure of some clinical trials with newer anti-cytokine agents. A potential approach to overcome this difficulty may involve careful selection of patients based on genetic or biochemical characteristics. Additionally, there are promising targets being explored for other fibrotic conditions that may be of benefit in CD and warrant investigation. Given the variables that contribute to fibrostenosis in CD, targeting of multiple culprits in the fibrotic process, in addition to the cytokines themselves, may be an option. Future investigations into novel fibrogenic pathways may lead to more selective therapeutic targets, as well as the identification of specific patient groups that could best benefit from precision treatment.

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References

1. Cosnes J, Gower-Rousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140:1785–94.
2. Latella G, Papi C. Crucial steps in the natural history of inflammatory bowel disease. World J Gastroenterol. 2012;18:3790–9.
3. Spinelli A, Correale C, Szabo H, et al. Intestinal fibrosis in Crohn's disease: medical treatment or surgery? Curr Drug Targets. 2010;11:242–8.
4. Theiss AL, Simmons JG, Jobin C, et al. Tumor necrosis factor (TNF) alpha increases collagen accumulation and proliferation in intestinal myofibroblasts via TNF receptor 2. J Biol Chem. 2005;280:36099–109.
5. Bahcecioglu IH, Koca SS, Poyrazoglu OK, et al. Hepatoprotective effect of infliximab, an anti-TNF-alpha agent, on carbon tetrachloride-induced hepatic fibrosis. Inflammation. 2008;31:215–21.
6. Khan SB, Cook HT, Bhangal G, et al. Antibody blockade of TNF-alpha reduces inflammation and scarring in experimental crescentic glomerulonephritis. Kidney Int. 2005;67:1812–20.
7. Trachtman H, Vento S, Herreshoff E, et al. Efficacy of galactose and adalimumab in patients with resistant focal segmental glomerulosclerosis: report of the font clinical trial group. BMC Nephrol. 2015;16:111.
8. Antoniou KM, Mamoulaki M, Malagari K, et al. Infliximab therapy in pulmonary fibrosis associated with collagen vascular disease. Clin Exp Rheumatol. 2007;25:23–8.
9. Bargagli E, Galeazzi M, Bellisai F, et al. Infliximab treatment in a patient with systemic sclerosis associated with lung fibrosis and pulmonary hypertension. Respiration. 2008;75:346–9.
10. Denton CP, Engelhart M, Tvede N, et al. An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. Ann Rheum Dis. 2009;68(9):1433.
11. Abraham DJ, Shiwen X, Black CM, et al. Tumor necrosis factor alpha suppresses the induction of connective tissue growth factor by transforming growth factor-beta in normal and scleroderma fibroblasts. J Biol Chem. 2000;275:15220–5.
12. Bitzer M, von Gersdorff G, Liang D, et al. A mechanism of suppression of TGF-beta/SMAD signaling by NF-kappa B/RelA. Genes Dev. 2000;14:187–97.
13. Mori R, Kondo T, Ohshima T, et al. Accelerated wound healing in tumor necrosis factor receptor p55-deficient mice with reduced leukocyte infiltration. FASEB J. 2002;16:963–74.
14. Di Sabatino A, Pender SL, Jackson CL, et al. Functional modulation of Crohn's disease myofibroblasts by anti-tumor necrosis factor antibodies. Gastroenterology. 2007;133:137–49.
15. Lichtenstein GR, Olson A, Travers S, et al. Factors associated with the development of intestinal strictures or obstructions in patients with Crohn's disease. Am J Gastroenterol. 2006;101:1030–8.
16. Sorrentino D, Avellini C, Beltrami CA, et al. Selective effect of infliximab on the inflammatory component of a colonic stricture in Crohn's disease. Int J Color Dis. 2006;21:276–81.
17. Pelletier AL, Kalisazan B, Wienckiewicz J, et al. Infliximab treatment for symptomatic Crohn's disease strictures. Aliment Pharmacol Ther. 2009;29:279–85.
18. Swaminath A, Lichtiger S. Dilation of colonic strictures by intralesional injection of infliximab in patients with Crohn's colitis. Inflamm Bowel Dis. 2008;14:213–6.

19. Jones DW, Finlayson SR. Trends in surgery for Crohn's disease in the era of infliximab. Ann Surg. 2010;252:307–12.
20. Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? Gut. 2011;60:1178–81.
21. Adelmann-Grill BC, Hein R, Wach F, et al. Inhibition of fibroblast chemotaxis by recombinant human interferon gamma and interferon alpha. J Cell Physiol. 1987;130:270–5.
22. Wynn TA, Cheever AW, Jankovic D, et al. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. Nature. 1995;376:594–6.
23. Gurujeyalakshmi G, Giri SN. Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. Exp Lung Res. 1995;21:791–808.
24. Oldroyd SD, Thomas GL, Gabbiani G, et al. Interferon-gamma inhibits experimental renal fibrosis. Kidney Int. 1999;56:2116–27.
25. Higashi K, Inagaki Y, Fujimori K, et al. Interferon-gamma interferes with transforming growth factor-beta signaling through direct interaction of YB-1 with Smad3. J Biol Chem. 2003;278:43470–9.
26. Higashi K, Tomigahara Y, Shiraki H, et al. A novel small compound that promotes nuclear translocation of YB-1 ameliorates experimental hepatic fibrosis in mice. J Biol Chem. 2011;286:4485–92.
27. Imai J, Hozumi K, Sumiyoshi H, et al. Anti-fibrotic effects of a novel small compound on the regulation of cytokine production in a mouse model of colorectal fibrosis. Biochem Biophys Res Commun. 2015;468:554–60.
28. King TE Jr, Albera C, Bradford WZ, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet. 2009;374:222–8.
29. Gasse P, Mary C, Guenon I, Noulin N, Charron S, Schnyder-Candrian S, Schnyder B, Akira S, Quesniaux VF, Lagente V, et al. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. J Clin Invest. 2007;117:3786–99.
30. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 2009;27:519–50.
31. Gasse P, Riteau N, Charron S, Girre S, Fick L, Pétrilli V, Tschopp J, Lagente V, Quesniaux VF, Ryffel B, Couillin I. Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis. Am J Respir Crit Care Med. 2009;179:903–13.
32. Lee KY, Ito K, Hayashi R, Jazrawi EP, Barnes PJ, Adcock IM. NF-kappaB and activator protein 1 response elements and the role of histone modifications in IL-1beta-induced TGF-beta1 gene transcription. J Immunol. 2006;176:603–15.
33. Szabo G, Csak T. Inflammasomes in liver diseases. J Hepatol. 2012;57:642–54.
34. Mancini R, Benedetti A, Jezequel AM. An interleukin-1 receptor antagonist decreases fibrosis induced by dimethylnitrosamine in rat liver. Virchows Arch. 1994;424:25–31.
35. Graham MF, Willey A, Adams J, Yager D, Diegelmann RF. Interleukin 1 beta downregulates collagen and augments collagenase expression in human intestinal smooth muscle cells. Gastroenterology. 1996;110(2):344–50.
36. Graham MF, Willey A, Zhu YN, Yager DR, Sugerman HJ, Diegelmann RF. Corticosteroids repress the interleukin 1 beta-induced secretion of collagenase in human intestinal smooth muscle cells. Gastroenterology. 1997;113(6):1924–9.
37. Dhimolea E. Canakinumab. MAbs. 2010;2(1):3–13.
38. Ali S. The dual function cytokine IL-33 interacts with the transcription factor NF-kB to dampen NF-kB-stimulated gene transcription. J Immunol. 2011;187:1609–16.
39. Hodzic Z, Schill EM, Bolock AM, Good M. IL-33 and the intestine: the good, the bad, and the inflammatory. Cytokine. 2017;S1043-4666(17):30189–8.
40. Mahapatro M, Foersch S, Hefele M, He G-W, Giner-Ventura E, et al. Programming of intestinal epithelial differentiation by IL-33 derived from pericryptal fibroblasts in response to systemic infection. Cell Rep. 2016;15(8):1743–56.

41. Pastorelli L, De Salvo C, Vecchi M, Pizarro TT. The role of IL-33 in gut mucosal inflammation. Mediat Inflamm. 2013;2013:608187.
42. Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. Am J Pathol. 2010;177(6):2804–15.
43. Groβ P, Doser K, Falk W, Obermeier F, Hofmann C. IL-33 attenuates development and perpetuation of chronic intestinal inflammation. Inflamm Bowel Dis. 2012;18(10):1900–9.
44. Sedhom MAK, Pichery M, Murdoch JR, Foligné B, Ortega N, Normand S, et al. Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. Gut. 2013;62(12):1714–23.
45. Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. J Gastroenterol. 2010;45(10):999–1007.
46. Masterson JC, Capocelli KE, Hosford L, Biette K, McNamee EN, de Zoeten EF, et al. Eosinophils and IL-33 perpetuate chronic inflammation and fibrosis in a pediatric population with stricturing Crohn's ileitis. Inflamm Bowel Dis. 2015;21(10):2429–40.
47. Liang H, Xu F, Wen XJ, Liu HZ, Wang HB, et al. Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion. Eur J Pharmacol. 2017;812:18.
48. Tan Z, Liu Q, Jiang R, Lv L, Shoto SS, et al. Interleukin-33 drives hepatic fibrosis through activation of hepatic stellate cells. Cell Mol Immunol. 2017. https://doi.org/10.1038/cmi.2016.63.
49. Vasseur P, Dion S, Filliol A, Genet V, Lucas-Clerc C, et al. Endogenous IL-33 has no effect on the progression of fibrosis during experimental steatohepatitis. Oncotarget. 2017. https://doi.org/10.18632/oncotarget.18335.
50. Doucet C, Brouty-Boye D, Pottin-Clemenceau C, et al. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. J Clin Invest. 1998;101:2129–39.
51. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol. 2004;4:583–94.
52. Jakubzick C, Kunkel SL, Puri RK, et al. Therapeutic targeting of IL-4- and IL-13-responsive cells in pulmonary fibrosis. Immunol Res. 2004;30:339–49.
53. Aoudjehane L, Pissaia A Jr, Scatton O, et al. Interleukin-4 induces the activation and collagen production of cultured human intrahepatic fibroblasts via the STAT-6 pathway. Lab Investig. 2008;88:973–85.
54. Hershey GK. IL-13 receptors and signaling pathways: an evolving web. J Allergy Clin Immunol. 2003;111:677–90; quiz 691.
55. Bailey JR, Bland PW, Tarlton JF, et al. IL-13 promotes collagen accumulation in Crohn's disease fibrosis by down-regulation of fibroblast MMP synthesis: a role for innate lymphoid cells? PLoS One. 2012;7:e52332.
56. Biancheri P, Di Sabatino A, Ammoscato F, et al. Absence of a role for interleukin-13 in inflammatory bowel disease. Eur J Immunol. 2014;44:370–85.
57. Fichtner-Feigl S, Strober W, Kawakami K, et al. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med. 2006;12:99–106.
58. Fichtner-Feigl S, Young CA, Kitani A, et al. IL-13 signaling via IL-13R alpha2 induces major downstream fibrogenic factors mediating fibrosis in chronic TNBS colitis. Gastroenterology. 2008;135:2003–13, 2013.e1-7.
59. Chiaramonte MG, Donaldson DD, Cheever AW, et al. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. J Clin Invest. 1999;104:777–85.
60. Maloy KJ. The Interleukin-23/Interleukin-17 axis in intestinal inflammation. J Intern Med. 2008;263:584–90.
61. Hata K, Andoh A, Shimada M, et al. IL-17 stimulates inflammatory responses via NF-kappaB and MAP kinase pathways in human colonic myofibroblasts. Am J Physiol Gastrointest Liver Physiol. 2002;282:G1035–44.

62. Meng F, Wang K, Aoyama T, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology. 2012;143:765–76.e1-3.
63. Mi S, Li Z, Yang HZ, et al. Blocking IL-17A promotes the resolution of pulmonary inflammation and fibrosis via TGF-beta1-dependent and -independent mechanisms. J Immunol. 2011;187:3003–14.
64. Baldeviano GC, Barin JG, Talor MV, et al. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. Circ Res. 2010;106:1646–55.
65. Khanna PV, Shih DQ, Haritunians T, et al. Use of animal models in elucidating disease pathogenesis in IBD. Semin Immunopathol. 2014;36:541–51.
66. Biancheri P, Pender SL, Ammoscato F, et al. The role of interleukin 17 in Crohn's disease-associated intestinal fibrosis. Fibrogenesis Tissue Repair. 2013;6:13.
67. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut. 2012;61:1693–700.
68. Wallace KL, Zheng L, Kanazawa Y, et al. TL1A modulates the differential effect of IL-17 blockade on mucosal inflammation. Gastroenterology. 2014;146:S-133.
69. Kitson J, Raven T, Jiang YP, et al. A death-domain-containing receptor that mediates apoptosis. Nature. 1996;384:372–5.
70. Chinnaiyan AM, O'Rourke K, Yu GL, et al. Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. Science. 1996;274:990–2.
71. Tan KB, Harrop J, Reddy M, et al. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells. Gene. 1997;204:35–46.
72. Bodmer JL, Burns K, Schneider P, et al. TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis factor receptor 1 and Fas(Apo-1/CD95). Immunity. 1997;6:79–88.
73. Al-Lamki RS, Wang J, Tolkovsky AM, et al. TL1A both promotes and protects from renal inflammation and injury. J Am Soc Nephrol. 2008;19:953–60.
74. Bamias G, Mishina M, Nyce M, et al. Role of TL1A and its receptor DR3 in two models of chronic murine ileitis. Proc Natl Acad Sci U S A. 2006;103:8441–6.
75. Prehn JL, Thomas LS, Landers CJ, et al. The T cell costimulator TL1A is induced by FcgammaR signaling in human monocytes and dendritic cells. J Immunol. 2007;178:4033–8.
76. Varfolomeev EE, Schuchmann M, Luria V, et al. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. Immunity. 1998;9:267–76.
77. Wang EC, Thern A, Denzel A, et al. DR3 regulates negative selection during thymocyte development. Mol Cell Biol. 2001;21:3451–61.
78. Wen L, Zhuang L, Luo X, et al. TL1A-induced NF-kappaB activation and c-IAP2 production prevent DR3-mediated apoptosis in TF-1 cells. J Biol Chem. 2003;278:39251–8.
79. Pappu BP, Borodovsky A, Zheng TS, et al. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. J Exp Med. 2008;205:1049–62.
80. Migone TS, Zhang J, Luo X, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity. 2002;16:479–92.
81. Meylan F, Davidson TS, Kahle E, et al. The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. Immunity. 2008;29:79–89.
82. Shih DQ, Zheng L, Zhang X, et al. Inhibition of a novel fibrogenic factor Tl1a reverses established colonic fibrosis. Mucosal Immunol. 2014;7:1492–503.
83. Picornell Y, Mei L, Taylor K, et al. TNFSF15 is an ethnic-specific IBD gene. Inflamm Bowel Dis. 2007;13:1333–8.
84. Michelsen KS, Thomas LS, Taylor KD, et al. IBD-associated TL1A gene (TNFSF15) haplotypes determine increased expression of TL1A protein. PLoS One. 2009;4:e4719.

85. Hirano A, Yamazaki K, Umeno J, et al. Association study of 71 European Crohn's disease susceptibility loci in a Japanese population. Inflamm Bowel Dis. 2013;19:526–33.
86. Shih DQ, Barrett R, Zhang X, et al. Constitutive TL1A (TNFSF15) expression on lymphoid or myeloid cells leads to mild intestinal inflammation and fibrosis. PLoS One. 2011;6:e16090.
87. Meylan F, Song YJ, Fuss I, et al. The TNF-family cytokine TL1A drives IL-13-dependent small intestinal inflammation. Mucosal Immunol. 2011;4:172–85.
88. Barrett R, Zhang X, Koon HW, et al. Constitutive TL1A expression under colitogenic conditions modulates the severity and location of gut mucosal inflammation and induces fibrostenosis. Am J Pathol. 2012;180:636–49.
89. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. FASEB J. 2004;18:816–27.
90. Wells RG. V. TGF-beta signaling pathways. Am J Physiol Gastrointest Liver Physiol. 2000;279:G845–50.
91. McKaig BC, McWilliams D, Watson SA, et al. Expression and regulation of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinases by intestinal myofibroblasts in inflammatory bowel disease. Am J Pathol. 2003;162:1355–60.
92. Mulsow JJ, Watson RW, Fitzpatrick JM, et al. Transforming growth factor-beta promotes profibrotic behavior by serosal fibroblasts via PKC and ERK1/2 mitogen activated protein kinase cell signaling. Ann Surg. 2005;242:880–7, discussion 887-9.
93. Flier SN, Tanjore H, Kokkotou EG, et al. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem. 2010;285:20202–12.
94. Tsukada S, Westwick JK, Ikejima K, et al. SMAD and p38 MAPK signaling pathways independently regulate alpha1(I) collagen gene expression in unstimulated and transforming growth factor-beta-stimulated hepatic stellate cells. J Biol Chem. 2005;280:10055–64.
95. Denton CP, Merkel PA, Furst DE, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007;56:323–33.
96. Rice LM, Padilla CM, McLaughlin SR, et al. Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. J Clin Invest. 2015;125:2795–807.
97. Medina C, Santos-Martinez MJ, Santana A, et al. Transforming growth factor-beta type 1 receptor (ALK5) and Smad proteins mediate TIMP-1 and collagen synthesis in experimental intestinal fibrosis. J Pathol. 2011;224:461–72.
98. Park SA, Kim MJ, Park SY, et al. EW-7197 inhibits hepatic, renal, and pulmonary fibrosis by blocking TGF-beta/Smad and ROS signaling. Cell Mol Life Sci. 2015;72:2023–39.
99. Moon JA, Kim HT, Cho IS, et al. IN-1130, a novel transforming growth factor-beta type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy. Kidney Int. 2006;70:1234–43.
100. Engebretsen KV, Skardal K, Bjornstad S, et al. Attenuated development of cardiac fibrosis in left ventricular pressure overload by SM16, an orally active inhibitor of ALK5. J Mol Cell Cardiol. 2014;76:148–57.
101. Koh RY, Lim CL, Uhal BD, et al. Inhibition of transforming growth factor-beta via the activin receptor-like kinase-5 inhibitor attenuates pulmonary fibrosis. Mol Med Rep. 2015;11:3808–13.
102. Iyer SN, Wild JS, Schiedt MJ, et al. Dietary intake of pirfenidone ameliorates bleomycin-induced lung fibrosis in hamsters. J Lab Clin Med. 1995;125:779–85.
103. Shimizu T, Kuroda T, Hata S, et al. Pirfenidone improves renal function and fibrosis in the post-obstructed kidney. Kidney Int. 1998;54:99–109.
104. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377:1760–9.
105. King TE Jr, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2083–92.
106. Mesa RA, Tefferi A, Elliott MA, et al. A phase II trial of pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone), a novel anti-fibrosing agent, in myelofibrosis with myeloid metaplasia. Br J Haematol. 2001;114:111–3.

107. Angulo P, MacCarty RL, Sylvestre PB, et al. Pirfenidone in the treatment of primary sclerosing cholangitis. Dig Dis Sci. 2002;47:157–61.
108. Burke JP, Watson RW, Murphy M, et al. Simvastatin impairs smad-3 phosphorylation and modulates transforming growth factor beta1-mediated activation of intestinal fibroblasts. Br J Surg. 2009;96:541–51.
109. Abe Y, Murano M, Murano N, et al. Simvastatin attenuates intestinal fibrosis independent of the anti-inflammatory effect by promoting fibroblast/myofibroblast apoptosis in the regeneration/healing process from TNBS-induced colitis. Dig Dis Sci. 2012;57:335–44.
110. Bataller R, Gines P, Nicolas JM, et al. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. Gastroenterology. 2000;118:1149–56.
111. Jaszewski R, Tolia V, Ehrinpreis MN, et al. Increased colonic mucosal angiotensin I and II concentrations in Crohn's colitis. Gastroenterology. 1990;98:1543–8.
112. Wengrower D, Zanninelli G, Pappo O, et al. Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. Inflamm Bowel Dis. 2004;10:536–45.
113. Wengrower D, Zanninelli G, Latella G, et al. Losartan reduces trinitrobenzene sulphonic acid-induced colorectal fibrosis in rats. Can J Gastroenterol. 2012;26:33–9.
114. Sullivan BP, Weinreb PH, Violette SM, et al. The coagulation system contributes to alphaVbeta6 integrin expression and liver fibrosis induced by cholestasis. Am J Pathol. 2010;177:2837–49.
115. Flynn RS, Murthy KS, Grider JR, et al. Endogenous IGF-I and alphaVbeta3 integrin ligands regulate increased smooth muscle hyperplasia in stricturing Crohn's disease. Gastroenterology. 2010;138:285–93.
116. Li C, Flynn RS, Grider JR, et al. Increased activation of latent TGF-beta1 by alphaVbeta3 in human Crohn's disease and fibrosis in TNBS colitis can be prevented by cilengitide. Inflamm Bowel Dis. 2013;19:2829–39.
117. Di Sabatino A, Jackson CL, Pickard KM, et al. Transforming growth factor beta signalling and matrix metalloproteinases in the mucosa overlying Crohn's disease strictures. Gut. 2009;58:777–89.
118. Latella G, Vetuschi A, Sferra R, et al. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colorectal fibrosis. Eur J Clin Investig. 2009;39:145–56.
119. Latella G, Vetuschi A, Sferra R, et al. Targeted disruption of Smad3 confers resistance to the development of dimethylnitrosamine-induced hepatic fibrosis in mice. Liver Int. 2009;29:997–1009.
120. Dooley S, Hamzavi J, Breitkopf K, et al. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rats. Gastroenterology. 2003;125:178–91.
121. Asseman C, Mauze S, Leach MW, et al. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med. 1999;190:995–1004.
122. Louis H, Van Laethem JL, Wu W, et al. Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. Hepatology. 1998;28:1607–15.
123. Nakagome K, Dohi M, Okunishi K, et al. In vivo IL-10 gene delivery attenuates bleomycin induced pulmonary fibrosis by inhibiting the production and activation of TGF-beta in the lung. Thorax. 2006;61:886–94.
124. Jin Y, Liu R, Xie J, et al. Interleukin-10 deficiency aggravates kidney inflammation and fibrosis in the unilateral ureteral obstruction mouse model. Lab Investig. 2013;93:801–11.
125. Aithal GP, Craggs A, Day CP, et al. Role of polymorphisms in the interleukin-10 gene in determining disease susceptibility and phenotype in inflamatory bowel disease. Dig Dis Sci. 2001;46:1520–5.
126. Marlow GJ, van Gent D, Ferguson LR. Why interleukin-10 supplementation does not work in Crohn's disease patients. World J Gastroenterol. 2013;19:3931–41.