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Stromal and immune cells in gut fibrosis: the myofibroblast and the scarface

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Abstract

Post-inflammatory scarring is the end-result of excessive extracellular matrix (ECM) accumulation and tissue architectural destruction. It represents a failure to effectively remodel ECM and achieve proper reinstitution and healing during chronic relapsing inflammatory processes. Scarring may affect the functionality of any organ, and in the case of inflammatory bowel disease (IBD)-associated fibrosis leads to stricture formation and often surgery to remove the affected bowel. The activated myofibroblast is the final effector cell that overproduces ECM under the influence of various mediators generated by an intense interplay of classic and non-classic immune cells. This review focuses on how proinflammatory mediators from various sources produced in different stages of intestinal inflammation can form profibrotic pathways that eventually lead to tissue scarring through sustained activation of myofibroblasts.

Keywords Intestinal fibrosis, inflammatory bowel disease, myofibroblast, extracellular matrix, Crohn's disease, ulcerative colitis

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Introduction

Inflammatory bowel diseases (IBDs) comprise ulcerative colitis (UC) and Crohn's disease (CD) and are characterized by chronic and recurrent inflammation of the intestinal tract [1]. Their pathophysiology is yet to be fully elucidated. Nonetheless, accumulated scientific data suggest that IBDs are caused by aberrant immune responses to the intestinal microflora, triggered by various environmental insults, in the context of a susceptible genetic background of the host [1]. The recurrent nature of IBD is characterized by repeated cycles of tissue inflammation, ulceration and repair that ultimately lead to tissue scarring [2]. Scarring is the end result of fibrosis, a process of excessive accumulation of extracellular matrix (ECM), and especially collagen, combined with the failure to effectively remodel ECM, that ultimately leads to the formation of strictures [2]. The clinical impact of this process is major in CD patients with the "fibrostenosing" phenotype, where symptoms and complications associated with small bowel obstruction predominate over those related to intestinal inflammation. In contrast, intestinal fibrosis and bowel obstruction rarely dominate the clinical picture in UC, as the inflammation is superficial and confined to the colon, which has a larger luminal diameter [3].

Although chronic intestinal inflammation is absolutely required for the development of fibrosis, it plays a minor role once fibrosis is established [4]. This is supported by the fact that anti-inflammatory medication has minimal or no effect on fibrostenotic CD, where surgery is still the only treatment option. Unfortunately, intestinal strictures are likely to recur following surgical treatment [4]. Specifically, almost 30% of patients with CD and 5% of patients with UC will have to undergo surgery for the treatment of intestinal strictures, with the risk of recurrence reaching 50% [5]. A considerable number of CD patients will be subjected to repeated surgeries, and occasionally, following resection of a significant part of the small bowel, will suffer from short bowel syndrome and malnutrition [6]. Therefore, there is an urgent need for the development of drugs that could prevent or even reverse intestinal fibrosis [5].

Under physiological conditions, acute inflammation leads to the activation of mesenchymal cells that increase the production of ECM in an effort to heal the wound. Chronic accumulation of immune cells and the constant release of inflammatory mediators lead to persistent tissue damage and excessive production of ECM [3]. The myofibroblast seems to be

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Conflict of Interest: None

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the protagonist in this process, being the leading mesenchymal cell in producing ECM components [7,8]. This review focuses on the interactions between immune cells and myofibroblasts that contribute to fibrosis and highlights pathways that could be potentially targeted.

Intestinal stricturing and ECM remodeling failure

Intestinal strictures are developed by thickening of all layers of the intestinal wall. This is mediated via hyperplasia and hypertrophy of smooth muscle cells, leading to hypertrophic and disordered muscularis layers, and increased production and disorganized deposition of ECM [9]. ECM consists of various structural proteins, such as collagen, glycoproteins and proteoglycans. Type I collagen is the major collagen subtype found in normal bowel, followed by type III and type V. Bowel fibrosis is characterized by an increase in the total collagen content, as well as an increase in the relative amounts of types III and V [10].

ECM remodeling is regulated by two different groups of proteins, the matrix metalloproteinases (MMPs) and their inhibitors. MMPs are proteolytic enzymes that cleave various ECM components. Different classes of MMPs cleave different ECM substrates, sometimes acting sequentially—for example in the case of collagen, initially cleaved by collagenases and then by gelatinases. MMPs can be regulated either transcriptionally, by various factors such as cytokines, growth factors, hormones, cell-cell and cell-matrix interactions, or post-transcriptionally and through epigenetics [11]. Among other important MMP regulators are the tissue inhibitors of metalloproteinases (TIMP)-1-4 [9]. It has been suggested that an imbalance between MMPs and TIMPs, due to a decrease in MMP activity and/or an increase in TIMP activity, may lead to ECM accumulation and eventually fibrogenesis [9]. The contribution of the imbalance between MMPs and TIMPs in tissue fibrosis has been demonstrated by studies in mice that lack TIMP-1. Specifically, TIMP-1 depletion in the chronic model of dextran sulfate sodium (DSS)-induced colitis resulted in reduced inflammation and collagen disposition, with elevated expression levels of MMP-9, but not MMP-2 [12].

The theory of MMP/TIMP imbalance contributing to intestinal fibrosis is also supported by descriptive studies in patients with fibrostenotic CD. Intestinal subepithelial myofibroblasts (SEMFs) isolated from fibrotic areas of patients with CD exhibited increased TIMP-1 transcripts and unchanged expression of MMPs compared to healthy controls or UC [13]. Treatment with transforming growth factor (TGF)-β1 on SEMFs isolated from healthy individuals resulted in TIMP-1 induction [13], whereas blockage of TGF-β in normal intestinal biopsies resulted in higher levels of MMP-3 but not TIMP-1 [14]. In addition, higher levels of TIMP-1, along with lower levels of MMP-12 and -3 transcripts were reported in mucosa overlying uninflamed strictured areas, when compared to mucosa lining uninflamed or inflamed non-strictured regions [15]. Resent studies indicate that elevated MMP-9 levels can be found in feces of patients with active UC; this correlates with clinical, endoscopic and histological activity, suggesting MMP-9 as a possible new fecal marker for monitoring disease activity [16]. However, alteration of the balance between MMPs and TIMPs cannot always predict ECM accumulation or degradation. In the case of MMP-7, which cleaves galectin-3, a crucial molecule of epithelial cell migration, the increase in MMP-7, and the subsequent degradation of galectin-3, inhibits re-epithelialization and eventually contributes to fibrosis [17,18]. MMP-10 has been found to be upregulated at the healing edges of ulcers in patients with UC, suggesting a possible role in disease resolution [19], while DSS treatment in mice lacking MMP-10 increased the severity of colitis [20]. Therefore, given the complex interactions between the various MMPs, their substrates and different TIMPs during ECM remodeling, targeting MMP/TIMP balance as a therapeutic strategy to prevent or treat fibrosis may produce highly unpredictable outcomes.

The activated myofibroblast

ECM production depends on the activation state of ECM-producing myofibroblasts. Activation and proliferation of myofibroblasts by inflammatory and profibrotic mediators is a central event in the process of normal wound healing after acute injury (Table 1). Following the resolution of an acute inflammation, the healing process is gradually terminated and the connective tissue gains its initial architecture. In the case of IBD, the ongoing and persistent inflammation leads to a sustained activation and proliferation of ECM-producing myofibroblasts [3].

Myofibroblasts are characterized by specific cellular markers that grant them an intermediate phenotype between fibroblasts and smooth muscle cells, their contractile shape and their ability to produce great quantities of ECM components when activated [9,21]. There are two main cell types of myofibroblasts in the intestine. The first ones are the intestinal cells of Cajal, which reside in the submucosa and the muscularis propria and provide signals that facilitate gastrointestinal motility [22]. The second type is the SEMFs, which express vimentin and α-smooth muscle actin (α-SMA) (Fig. 1). SEMFs are located within the lamina propria underneath the epithelial cells, forming an anastomosing syncytium that is dense in the crypt region and sparse at the colonic surface or the villi of the small bowel [7]. Gene expression profiles of pericryptal myofibroblasts suggest their participation in the intestinal stem cell niche through the production of bone morphogenetic protein (BMP) antagonists and R-spondins, well-described Wnt agonists, that maintain epithelial stem cell Wnt signaling and therefore self-renewal and expansion [23]. A recent study showed that SEMFs produce angiopoietin-like protein 2, an essential factor for epithelial regeneration that acts on epithelial cells through the BMP/β-catenin signaling pathway [24]. In addition, they secrete growth factors such as TGF-β [25], cyclooxygenase products [26] such as prostaglandin E2 (PGE2) [27], and ECM-related proteins [26], which suggests a primary role in epithelial reinstitution upon injury.

Table 1 Factors with a profibrotic effect and their mechanisms of action in myofibroblast biology. Data from preclinical studies and experimental models

Profibrotic factors	Effect on myofibroblasts
Platelet-derived growth factor	Enhances myofibroblast chemotaxis and collagen production [55]
Activated coagulation factors (thrombin, activated coagulation factor X and endothelin-1)	Myofibroblast differentiation, migration and pro-collagen production [45-47]
Transforming growth factor β	Differentiates fibroblasts to myofibroblasts, enhances their proliferation and migration, increases production of collagen and TIMP-1 [13;35;51]
Connective tissue growth factor	Promotes fibroblast proliferation and production of ECM proteins [60;61]
Tumor necrosis factor-α	Induces myofibroblast proliferation and migration, over-expression of collagen and TIMP-1 and inhibition of MMP-2 activity [90;91]
IL-1α	Indirect effect via the induction of IL-6, IL-8, and CTGF expression [74;75]
IL-17	Regulates the expression of TGF-β and CTGF in myofibroblasts and upregulates collagen production [107]
IL-33	Indirect effect via induction of alternative (M2) macrophage polarization and strong Th2-associated responses [81-83]
IL-4	Induces fibroblast activation and proliferation and to enhance expression of collagen and fibronectin [99]
IL-13	Enhances TGF-β production by macrophages, but exerts a direct, TGF-β-independent fibrogenic effect and acts through different pathways according to tissue and profibrotic triggers [92]

TIMP, tissue inhibitors of metalloproteinase; ECM, extracellular matrix; MMP, matrix metalloproteinase; IL, interleukin; CTGF, connective tissue growth factor; TGF, transforming growth factor; M2, alternative macrophage polarization

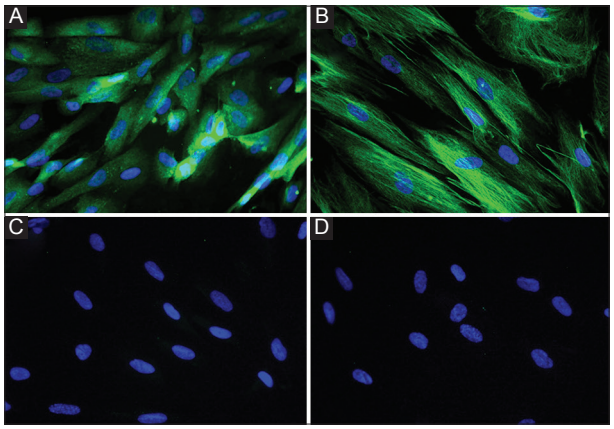

Figure 1 Human intestinal subepithelial myofibroblasts. Immuno-fluorescence staining of subepithelial myofibroblasts isolated from human colonic biopsies, expressing α-smooth muscle actin (A) and vimentin (B), negative for desmin (C), and isotype matched control antibody (D) (unpublished data)

There are accumulating data that incriminate SEMFs in autoimmune inflammation and post-inflammatory fibrosis. Specifically, SEMFs respond to proinflammatory cytokines by upregulating the expression of interleukin (IL)-8, IL-6, monocyte chemotactic protein-1 (MCP-1/CCL-2), granulocyte macrophage colony-stimulating factor and macrophage colony-stimulating factor [28,29]. They also express Toll-like receptor (TLR)-2 and TLR-4, which induce MCP-1, growth-related oncogene-α (GRO-α), IL-6 and IL-8 upon stimulation with lipopolysaccharide [30,31] and TLR-5, which induce IL-6 and IL-8 in response to flagellin [31]. Furthermore, they have been found to constitutively express class II major histocompatibility complex, and possibly act as non-professional antigen-presenting cells to promote $CD4^+$ T cell differentiation [32]. Recently, we have shown that stimulation of human SEMFs with proinflammatory cytokines results in production of tumor necrosis factor (TNF)-like ligand 1A (TL1A), a molecule recently implicated in both T-cell differentiation and intestinal fibrosis [33]. Concerning their possible participation in post-inflammatory intestinal fibrosis, SEMFs have been found to respond to proinflammatory and profibrotic cytokines, such as IL-17A [34] and TGF-β [35], by increasing production of collagen and MMPs. Finally, we have shown that conditioned medium from epithelial cell cultures stimulated with proinflammatory cytokines can enhance SEMF migration, and production of collagen and MMPs, suggesting the existence of a crosstalk between SEMFs and overlying epithelial cells during intestinal inflammation that can generate profibrotic responses [35].

Other stromal cells capable of transforming into activated ECM-producing intestinal myofibroblasts include fibroblasts and pericytes [36]. Fibroblasts are potent ECM-producing cells, present in small numbers in almost every tissue. They express specific cellular markers, such as CD90 and vimentin, and they are negative for α-SMA and desmin expression [21]. In case of acute injury or inflammation, fibroblasts are activated, migrate and proliferate at the site of the wound and contribute to ECM production [37]. It has also been reported that upon stimulation with specific growth factors, such as TGF-β1, fibroblasts express α-SMA and differentiate into myofibroblast-like cells [38].

Pericytes are mainly found in capillaries and small blood vessels surrounding endothelial cells [36] and play a major role

in angiogenesis, as they express receptors for platelet-derived growth factor (PDGF), endothelin-1 and angiotensin II [39]. Pericytes are also implicated in fibrosis, as they are characterized as α-SMA and desmin positive cells and upon acute or chronic inflammation they differentiate into fibroblast-like cells, producing large quantities of ECM components. In a recent study, sections from inflamed mucosa from patients with long-lasting UC showed an increased population of active pericytes compared to controls [40]. However, their contribution to intestinal fibrosis has not yet been clearly defined [36,41].

Another source of ECM-producing cells is the bone marrow-derived fibrocytes, which originate from multipotent bone marrow mesenchymal stem cells. Under certain circumstances, bone marrow mesenchymal stem cells differentiate and give rise to fibrocytes, which in turn travel through the bloodstream to the affected tissue. Fibrocytes express specific markers of hematopoietic origin, such as CD45, CD34, CD11, CD13, CD14, CD80 and CD86, and they are also positive for collagen I and α-SMA expression [36]. Fibrocytes' implication in fibrosis has been indicated by a study showing an increased fibrocyte population in inflamed specimens and peripheral blood from patients with CD. In the same study, peripheral blood-isolated fibrocytes expressed increased collagen type I and TNF-α mRNA levels in response to lipopolysaccharide, indicating that fibrocytes may actively contribute to fibrosis upon injury [42].

Additionally, non-mesenchymal cells, such as epithelial and endothelial cells, are able to contribute to fibrosis via epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition [43]. Both are common events in embryogenesis and carcinogenesis, but have also been observed during fibrogenesis. In this process, epithelial or endothelial cells lose their initial markers, rearrange their cytoskeleton, change morphology and acquire a fibroblast-like phenotype and function. Eventually, cells express α-SMA or vimentin and acquire the ability to produce ECM components, such as collagen and fibronectin [44].

From endothelial and epithelial injury to myofibroblast activation

Wound-healing responses are initiated by the activation and proliferation of ECM-producing myofibroblasts in response to acute epithelial and/or endothelial injury. Endothelial injury activates coagulation cascades as a part of the normal wound-healing response. We and others have shown that activated coagulation factors, such as thrombin, activated coagulation factor X and endothelin-1, potentially produced by endothelial cells, can induce myofibroblast differentiation, migration and pro-collagen production (Fig. 2) [45-47]. Interactions of platelet glycoprotein receptors Ia/Ib/V/VI/IX with von Willebrand factor and exposed collagen induce the arrest and activation of adherent platelets [48]. Activated platelets secrete many profibrotic factors, including TGF-β, connective tissue growth factor (CTGF) and PDGF, that act upon precursors of myofibroblasts to drive differentiation to activated myofibroblasts with profibrotic phenotype.

TGF-β is a pluripotent cytokine implicated in many cellular and immunological processes. The TGF-β/SMAD pathway is a major "core" pathway for fibrosis that includes IL-13 and CTGF as upstream and downstream mediators, respectively, and can be enhanced or inhibited by various pro- or antifibrotic molecules [49]. Activated TGF-β1 differentiates human fibroblasts to myofibroblasts, via upregulation of α-SMA expression, enhances their proliferation and induces resistance to apoptosis [50]. Acting upon human SEMFs, TGF-β enhances their migratory capacity and increases production of collagen and TIMP-1 [13,35,51]. TGF-β blockade ameliorated fibrosis in animal models of chronic colitis [52], whereas overexpression of TGF-β leads to intestinal stricture formation [53]. In a newly established model for fibrosis using human intestinal organoids, TGF-β treatment resulted in upregulation of fibrosis-related factors, such as collagen type I, fibronectin, α-SMA, actin contractile gene MYLK and fibrogenic transcription factor MLK1 [54]. Despite the undisputable contribution of TGF-β to intestinal fibrosis, the optimal TGF-β inhibition strategy is still under investigation so as to selectively block profibrotic, but not immune-regulatory pathways that are vital to mucosal homeostasis [9].

PDGF is a potent mitogen for myofibroblasts, enhancing chemotaxis and collagen production [55]. However, evidence for a role of PDGF in intestinal fibrosis is still sparse and indirect. Specifically, increased PDGF-A transcripts have been detected in the colonic mucosa in UC. SEMFs isolated from UC patients have been found to express the PDGF receptor β (PDGFRβ), while isolated intestinal SEMFs have been found to respond to PDGF-AB and PDGF-BB by increasing migration and proliferation [51,56]. Kurahashi *et al* identified a subpopulation of intestinal fibroblasts that express PDGFR and present distinct characteristics [57]. In another study, stimulation of CCD-18Co colonic fibroblasts or colonic epithelial cell line T84 with PDGFA led to an increased proliferation rate. Furthermore, PDGFA activity was found to be regulated by vasopressin, a neurohormone mainly involved in the regulation of water reabsorption in the kidney and large intestine, indicating a possible mechanism in wound repair upon epithelium injury [58]. Therefore, further investigation is warranted of the role for PDGF in intestinal fibrosis.

CTGF is a cysteine-rich peptide, induced by TGF-β, PDGF and basic fibroblast growth factor in fibroblasts, epithelial and endothelial cells, and is crucial for angiogenesis and wound healing [59]. It is a heparin-binding ECM-associated protein that regulates cell adhesion, migration, proliferation, and differentiation through integrin receptors on responding cells that include endothelial cells, platelets and fibroblasts [60]. Being a downstream mediator of the TGF-β pathway, CTGF acts upon stromal cells to promote fibroblast proliferation and production of ECM proteins and MMPs [60,61]. CTGF expression has been found to increase progressively along with the development of intestinal fibrosis in chronic 2,4,6-Trinitrobenzenesulfonic acid (TNBS) colitis, whereas knocking-down Smad3 decreased CTGF production and fibrosis development [62,63]. Increased expression of CTGF has been found in intestinal strictures of CD patients [64-66].

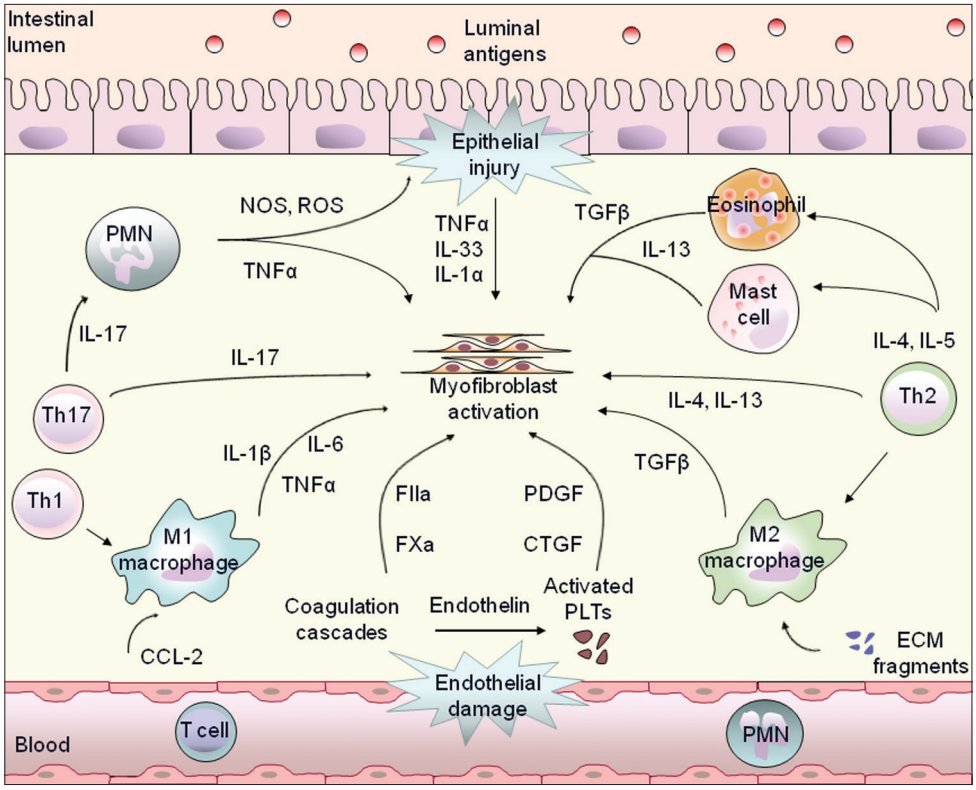

Figure 2 Immune pathways that drive myofibroblast activation. Classic and non-classic immune cells participating in the different stages of tissue inflammation, from initial endothelial or epithelial injury to innate and subsequent adaptive immune activation, drive myofibroblast activation. These include coagulation factors, growth factors (PDGF and CTGF), chemokines (CCL-2), cytokines (TGF-β, IL-13, TNF-α, IL-17) and reactive oxygen (ROS) and nitrogen (NOS) species

Further studies using *in situ* hybridization have demonstrated localization of CTGF transcripts primarily in fibroblasts within the submucosal layer [66].

Increased expression of IL-1β and IL-6 has been detected in inflamed tissue from IBD patients, produced mostly by lamina propria mononuclear cells [67] Okuno *et al* showed that intestinal SEMFs overexpress collagen type I and type IV, as well as MCP-1 and MMP-1, in response to IL-1β stimulation [28]. In a more recent study, IL-1β upregulated the mRNA levels of IL-36γ in human SEMFs [68], a cytokine belonging to the IL-1 family that was recently found to be elevated in active inflamed tissues of patients with IBD [69]. The role of IL-1β in intestinal wound repair is highlighted by an *in vitro* study where conditioned media from CCD-18Co previously stimulated with IL-1β induced the migration and differentiation of epithelial cell line Caco-2. Furthermore, IL-1β induced the overexpression of Wnt5a in CCD-18Co, an essential molecule of epithelium integrity and regeneration. Conditioned media depleted from Wnt5a failed to promote epithelial migration and differentiation, indicating that IL-1β indirectly induces epithelial migration through its direct action on myofibroblasts' ability to express Wnt5a [70]. However, increased local and systemic IL-6 levels have been mainly associated with disease activity and severity. SEMFs isolated either from inflamed or non-inflamed tissue of patients with CD expressed elevated levels of IL-6, compared with SEMFs isolated from non-IBD patients [71]. Nonetheless, a link between IL-6 and fibrosis in IBD has not yet been established.

Intestinal epithelial necrosis release damage-associated molecular patterns (DAMPs) as part of a sterile inflammatory response, which takes place in parallel with an aberrant response to pathogen-associated molecular patterns (PAMPs) and contributes to the development of chronic autoimmune intestinal inflammation. IL-1α is part of the DAMPs released from damaged intestinal epithelium, and neutralization of IL-1α or its receptor has been found to downgrade experimental intestinal inflammation [72,73]. IL-1α has been found to amplify mucosal and skin inflammation, partly by acting on intestinal subepithelial fibroblasts to induce production of IL-6 and IL-8 [74]. Furthermore, IL-1α upregulates MMP-9 and promotes contractility and CTGF expression on skin fibroblasts and intestinal myofibroblasts [35,75]. Despite the paucity of data regarding intestinal fibrosis, IL-1α is considered a profibrotic cytokine, as IL-1α depletion or deficiency reduced collagen deposition and expression of fibrosis-associated genes in bleomycin-induced lung fibrosis and in models of liver fibrosis [76,77]. IL-33 is another DAMP that is released upon epithelial or endothelial injury and its role in fibrosis has not been adequately studied [78]. Epithelial-derived IL-33 has been found to promote regulatory T cell responses and to antagonize IL-23 in experimental colitis [79]. Intestinal SEMFs and hepatic stellate cells have been identified as important

additional sources of IL-33 [80]. IL-33 has been associated with fibrosis in lung and skin animal models, mainly because of its ability to induce alternative (M2) macrophage polarization and strong Th2-associated responses by innate lymphoid and CD4 helper T cells [81-83].

Glycosaminoglycan hyaluronan (HA) is a large linear polymer, composed of repeating disaccharide units of glucuronic acid and N-acetylglucosamine, and it is a component of ECM, implicated in both inflammation and fibrosis. Fragments of HA are considered DAMPs and are recognized by various cell types through receptors, including CD44, TLR2, and TLR4 [84]. HA degradation into fragments seems to be regulated by a recently discovered protein, KIAA1199, and this is supported by a study showing that skin fibroblasts lacking this protein are unable to catalyze HA [85]. In a recent study, KIAA1199 was found to be highly elevated in submucosal regions of inflamed and fibrotic colon sections from patients with CD, with its substrate, HA, being in close proximity. In addition, intestinal fibroblasts isolated from patients with CD expressed high levels of KIAA1199 in ECM, and its expression was specifically regulated by IL-6. Silencing KIAA1199 in intestinal fibroblasts resulted in failure of HA degradation, indicating that intestinal fibroblasts are important players in HA degradation [86].

Immune responses in fibrotic process

Innate immune responses initiate profibrotic cascades

Epithelial injury and pathogen entry release DAMPs and PAMPs, resulting in activation of TLRs, NOD-like receptors, c-type lectin receptors and inflammasomes on resident macrophages that drive the secretion of proinflammatory cytokines and chemokines [87]. Complex chemokine gradients are formed via the cooperation of immune and non-immune cells to recruit innate immune cells. Platelet adhesion to the damaged endothelium and subsequent PDGF production drives MCP-1/CCL-2 production by endothelial cells, mesenchymal cells and monocytes, which in turn induces monocyte migration and infiltration at the site of injury [88]. This initial early phase of tissue injury is characterized by local infiltration by classically activated (M1) macrophages that secrete IL-1, IL-12, IL-23, TNF-α and reactive oxygen and nitrogen species [87]. M1 products, such as TNF-α, participate in myofibroblast activation as part of the normal wound healing response, but also link persistent inflammation to fibrosis (Fig. 2) [87].

Accumulating evidence from animal and *in vitro* studies suggests that TNF-α acts as a profibrotic molecule. In a rat experimental bowel fibrosis model, treatment with anti-TNF-α resulted in decreased inflammation and fibrosis accompanied by reduced expression of pro-collagen, TGF-β and insulin-like growth factor (IGF) [89]. TNF-α has a direct profibrotic effect on intestinal myofibroblasts via its receptor, TNFR2. TNFR2 signaling through ERK1/2 and STAT3 induced myofibroblast proliferation, over-expression of collagen and TMP-1 and inhibition of MMP-2 activity [90]. Recent studies have shown that TNF-α can enhance myofibroblast migration through the P38 MAPK-regulated cyclooxygenase (COX)-2, heat shock protein 27 and protein kinase D signaling pathways [91]. Indirect actions of TNF-α include upregulation of IL-13Rα2 expression on intestinal macrophages in order to increase TGF-β production in response to IL-13 [92]. Furthermore, TNF-α enhances nuclear factor (NF)-κB translocation that upregulates production of IL-1 and IL-6 by lamina propria mononuclear cells and intestinal epithelium [93]. Studies using a chronic TNBS colitis model have shown that NF-κB blockade could ameliorate inflammation-associated intestinal fibrosis [94]. However, despite the undisputable benefits of anti-TNF-α antibodies in the treatment of the inflammatory component of IBDs, there is still not enough evidence that TNF-α neutralization can prevent or even ameliorate intestinal fibrosis [95,96].

At the stage where inflammation resolves or becomes chronic, apoptosis and death with release of extracellular traps (ETosis) of immune cells become central elements of the inflammatory processes [97]. Enhanced phagocytosis shifts macrophages to the alternatively-activated M2 phenotype that release mediators of anti-inflammatory and profibrotic functions, such as IL-4, IL-13, IL-10 and TGF-β (Fig. 2) [87]. Adipose macrophages from active lesions of IBD patients exhibited an alternatively-activated phenotype with increased production of Th2 cytokines, including IL-4 and -13. Furthermore, a direct implication of alternatively activated macrophages in fibrosis has been proposed, through the induction of Arginase-I synthesis, an enzyme necessary for the supply of metabolites used in collagen synthesis [98]. Stimulation of myofibroblasts with IL-4 results in increased collagen production [99], whereas IL-13 regulates their TGF-β expression levels [100]. Increased collagen deposition in CD tissue samples correlated with elevated IL-13 and TIMP-1 expression from mononuclear cells [101].

Adaptive immune responses perpetuate fibrosis through the sustained activation of myofibroblasts

Apart from innate immunity, the aberrant adaptive immune responses that characterize IBD are also implicated in fibrosis. Interferon (IFN)-γ, the hallmark of a Th1 immune response, seen in the early stages of CD [102], has been considered as an antifibrotic cytokine. IFN-γ interferes with the TGF-β signaling pathway, blocking both TGF-β and CTGF expression [103]. IFN-γ knockout mice are more susceptible to liver fibrosis, while treatment with IFN-γ has been beneficial [104]. In experimental renal fibrosis, the selective delivery of IFN-γ to myofibroblasts resulted in amelioration of fibrosis with reduced collagen synthesis and α-SMA production [104]. IFN-γ therapy has been used to treat idiopathic pulmonary fibrosis patients who have low IFN-γ levels, with variable success [105].

In contrast, Th17 immune responses that also characterize CD have been associated with fibrogenesis. Biancheri *et al* reported that IL-17A was found to be considerably elevated in

strictured CD tissues, along with elevated collagen, MMP-3, MMP-12 and TIMP-1 [106]. In the same study, myofibroblasts isolated from intestinal biopsies from either patients with fibrostenotic CD or healthy individuals expressed both receptors for IL-17, and upon stimulation with IL-17A there was an upregulation of MMP-3, -12, TIMP-1 and collagen [106]. Additional studies suggest that IL-17 is implicated in fibrosis by regulating the expression of TGF-β and CTGF in myofibroblasts and upregulating collagen production [107]. In addition, IL-22, also produced by Th17 T cells, seems to play a protective role against liver and lung fibrosis [107,108]. Although secukinumab, a monoclonal antibody against IL-17, has proven ineffective for the treatment of inflammation in active CD, it has not been tested in the prevention or treatment of CD-associated intestinal fibrosis [109].

Atypical Th2 immune responses mostly characterize UC. However, Th2 have also been detected during late stages of experimental and human CD [110,111]. Th2-associated cytokines, mainly IL-4 and IL-13, have been reported to directly stimulate myofibroblast activation (Fig. 2). Specifically, IL-4 has been found to induce fibroblast activation and proliferation and to enhance expression of collagen and fibronectin [112]. IL-4 and IL-13 share a common IL-4Rα receptor and both signal through STAT6 [113]. However, experimental schistosome infection of double IL-4/IL-13 knockout mice has demonstrated that IL-13 is more fibrogenic than IL-4 [114]. In the colon, binding of IL-13 to its receptor induces TGF-β in intestinal macrophages, which in turn results in upregulation of profibrotic factors, such as IGF-1 and early growth response protein (Egr)-1, that stimulate collagen production by intestinal myofibroblasts. Inhibition of either IL-13 or TGF-β reduced levels of IGF-1 and Egr-1, an early transcription factor that transduces the fibrogenic activities of TGF-β, resulting in decreased collagen deposition [92]. Taken together, the above experimental evidence suggests that IL-13 is an important mediator of fibrosis that acts through multiple pathways.

Molecules and mechanisms of anti-fibrotic pathways

Anti-fibrotic molecules can be produced by a variety of immune and non-immune cells and play a major role in ECM-remodeling (Table 2). Their relative deficiency has been considered to enhance ECM accumulation and fibrosis. Unfortunately, most of the information on the role of these molecules in fibrosis comes from other tissues. PGE2 can induce myofibroblast dedifferentiation, inhibit proliferation and reduce collagen and ECM production. PGE2 is a bioactive lipid mediator produced by many different cell types, including fibroblasts, and is activated through catalyzation by COX-2 [115]. PGE2 in lung epithelial cells can limit TGF-β-induced myofibroblast differentiation [116,117]. Furthermore, PGE2 signaling through the cAMP-coupled E prostanoid 2 and 4 receptors inhibited fibroblast proliferation and pro-collagen expression [118] and promoted fibroblast apoptosis [119]. PGE2 supplementation was able to rescue murine lungs from bleomycin-induced [120] and colon from TNBS-induced fibrosis [121]. It has been speculated that there is an altered balance of TGF-β and PGE2 in fibrosis, as TGF-β has been found to downregulate COX-2 expression and PGE2 production [122].

Although fibroblast growth factors (FGFs) are thought to be potent mediators of fibrosis, FGF-1, a member of the FGF family, has been shown to possess anti-fibrotic properties. FGF-1 is able to decrease collagen production and α-SMA expression on fibroblasts, partially inhibit TGF-β and reverse TGF-β-induced EMT [123,124], Grb2-associated binder 1 (Gab1) adaptor protein is a docking protein that interacts with receptor tyrosine kinases, amplifying growth factor signals [125]. In an animal model of liver fibrosis, Kizu *et al* showed that Gab1-conditional knockout mice developed severe liver fibrosis, indicating a possible antifibrotic or homeostatic role of Gab-1 [126].

Ghrelin is a polypeptide expressed mostly in the stomach and its main role is to increase appetite and enhance food intake [127]. Apart from these well-known actions, ghrelin has been characterized as an anti-fibrotic factor, as it prevents doxorubicin-induced myocardial fibrosis [128] and bleomycin-induced dermal fibrosis [129], reduces collagen production in fibroblasts from patients with system sclerosis [130], and attenuates renal fibrosis in a rat animal model [131]. Another antifibrotic factor is the product of polyamine biosynthesis, the 5′-methylthioadenosine (MTA). MTA is a sulfur-containing adenine nucleoside, present in all mammalian tissues, that has been shown to possess anti-inflammatory properties [132]. Oral administration of MTA reduced colonic inflammation in the DSS colitis model [133], and ameliorated murine liver fibrosis via inhibition of myofibroblast activation, proliferation and collagen production [134].

In addition to extracellular antifibrotic factors that act via interaction with membrane receptors, intracellular mediators can also exhibit antifibrotic effects. Peroxisome proliferator-activated receptor (PPAR)-γ is a ligand-activated nuclear receptor with an important role in fatty acid storage and glucose metabolism. Being expressed in a wide variety of immune and non-immune cells, including macrophages and fibroblasts, it has been shown to decrease inflammatory responses in the intestine and many tissues [135]. PPAR-γ agonists are currently used for the treatment of obesity-associated type 2 diabetes and are currently under investigation in clinical trials for inflammatory diseases, asthma and cancer [136]. PPAR-γ inhibits profibrotic signaling by TGF-β and Wnt-β-catenin [137,138] via distinct tissue or cell-specific pathways [139]. It downregulates TGF-β and actin α1 expression by human intestinal fibroblasts from UC patients, antagonizes TGF-β profibrotic effects, such as collagen and fibronectin production [137], and suppresses TGF-β-induced EMT [140]. A novel PPAR-γ agonist has recently been shown to ameliorate intestinal fibrosis in a chronic DSS colitis model [141].

Finally, vitamins A, E, K1 and D have been reported to exhibit antifibrotic properties. Vitamin A administration in the bile duct ligation model of hepatic injury resulted in amelioration of fibrosis and reduced expression of fibrotic markers, including α-SMA, keratinocyte growth factor and

Table 2 Factors with an antifibrotic effect on the implication of myofibroblasts in the fibrotic process. Data from preclinical studies and experimental models

Antifibrotic factors	Effect on myofibroblasts
Interferon-γ	Blocks both TGF-β and CTGF expression [103], reduces collagen synthesis and α-SMA production [104]
Prostaglandin E2	Induces myofibroblast dedifferentiation, inhibits proliferation and reduces collagen and ECM production [116-118]
Fibroblast growth factor-1	Decreases collagen production and α-SMA expression on fibroblasts, partially inhibits TGF-β and reverses TGF-β-induced EMT [123,124]
Grb2-associated binder 1	Gab1-conditional knockout mice developed severe liver fibrosis, indicating a possible anti-fibrotic or homeostatic role of Gab-1 [126]
Ghrelin	Prevents doxorubicin-induced fibrosis [128], reduces collagen production in fibroblasts from patients with system sclerosis [130], and attenuates renal fibrosis in a rat animal model [131]
5′-methylthioadenosine	Inhibits myofibroblast activation, proliferation and collagen production [134]
Peroxisome proliferator-activated receptor-γ	Inhibits profibrotic signaling by TGF-β and Wnt-β-catenin [137,138], reduces expression of TGF-β antagonizes TGF-β profibrotic effects [137], suppresses TGF-β-induced EMT[140] and ameliorates intestinal fibrosis in a chronic DSS-colitis model [141]
Vitamin A	Vitamin A ameliorates fibrosis and reduces expression of fibrotic markers, including α-SMA, keratinocyte growth factor and glial fibrillary acidic protein [142]
Vitamin E	Reduces liver fibrosis in murine models via downregulating hepatic TGF-β levels [143]
Vitamin K1	Reduces production of collagen [146]
Vitamin D	Inhibits TGFβ-induced EMT[147] and suppresses expression of PDGF, TGF-β, collagen I, TIMP-1 and α-SMA, it also inhibits the upregulation of TGF-β, the accumulation of collagen, the expression of α-SMA and the production of collagen I by myofibroblasts [148,149]

TGF, transforming growth factor; CTGF, connective tissue growth factor; α-SMA, α-smooth muscle actin; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; Gab1, Grb2-associated binder 1; DSS, dextran sulfate sodium; PDGF, platelet derived growth factor; TIMP, tissue inhibitors of metalloproteinase

glial fibrillary acidic protein [142]. Vitamin E reduced liver and lung fibrosis in murine models and has been found to improve liver fibrosis in non-alcoholic steatohepatitis patients, possibly via downregulating hepatic TGF-β levels [143-145]. Vitamin K1 reduced production of collagen and ameliorated bile duct ligation-induced liver fibrosis in rats [146]. Finally, vitamin D inhibited TGF-β-induced EMT in human airway epithelial cells [147], and suppressed expression of PDGF, TGF-β, collagen I, TIMP-1 and α-SMA in an animal model of liver fibrosis [148]. Furthermore, it inhibited the upregulation of TGF-β, the accumulation of collagen and the expression of α-SMA and the production of collagen I by myofibroblasts isolated from TNBS-colitis mice [149].

Concluding remarks

Tissue inflammation can initiate multiple pathways, involving a variety of immune and non-immune cells, that result in the activation of myofibroblasts, a central element in ECM remodeling processes. The end result varies from reinstitution, repair and normal tissue healing to excessive collagen accumulation, fibrosis, permanent structural destruction and functional failure. The determinants of tissue fate are far from being clear, because of the multiplicity of cells and the complexity of the pathways involved. This is especially true for intestinal fibrosis, where available information is largely extrapolated from findings in other tissues and *in vitro* studies. However, although the end result of fibrotic processes is similar in all organs, differential profibrotic pathways have been found to prevail, depending on the tissue or the trigger. Therefore, experimental work focused on post-inflammatory intestinal fibrosis, together with data integration from other organs, are warranted in order to clearly define targets for intervention in the field of IBD.

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