Cellular and molecular mechanisms of intestinal fibrosis

Silvia Speca, Ilaria Giusti, Florian Rieder, Giovanni Latella

Silvia Speca, Ilaria Giusti, Giovanni Latella, Gastroenterology Unit, Department of Internal Medicine and Public Health, University of L’Aquila, 67100 L’Aquila, Italy
Florian Rieder, Department of Gastroenterology and Hepatology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 73051, United States
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Correspondence to: Giovanni Latella, MD, Gastroenterology Unit, Department of Internal Medicine and Public Health, University of L’Aquila, Piazza S Tommasi, 1-Coppito, 67100 L’Aquila, Italy. giolatel@tin.it
Telephone: +39-862-434735 Fax: +39-862-433425
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Abstract
Fibrosis is a chronic and progressive process characterized by an excessive accumulation of extracellular matrix (ECM) leading to stiffening and/or scarring of the involved tissue. Intestinal fibrosis may develop in several different enteropathies, including inflammatory bowel disease. It develops through complex cell, extracellular matrix, cytokine and growth factor interactions. Distinct cell types are involved in intestinal fibrosis, such as resident mesenchymal cells (fibroblasts, myofibroblasts and smooth muscle cells) but also ECM-producing cells derived from epithelial and endothelial cells (through a process termed epithelial- and endothelial-mesenchymal transition), stellate cells, pericytes, local or bone marrow-derived stem cells. The most important soluble factors that regulate the activation of these cells include cytokines, chemokines, growth factors, components of the renin-angiotensin system, angiogenic factors, peroxisome proliferator-activated receptors, mammalian target of rapamycin, and products of oxidative stress. It soon becomes clear that although inflammation is responsible for triggering the onset of the fibrotic process, it only plays a minor role in the progression of this condition, as fibrosis may advance in a self-perpetuating fashion. Definition of the cellular and molecular mechanisms involved in intestinal fibrosis may provide the key to developing new therapeutic approaches.

INTRODUCTION
Fibrosis is a chronic and progressive process characterized by an excessive deposition of extracellular matrix (ECM) components, such as collagens. It is believed to follow chronic tissue inflammation and ultimately leads to organ scarring and subsequent loss of function. Fibroproliferative disease may affect almost all tissues and organs, including the skin, kidneys, lungs, cardiac and vascular systems, eyes, liver, pancreas and intestine. Tissue fibrosis is a leading cause of morbidity and mortality. It has been estimated that 45% of deaths in the United States can be attributed to fibrotic disorders[1].

Intestinal fibrosis is usually considered to be a com-
mon complication of several enteropathies with distinct initiating pathophysiology, such as inflammatory bowel disease (IBD), radiation enteropathy, graft-versus-host disease, collagenous colitis, cosinophilic enteropathy, drug-induced enteropathy, sigmoid diverticulitis, solitary rectal ulcer, cistic fibrosis, intra-territorial fibrotic adhesions, desmoplastic reaction in gastrointestinal tumors (familial adenomatous polyposis-FAP), desmoid tumors, gastrointestinal (GI) stromal tumors (GISTs) and post-surgical intestinal adhesions and strictures leading to intestinal stenosis and obstruction\textsuperscript{1,3}.

Of these enteropathies, IBD is the main cause of intestinal fibrosis since this disease is characterized by a persistent immuno-mediated intestinal inflammation. In IBDs, both ulcerative colitis (UC) and Crohn’s disease (CD), fibrosis follows the distribution and location of inflammation. In UC, the deposition of ECM is restricted to the mucosal and submucosal layers of the large bowel. In CD, fibrosis can involve the entire bowel wall of the GI tract including the mucosa, submucosa, muscularis mucosa, muscularis propria and serosa layers\textsuperscript{3}. The increase of ECM in the tissue, with collagens and fibronectins being the major components, can ultimately lead to development of intestinal strictures and obstruction. Fibronectin has been shown to co-localize with aggregations of fibroblasts\textsuperscript{3}. Intestinal strictures may be composed of a combination of fibrosis and inflamed tissue. In CD, these may occur anywhere in the GI tract, but most frequently affect the terminal ileum. The increased rate of clinical complications related to small bowel fibrosis in CD is likely related to the smaller diameter of the bowel lumen, rather than to more severe fibrosis.

Intestinal injury is almost invariably followed by an acute inflammatory response. This is usually followed, in turn, by physiologic healing of the damaged tissue and restoration of the normal structure and function of the intestine. If this does not occur, chronic inflammation can develop, characterized by continuous events of injury and repair that may lead to the development of fibrosis. Injury to the intestine is not an uncommon phenomenon, even in otherwise healthy individuals. In most instances, wound healing leads to normal restitution and resolution of the tissue damage. In IBD, it is still unclear which factor triggers the road to chronicity. In addition, once intestinal inflammation is chronic, it is not yet understood what sets the stage for the later development of intestinal strictures.

In contrast to the intensive investigations focusing on the immunological mechanisms related to the early phases of intestinal inflammation and repair, the pathophysiology of chronic mucosal wound healing and the late events of repair leading to fibrosis remain largely unexplored.

It appears to be widely accepted that chronic intestinal inflammation invariably leads to fibrosis. However, this process does not occur in all patients. Chronic intestinal inflammatory diseases exist, such as celiac disease or lymphocytic colitis, that are not complicated by fibrosis and stricture formation. These findings indicate that distinct mechanisms of inflammation and restitution/fibrosis exist. It is crucial to explore this area since various pathways could be targeted separately, which would thus allow tailored treatment for wound healing abnormalities, especially in IBD.

Several lines of evidence suggest that inflammation is necessary to trigger the onset of the fibrotic process, but subsequently plays a minor role in progression of the disease\textsuperscript{10}. Anti-inflammatory treatment in IBD and in other chronic inflammation-associated fibrotic conditions in various organs (lung, liver, kidney) does not prevent evolution of fibrosis once the process of excessive ECM deposition has started. Mechanisms that regulate fibrosis, therefore, appear to be distinct from those regulating inflammation. The lack of efficient and well-tolerated anti-fibrotic drugs is partly due to the fact that the main and specific cellular and molecular pathways leading to fibrosis remain to be identified.

Various findings suggest that innate and adaptive immune mechanisms acting in chronic inflammation may divert the healing process towards fibrogenesis. A potential mechanism has been attributed to plasticity of the adaptive CD4 T cell immune response. Although CD4 T helper (Th) 1 and Th2 cells are reasonably stable after differentiation occurs, mature Th17 and Foxp3 regulatory T cells (Tregs) can transform into other subsets, i.e., exhibit plasticity\textsuperscript{10}. This appears to be driven by the transcription factors related orphan receptor (ROR) \(γ\) for Th17 cells and Foxp3 for Treg cells. A potential escape mechanism could be the micro RNA system: small sequences of RNA that interact with genes and alter their transcription\textsuperscript{11}. Changes in microRNA expression, induced by biological treatment, may result in non-response to the therapeutic agents.

Fibrogenesis is a “physiological process” triggered by the onset of inflammation that may lead either to tissue repair or fibrosis depending on the balance between production of ECM proteins and enzymatic degradation. Intestinal fibrosis is related to the abnormal function of activated intestinal mesenchymal cells (proliferation, migration, contraction, ECM production) namely, ECM-producing cells or myofibroblasts. Myofibroblasts are activated by a variety of mechanisms including paracrine signals derived from immune and non-immune cells, autocrine factors secreted by myofibroblasts, and pathogen-associated molecular patterns (PAMPs) derived from micro-organisms that interact with pattern recognition receptors such as the toll-like receptors (TLRs)\textsuperscript{12}. The most investigated soluble factors that regulate the activation of the ECM-producing cells include cytokines [interleukin (IL) -6, IL-13, IL-17, IL-21, tumor necrosis factor (TNF-\(α\)], chemokines [monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1], growth factors [transforming growth factor (TGF)-\(\beta\), connective tissue growth factor (CTGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF)-1 and 2, epidermal growth factor (EGF)], components of the renin-angiotensin (ANG) system (RAS), angiogenic
factors [e.g., vascular endothelial growth factor (VEGF)], peroxisome proliferator-activated receptors (PPARs), mammalian target of rapamycin (mTOR), and products of oxidative stress[10]. All these molecules are being investigated as potential targets of anti-fibrotic drugs. Pharmacological modulation of tissue ECM deposition by reducing activated ECM-producing cells and their profibrogenic effects (proliferation, motility, contraction, ECM production) could be useful in the prevention and treatment of intestinal fibrosis[1].

ECM degradation is mediated by the matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The fine balance between MMPs and TIMPs appears to be disturbed in chronically impaired wound healing in IBD. It is unclear which specific MMPs and TIMPs are involved and how they are regulated in this process. Nevertheless, effective pharmacological modulation of the MMP/TIMP-system could be helpful in the reversal of already established tissue fibrosis[10].

In IBD, current therapeutic agents, mainly corticosteroids, aminosalicylates, immunosuppressants (e.g., azathioprine and methotrexate), and even the more recent biologic drugs, such as anti-TNF antibodies, can improve intestinal inflammation but seem unable to significantly improve fibrosis[7-9]. Surgical correction, by means of intestinal resection or stricturoplasty, is necessary in up to 75% of CD patients during the course of their disease[10-12]. However, surgical resection is associated with a high rate of recurrent strictureing disease and the need for repeated surgery is high; therefore, exploration of new therapeutic approaches has now become mandatory[1,13,14].

**RELATIONSHIP BETWEEN INFLAMMATION AND FIBROSIS**

Wound healing is an important biological process that allows the replacement of dead or damaged cells after tissue injury and involves a regenerative phase, where injured cells are replaced by cells of the same type, thus leaving no evidence of damage, and a phase known as fibroplasia, where normal parenchymal tissue is replaced by the deposit of new ECM components[1].

In physiological conditions, the normal turnover of ECM, in each tissue, is regulated by several factors that influence synthesis and degradation. Indeed, under normal physiological conditions, continuous ECM remodeling occurs consisting of the production and balanced degradation of ECM components[10]. Myofibroblasts are the predominant mucosal cells that synthesize components of the ECM and ECM breakdown is mediated by proteolytic enzymes derived from various cell types of which the MMP family represent a large and important component[15,16].

Fibrosis is progressive but considered reversible. Several studies have shown that fibrosis may progress independently of inflammation[10,11].

The inflammatory process may be acute or chronic based on its duration and the cells involved. Acute inflammation is a rapid response to injurious agents while chronic inflammation is a process of prolonged duration in which the inflammatory cascade lasts for weeks or months and under certain circumstances a lifetime[18].

During a chronic inflammatory process, the physiological sequence of events related to inflammation (coagulation cascade, inflammatory response, and fibroproliferative response) is activated for a prolonged time period. If the network of negative feedback mechanisms that terminate the proliferative and fibrotic response fails to operate, the healing process can become pathologic causing continuous activation of the fibroproliferative response and excess of ECM deposit that progressively leads to substantial changes in normal tissue architecture with loss of organ function[13,15,17].

Inflammation is induced and sustained by an infiltrate of immune cells, such as T cells, macrophages and neutrophils in the intestinal mucosa, which determine tissue damage characterized through a loss of epithelial cells and degradation of ECM in the lamina propria leading to the formation of ulcerations. Subsequently, release of inflammatory mediators from damaged epithelial and/or endothelial cells leads to an anti-fibrinolytic-coagulation cascade and formation both of blood clots and a provisional ECM[15,17,18].

Clotting releases thrombin, a potent inducer of platelet degranulation through which a vast store of bioactive mediators (including thromboxanes, prostaglandins, chemokines and cytokines) are secreted promoting further clot formation and stimulation of the inflammation process. Another consequence of platelet degranulation is the release of potent chemotactic and growth-promoting cytokines at the site of inflammation which enhances inflammatory responses by recruitment of immune cells and activation of immune and non-immune cells and the release of oxygen radicals, cathepsins and ECM-degrading enzymes. Neutrophils are the first cell type recruited and represent the most abundant inflammatory cell in the early stages of wound healing. Activated neutrophils degranulate, releasing inflammatory and pro-fibrogenic cytokines, and subsequently die followed by the recruitment of macrophages. During this initial leukocyte migration phase, the activated macrophages and neutrophils eliminate tissue debris, dead cells, and any invading organisms. They also produce cytokines and chemokines, which initiate and amplify the wound-healing response. These factors are also mitogenic and chemotactic for endothelial cells, which surround the injury and form new blood vessels as they migrate toward the center of the ulceration[18].

When inflammation becomes chronic it induces myofibroblast activation that can determine an excessive accumulation of ECM, which promotes the formation of a permanent fibrotic scar. ECM consists of several molecular components including collagens (1-Ⅵ), elastin, glycoproteins, glycosaminoglycans and proteoglycans (Table 1). ECM is not an inactive structure, but directly regulates the inflammatory response and the process of healing and fibrosis by focal adhesion with immune and...
Table 1  Extracellular matrix molecules involved in wound repair and fibrosis

| Collagens       | Fibrillar type collagens 1-III-V |
|-----------------|----------------------------------|
| Non-fibrillar collagen type IV |                     |
| Glycoproteins   | Laminin                          |
|                 | Entactin/nidogen                 |
|                 | Fibronectin                      |
|                 | Tensin                           |
|                 | Sparc/BM40                       |
|                 | Thrombospondin                   |
| Proteoglycans   | Glycosaminoglycans (hyaluronic acid) |
|                 | Heparan sulfate                   |
|                 | Chondroitin sulfate               |
| ECM-modifying proteins | Matrix metalloproteinases       |
|                 | Tissue inhibitor of metalloproteinases |

ECM: Extracellular matrix.

non-immune cells as well as myofibroblasts[19].

In summary, the events triggered by chronic injury that are involved in fibrogenesis are: (1) immediate damage to the epithelial/endothelial barrier; (2) release of chemokines and cytokines; (3) recruitment of inflammatory cells (immune and non-immune); (4) release of reactive oxygen species (ROS) and pro-fibrogenic cytokines and growth factors; (5) activation of ECM-producing cells; and (6) accumulation of ECM proteins.

**Innate immune mechanisms**

The causes leading to chronic intestinal inflammation in IBD are unknown. It has been suggested that, in genetically predisposed subjects, it could be the result of a dysregulated immune response to intra-luminal antigens, such as resident luminal bacteria, bacterial products, or dietary antigens; in a chronic defective mucosal barrier, the continuous exposure of lamina propria immune cells to the luminal antigens may trigger, amplify and maintain the local inflammation.

Genetic factors and luminal microbes, in addition to their role in triggering the IBD, are also directly, or indirectly, involved in the development of intestinal fibrosis in IBD.

The luminal bacteria express PAMPs, including lipopolysaccharide (LPS) components of bacterial cell walls, bacterial DNA, and double-stranded RNA, able to bind to pattern recognition receptors expressed by a wide variety of immune and non-immune cells, such as the extracellular TLRs and the intracellular nucleotide oligomerization domain/caspase recruitment domain (NOD/CARD)-like receptors[20].

TLRs are expressed on immune cells as well as non-immune cells, and are involved in innate immunity by recognizing specific patterns of microbial components.

The TLR family, comprised of 10 members, are able to act as primary sensors of microbial products triggering a host defence response against invading pathogens and activating signaling pathways that induce the expression of immune and pro-inflammatory genes[19].

Bacterial components induce the activation of an intracellular signaling cascade via TLRs that involves the preserved intra-cytoplasmic Toll/interleukin-1R (TIR) domain and TIR domain-containing adaptors, such as MyD88. A MyD88/interleukin-1 receptor-associated kinase signaling is thus generated, which regulates innate immunity and can strongly influence the course of the inflammatory response. An increased mucosal expression of TLRs in the intestine of patients with IBD has been reported[21,22]. The TLR activation can be considered a pivotal event in the immunological response in IBD patients[21], suggesting that over-expression of certain TLRs could be one of the underlying mechanisms leading to the abnormal host reaction to commensal bacteria, as seen in IBD[22]. Another important aspect of this defective immune tolerance to commensal bacteria seems to be TLR polymorphisms. Various studies have demonstrated the correlation between TLR4 polymorphisms, at Asp299Gly and Thr399Ile, and the development of IBD[23,24]. Indirect evidence also suggests the involvement of an aberrant innate immune response in intestinal fibrogenesis. Antibodies directed against microbial peptides represent good serological markers that could help in the identification of fibrostenosing CD. Patients with a stronger immune response to microbial peptides are more likely to develop earlier complicated CD, including the earlier occurrence of fibrostenotic CD[25,26].

Nevertheless, the exposure of subepithelial cells to bacterial ligands seems not only to lead to immune-mediated inflammation, but also to direct mesenchymal cell activation.

Studies performed on animal models of IBD have demonstrated not only the presence of TLRs in intestinal fibroblasts and the role of PAMPs in their activation, but have also revealed a close correlation between pathogen-mediated fibroblast activation and the progression of fibrosis. The TLR ligands could directly lead to differentiation of fibroblasts into activated myofibroblasts and TLR expression in non-immune cells can be considered another key event leading to tissue scarring and to the development of fibrosis[27-29].

The role of TLRs in the fibrosis-promoting signals has been frequently observed in the liver in which hepatic stellate cells (HSCs), the main precursors of ECM-producing cells, are sensitized by TLR4 activation; this receptor is functionally expressed by HSCs and directly stimulates myofibroblasts to enhance TGF-β signaling[30]. An increase of LPS during hepatic fibrogenesis induces a TLR4-mediated downregulation of bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI), a pseudoreceptor that decoys TGF-β by lacking an intracellular kinase domain[31]. Thereby, the TLR4-MyD88-dependent downregulation of BAMBI induces an unrestricted activation of the TGF-β pathway in HSCs. The LPS-TLR4-MyD88 signaling cascade results are thus closely linked with TGF-β-mediated collagen deposition and promotion of liver fibrosis[30,31].

The role of TLRs has been investigated in the dam-
aged tissue of several organs, including the intestine, even if their involvement in fibrogenic progression has not been completely elucidated.

An increased expression of TLR 2, 3, 4, 6 and 7 has been observed in the intestinal fibroblasts isolated from patients with CD[21,22,32]. These receptors are activated by their respective microbial ligands and promote the differentiation of fibroblasts into collagen-producing myofibroblasts to induce a fibrogenic response[32]. In addition, an increased production of fibronectin and upregulated levels of α-smooth muscle actin (α-SMA) in human intestinal myofibroblasts exposed to TLR ligand have been reported, thus confirming the pro-fibrogenic activity of TLRs and the direct link between bacterial innate immunity and intestinal fibrosis in IBD in man[33]. Furthermore, TLR activation of gut myofibroblasts induces an increased secretion of CXC chemokine ligand 8, a ligand that was initially characterized as a neutrophil chemotactic factor, but seems to exhibit both an angiogenic and angiostatic activity that can lead to the progression of intestinal fibrosis[34].

Another key factor able to activate an innate immune response by intracellular recognition of PAMPs of selected microorganisms is NOD2, an intracellular peptidoglycan receptor for muramyl dipeptide encoded by the NOD2/CARD15 gene present on chromosome 16. NOD2/CARD15 polymorphisms seem to be, in part, involved in the pathogenesis of CD. Considerable evidence confirms, indeed, a relationship between the mutation of NOD2/CARD15 genes and CD, either alone or in combination with mutation of TLR (especially TLR4) or ATG16L1 (an autophagy gene)[33].

The major NOD2/CARD15 polymorphisms associated with CD are Arg702Trp, Gly908Arg and Leu1007fsinsC and these appear to produce defects in the host defence response against invading bacteria that can lead to a persistent intracellular infection with chronic stimulation of inflammatory cells[35]. NOD2 is present in several cell types such as monocytes and macrophages where it seems to decrease the TLR-induced production of pro-inflammatory Th1 cytokines[35]. NOD2 is also expressed in Paneth cells. A correlation between NOD2/CARD15 mutations and the downregulation of mucosal α-defensin has been repeatedly proven[36,37]. Furthermore, NOD2 can induce autophagy by recruiting the critical autophagy protein ATG16L1 on the plasma membrane during bacterial internalization.

In addition, there is a growing body of evidence proving that the main NOD2/CARD15 variants are closely related to ileal disease, a stenosing phenotype and to a greater need for abdominal surgery in CD patients[38]. Cells carrying NOD2 variants show an enhanced pro-inflammatory response to various intestinal microbes and lead to an increased TGF-β production and collagen deposition by T cells[39]. All these findings provide evidence that may encourage the clinical use of NOD2/CARD15 genotyping, both as a marker of CD and as a prognostic factor of the need for early surgery due to strictureting and fibrostenosing disease[39].

IBD, however, is a polygenetic disease. The development of genome-wide association scanning technologies has led to the discovery of more than 100 confirmed IBD loci[41-43]. Some, such as the Th 17 pathway genes (IL23R, IL12b, Janus kinases 2 (JAK2), signal transducers and activators of the transcription 3 (STAT3), are shared between CD and UC, others are phenotype-specific (autophagy genes such as ATG16L1, IRGM and NOD2 for CD; epithelial barrier genes HNF4a, E-Cadherin, LAMB1 and IL-10 for UC). Variants of some of these genes would also be excellent candidates involved in the development of fibrosis in IBD[44-46].

Most studies have focused on the involvement of non-immune cell types in the progression of fibrosis in IBD, including mesenchymal cells (smooth muscle cells, fibroblasts, myofibroblasts), epithelial cells, nerve cells and platelets[47,48]. In addition, mucosal microvascular cells can play a central role in the microcirculation regarding the onset and maintenance of IBD[49]. Much evidence indicates that neoangiogenesis, the growth of blood vessels from those already present, plays a crucial role in IBD[50]. The microcirculation and endothelial cells perform a crucial task in intestinal immune homeostasis: endothelial cells regulate the type and the number of leukocytes that migrate from the blood flow to the interstitial space and specialized vascular cells, such as those found in the endothelium of venules, control selectively the influx of a specific subset of T-cells[51].

Despite the evidence regarding the role of endothelial cells and neoangiogenesis in the inflammatory process, very limited data are available on their role in intestinal fibrosis.

**CELLULAR MECHANISMS**

The key event that leads to the development of intestinal fibrosis is not only recruitment of immune and inflammatory cells, but also the exposure of mesenchymal cells to a variety of inflammatory mediators that are able to maintain these cells in a persistent state of trans- and de-differentiation between fibroblasts, myofibroblasts and smooth muscle cell phenotypes. All mesenchymal cell types can directly or indirectly contribute to intestinal fibrosis by producing large amounts of ECM proteins with different architectural and barrier functions, and are able to regulate several growth factors acting in a paracrine and autocrine fashion[40-53]. Each single mesenchymal cell type can be identified by the expression of specific cellular markers, as reported in Table 2.

Several conditions (such as ischemia, radiation, chemicals, microbes, products of oxidative stress, drugs, autoimmunity and allergies) promote the release of inflammatory mediators during tissue injury, leading to activation of mesenchymal and non-mesenchymal cells that produce ECM, also referred to as ECM-producing cells (Figure 1).

Cellular processes that lead to abnormal wound healing are mainly represented by activation (proliferation,
migration, contraction, ECM production) of fibroblasts and myofibroblasts. Nevertheless, it has become clear that the cellular source of the ECM proteins not only involves mesenchymal cells, such as fibroblasts, myofibroblasts and smooth muscle cells, but also other cell populations, such as stellate cells, pericytes, as well as bone-marrow and intestinal stem cells. Recently, it has also been observed that myofibroblasts may derive from non-mesenchymal cell transformation such as epithelial-to-mesenchymal transition and endothelial-to-mesenchymal transition.

### Fibroblasts

Fibroblasts are a heterogeneous population of cells present in the interstitium of all normal tissues and organs where they play a pivotal role in maintaining structural integrity, regulating matrix homeostasis and taking part in healing and regenerative processes and/or in pathogenesis of scarring.

An acute injury or acute inflammation can launch activation of fibroblasts driving them towards a fibrogenic phenotype. This activated fibroblast can be engaged either in normal wound healing or fibrosis. Fibroblast activation is characterized by a post-transcriptional or post-translational up-regulation of ECM secretion.

Fibroblasts co-express vimentin, fibroblast-specific protein 1 (FSP-1, S100A4), N-cadherin, and prolyl 4-hydroxylase (Table 2).

The increase in the resident fibroblast population is a pivotal mechanism for the development of intestinal fibrosis. Several growth factors found in the inflamed gut, such as IGF-1, basic fibroblast growth factor (bFGF), EGF, CTGF, PDGF, and pro-inflammatory cytokines, such as interleukins (IL-1β, IL-6) and TNF-α, increase their proliferation rate.

TGF-β1, one of the most potent profibrogenic factors, shows a bifunctional role in fibroblast proliferation with either growth stimulation or growth inhibition and blocking of cell differentiation depending upon the concentration used or the organ from which the cells are derived. TGF-β1 can also act in an indirect mode by up-regulation of the PDGF receptor, increasing synthesis of CTGF and promoting expression of IGF-1, all factors that directly affect proliferation.

Another mechanism that further increases the number of activated fibroblasts is the migration from non-affected tissue areas into and through the surrounding ECM, under the effect of a chemotactic gradient (chemotaxis) or with active random movement (chemokinesis) to the wound area. In the intestine, the cell migration is generally an essential factor for physiological wound repair, and is a fundamental process during the progression of pathological conditions such as fibrosis in IBD. Migration of fibroblasts can be induced by autocrine stimuli, like fibronectin, and paracrine factors, such as PDGF-AB, IGF-1, EGF and TGF-β1, all of which appear to be fibronectin-dependent. Mediators of active inflammation, such as TNF-α, IFN-γ or PGE2, can inhibit fibroblast migration further indicating distinct mechanisms of inflammation and fibrogenesis.

### Myofibroblasts

Myofibroblasts represent a highly contractile cell type that is thought to be critical for tissue repair and the pathogenesis of fibrogenetic diseases. These cells exhibit a “hybrid” phenotype between fibroblasts and smooth muscle cells and, when activated, synthesize high levels of ECM, particularly collagen, glycosaminoglycans, tenasin and fibronectin. Besides their normal activities in growth and differentiation of tissues, the myofibroblasts play a central role in wound healing. During normal wound repair of a tissue injury, a closely regulated sequence is induced, including activation and proliferation of myofibroblasts that are present in the wound bed where they play essential roles in wound contraction and connective tissue restoration, e.g., through the production of ECM and basement membrane molecules.

Myofibroblasts can also play a role in the up- or down-regulation of the inflammatory response by the secretion of cytokines and chemokines. When these processes are not controlled, deranged, or repeated, as occurs in several fibroproliferative diseases, the normal resolution stages are abrogated and the proliferation of myofibroblasts continues, inducing excessive accumulation of the ECM and leading to alterations in the tissue architecture and ultimately to organ failure. Therefore, fibrotic disease is a major pathological end point of activated and prolif-

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**Table 2  Cell types involved in intestinal fibrosis**

| Cell type                             | Positive markers                      |
|---------------------------------------|---------------------------------------|
| Fibroblasts                           | Vimentin, FSP-1, N-cadherin (high),   |
| Intestinal subepithelial myofibroblasts| Vimentin, α-SMA, collagen type 1,     |
| Intestinal cells of Cajal              | Vimentin, α-Ki receptor                |
| Smooth muscle cells                   | Vimentin (low), α-SMA, Desmin,        |
| Stellate cells                        | Vitamin A, GFAP, α-SMA,               |
| Pericytes                             | α-SMA, desmin (low), MCSP,            |
| Endothelial cells                     | ANG I and II, ET-1, collagen type 1   |
| Bone marrow stem cells                | E-cadherin, cytoketatin               |
| Hematopoietic stem cells              | CD31, vWF, VE-cadherin,              |
| Mesenchymal stem cells                | N-cadherin (low), vimentin (low)      |
| Fibrocytes                            | CD45, CD34, CD14                      |
| Fibrocytes                            | CD105, CD73, CD44, CD71, CD90        |

FSP-1: Fibroblast specific protein 1; MCSP: Melanoma chondroitin sulfate proteoglycan; RG55: Regulator of G protein signaling-5; GFAP: Glial fibrillary acidic protein; α-SMA: α-smooth muscle actin; VEG: von Willebrand factor; Vascular endothelial cadherin; vWF: von Willebrand factor; cKit: Tyrosine-protein kinase kit or CD117; ANG I and II: Angiotensin I and II; ET-1: Endothelin-1; CD: Cluster of differentiation. High and low indicate level of expression.
Erating myofibroblasts in most, if not all, tissues\[1,17\].

Myofibroblastic cells contain smooth muscle cytoskeletal markers (in particular α-SMA) together with three filaments (vimentin, desmin or myosin), with variable expression depending on tissue, species and environmental factors\[61,62\]. Based on immunohistochemical staining of these filaments in a given tissue, a classification system has been proposed\[61,62\]. Myofibroblasts express only vimentin (V-type myofibroblasts), those that express vimentin and desmin (VD-type), those that express vimentin, α-SMA and desmin (VAD-type), those that express vimentin and α-SMA (VA-type), and those that express vimentin and myosin (VM-type)\[61,62\].

Two types of myofibroblasts are present in the intestinal mucosa in physiological conditions, the interstitial cells of Cajal (ICC) and the intestinal sub-epithelial myofibroblasts (SEMFs)\[61,62\].

ICC are located in the submucosa and muscularis propria in association with the smooth muscle layer of the gut, while SEMFs are mainly located at the base of the intestinal crypts in the lamina propria. ICC are pacemaker cells which regulate gastrointestinal smooth muscle motility, facilitate the propagation of electrical events and modulate neurotransmission\[63\]. ICC express vimentin and the c-Kit receptor (Table 2).

SEMFs, also called pericryptal fibroblasts, are a syncytium of α-SMA-positive mesenchymal cells, which reside subjacent to the basement membrane of the small and large intestines\[61,62\]. SEMFs form a three-dimensional network and are in connection with each other by gap and adherent junctions, but also maintain connections with epithelial cells through fenestrations in the basement membrane; they also interact with intestinal macrophages. SEMFs express α-SMA and vimentin suggesting that they are members of the VA class of myofibroblasts. They may also express smooth muscle myosin (and thus may be referred to as VAM-type myofibroblasts), although the expression of myosin is less than that observed in the corresponding smooth muscle cells in the same tissue\[61,62\].

It is unknown whether SEMFs and ICC differentiate from a common precursor. Instead, it has been clearly demonstrated that mediators which act on myofibroblasts, promoting their proliferation and ECM production, are numerous and include: PDGF, EGF, IGF-1 and 2, CTGF, IL-1, IL-13, stem cell factor (SCF), endothelins (ET-1, -2, -3), ANG II, TGF-α, TGF-β, bFGF and PPARs; these factors are all relevant for the transdifferentiation of fibroblasts to myofibroblasts\[15\].

Smooth muscle cells
In physiological conditions when there is a need for replacement of connective tissue following injury, the production of collagen is devolved to cells of mesodermal origin. Other cells do not synthesize collagen or may produce only minute quantities; however, under abnormal stimuli collagen expression may become considerably enhanced in these cells\[61,62\]. Airway smooth muscle cells (SMCs) appear to play an important role in controlling and perpetuating airway inflammation and fibrosis in chronic airway diseases\[64\].

SMCs are one of the three interrelated cell phenotypes into which intestinal mesenchymal cells can differentiate (the other two being fibroblasts and myofibroblasts) and are mainly identified by α-SMA, desmin and collagen type I \[27\] (Table 2).

Several studies have shown that SMCs are an important cellular component of IBD. In UC, their behavior leads to a considerable thickening of the muscularis mucosa and, in CD, to a remarkable thickening of the bowel wall, with subsequent stricture formation and obstruction. These cells actively contribute to the development of fibrosis in IBD by inducing production of collagen and MMPs in response to several inflammatory mediators, such as TGF-β and IL-1β. SMCs are also able to release significant amounts of IL-6, contributing to the...
inflammatory process.\[66\]

**Stellate cells**

Stellate cells were initially described in the liver as mesenchymal cell precursors important in retinoic acid metabolism, where they represent the site of fat or vitamin A storage. Thereafter, the important contribution of these cells to hepatic and pancreatic fibrosis has been shown.\[66,67\]

These cells are identified through the expression of vitamin A, glial fibrillary acidic protein (GFAP) and \(\alpha\)-SMA (Table 2).

In the liver, it has been clearly shown that HSCs are the main contributors to fibrogenesis. Acute or chronic inflammation can promote activation of HSCs through transdifferentiation from quiescent vitamin A-rich cells into myofibroblast-like cells with strong proliferative, fibrogenic and contractile ability. Moreover, the presence of stellate cells has been detected also in other organs, such as the pancreas, gut, lung, uterus, kidney and deferent duct, even if their functions remain to be fully elucidated.\[51,66,67\]

Interestingly, there is very limited information regarding intestinal stellate cells, although recently these have been isolated and cultured from the human intestinal mucosa.\[51\]. In IBD, these cells show a higher proliferation rate, faster differentiation into myofibroblasts, and an earlier and higher collagen production than those from the normal non-IBD mucosa.\[51\]

**Pericytes**

Pericytes are cells derived from non-differentiated mesenchymal cells. They surround the endothelial cells of capillaries and small blood vessels.

Pericytes stain positive for \(\alpha\)-SMA, desmin, melanoma chondroitin sulphate proteoglycan, platelet-derived growth factor receptor (PDGFR)-\(\beta\), and regulator of G protein signaling-5. Pericytes also express costimulatory molecules such as CD80, CD86, CD13 and CD90, suggesting that they may be replenished by circulating fibrocytes. Furthermore, pericytes can express ANG I and II, endothelin-1 and collagen type I (Table 2). The pattern of these pericyte markers differs according to the type of vessel, organ and also pathological condition.

Pericytes control endothelial cell differentiation, endothelial signaling, angiogenesis and ECM degradation. These multiple functions can be exerted due to their location between the interstitium and endothelium.\[64\]. Moreover, on account of their intermediate phenotype between vascular-SMC and fibroblasts, pericytes represent a useful reserve of fibroblasts during wound healing and are, therefore, considered an excellent contributor to inflammation-associated fibrosis on account of their ability to differentiate into collagen-producing fibroblast-like cells.\[51\]. Pericytes increase the deposition of ECM proteins in proximity to the blood vessels during the initial phase of fibrotic processes. In addition, the involvement of pericytes in intestinal fibrogenesis has been recently highlighted, even if their contribution remains to be defined.\[51\].

**Epithelial and endothelial cell transformation**

The main fibrogenic cells (fibroblasts, myofibroblasts) may also derive from non-mesenchymal cells, including epithelial and endothelial cells, via transformation.

Epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition (EndoMT) is a key process in tissue development, carcinogenesis and organ fibrosis, and is characterized by dramatic changes in cell phenotype and function. Both are a consequence of disruption of the local basement membrane, loss of epithelial cell adhesion, reprogramming of the signaling machinery, de novo synthesis of \(\alpha\)-SMA, rearrangement of cytoskeletal proteins and transmigration of the epithelial cells through the basement membrane into the interstitial space. Epithelial or endothelial cells assume a spindle-shape morphology, lose classical cell markers and gain typical fibroblast or myofibroblast markers, such as FSP-1, \(\alpha\)-SMA or vimentin, and show the capacity to produce interstitial collagens and fibronectin (Table 2). All these changes are due to the high plasticity of epithelial and endothelial cells. Therefore, these cell types can be considered a multipotent progenitor tissue, which can display alternative developmental pathways following injury.

Recent data on the contribution of EMT and EndoMT in intestinal fibrosis are now available. It has been shown in animal models and in human primary cells that EMT and EndoMT can contribute to intestinal fibrogenesis.\[51,67\].

**Bone marrow and intestinal stem cells**

Stem cells are a non-differentiated cell type that do not increase in number but generate a range of differentiated progeny that may continue to be divided via a process of clonal growth. They are also defined as non-specialized cells that reside in a particular tissue and renew themselves thus becoming capable of producing all the specialized cell types of the tissue.\[73\].

Reserves of adult stem cells include the bone marrow, blood stream, cornea and retina, dental pulp, liver, skin, gastrointestinal tract and pancreas. Bone marrow contains hematopoietic and mesenchymal stem cells, which are able to migrate to the majority of organs and differentiate into various cell types. These cells may represent a source of fibroblasts during inflammatory and fibrotic disease; indeed, stem cells can migrate from the bone marrow to sites of injury, during pathological conditions, and differentiate to mesenchymal cells able to express tissue connective proteins.\[51\].

While HSCs give rise to 3 classes of blood cells (leukocytes, erythrocytes and thrombocytes), and are characterized by several specific markers, including CD45, CD34 and CD14, the mesenchymal stem cells (MSCs) are multipotent and can differentiate into several cell types such as osteoblasts, chondrocytes, myocytes and myofibroblasts. MSCs express CD105, CD73, CD44, CD71 and CD90. Both HSCs and MSCs express vimentin, che-
mokines and chemokine receptors, adhesion molecules and integrins[8].

A class of bone marrow-derived cells that become progenitors for mesenchymal cells is represented by fibrocytes that circulate in the peripheral blood. These cells appear to be involved in intestinal repair and fibrosis in IBD[69,317,324,74]. Fibrocytes express hematopoietic markers (CD45, CD34, CD11, CD13, CD14, CD80, CD86), as well as collagen 1 and α-SMA[27,317,74-76] (Table 2).

The ability of fibrocytes to differentiate into fibroblasts and contractile myofibroblasts has been highlighted. This leads to the production of ECM components and ECM-modifying enzymes by differentiated fibrocytes that migrate to the affected tissues during both normal tissue repair and inflammatory fibrotic processes. Under physiological conditions, fibrocytes, after their maturation in the bloodstream, may contribute to the local population of macrophages and dendritic cells, whereas during an inflammatory process, numerous fibrocytes differentiate into mesenchymal cells synthesizing ECM. This behavior demonstrates the close relationship between fibrocyte proliferation, migration and differentiation, as well as tissue fibrogenesis[25].

Fibrocyte functions that lead to tissue fibrosis are modulated by IL-1, TGF-β and serum amyloid P (SAP), a serum protein that inhibits the maturation process in the circulation but promotes differentiation of fibrocytes within the tissue[29]. Furthermore, fibrocytes themselves produce growth factors, such as CTGF and TGF-β, inflammatory cytokines and chemokines, that in turn promote the proliferation of resident fibroblasts and their differentiation into myofibroblasts[79].

The contribution of fibrocytes to tissue damage and progression of fibrosis has been shown in several pathological conditions, including asthma, nephrogenic fibrosis, systemic sclerosis, atherosclerosis, chronic pancreatitis, chronic cystitis and tumor-associated stromal reactions[77].

Another aspect of intestinal fibrosis under investigation is the presence and the involvement of local stem cells in the fibrotic process. Undifferentiated intestinal stem cells (ISCs) give rise to daughter or progenitor cells, which can subsequently differentiate into mature cell types such as columnar cells, goblet cells, neuroendocrine cells and Paneth cells[27,74,76].

Differentiation of ISCs is induced by various transduction pathways and appears to be involved in the pathogenesis of IBD[78]. It has been highlighted that a potential mechanism linked to the development and progression of Crohn’s disease is the differentiation of ISCs into Paneth cells[78]. These cells produce different broad spectrum antimicrobial peptides, principally the α-defensins HD-5 and HD-6. In small intestinal Crohn’s disease, both these Paneth cell products are specifically reduced. Mechanisms for defective antimicrobial Paneth cell function are complex due to the impaired functions of various pathways including the NOD2, Wnt pathway transcription factor TCF7L2 (also known as TCF4), autophagy factor ATG16L1, endosomal stress protein XBP1, toll-like receptor TLR9, calcium-mediated potassium channel KCNN4, as well as to the inactivation of HD-5.

A better understanding of all these mechanisms may lead to the development of new therapeutic approaches for the prevention and/or treatment of intestinal fibrosis progression.

**MOLECULAR MECHANISMS**

Intestinal fibrosis results from the activation of a large variety of cell types that act synergistically and are exposed to an extremely complex microenvironment, under the control of various biological mediators, such as growth factors, cytokines, chemokines, proteolytic enzymes, complement components, vasoactive amines and peptides. The most important of these molecules include, specifically, TGF-β, activins, CTGF, PDGF, IGF-1 and 2, EGF, ET-1, -2, -3, RAS, various cytokines such as IL-1, -4, -6, -13, -17, -21, -22, -23, TNF-α, ROS, PPARγ, mTOR, MMPs and TIMPs. All these molecules play an important role in the activation of the acute and chronic inflammatory response. In addition, they also regulate the fibrogenic processes stimulating ECM accumulation, independently of inflammation[1,15,25], acting in autocrine, paracrine, or endocrine pathways. All these profibrotic molecules may be considered as a target for new anti-fibrotic treatment approaches.

**TGF/Smad proteins**

Studies on transgenic mice over-expressing TGF-β have revealed the development of fibrosis in several organs, including skin, kidney, lung, heart, blood vessels, liver, pancreas and intestine[10].

TGF-β is a multifunctional polypeptide hormone acting in essentially all cells, influencing distinct functions including proliferation, differentiation, apoptosis, immunoregulation, regulation of the inflammatory response, restitution and healing, as well as fibrosis[79]. At a cellular level, TGF-β affects virtually all stages of the chronic inflammatory and fibrotic disease processes.

The profibrotic effects of TGF-β are numerous, including influx and activation of ECM-producing cells, as well as promoting EMT and EndoMT. The biological action of TGF-β that contributes to several fibrotic diseases is the regulation not only of the synthesis but also the breakdown of ECM proteins, including collagens, fibronectins and proteoglycans[77].

Multicellular organisms present more than 60 TGF-β family members. These include 3 TGF-βs, 5 activins and at least 8 bone morphogenetic proteins (BMPs), all encoded by distinct genes. The three mammalian TGF-β isoforms, TGF-β1, 2 and 3, are secreted as latent precursor molecules (LTGF-β) containing an amino-terminal hydrophobic signal peptide region, the latency associated peptide (LAP) region and the C-terminal potentially bioactive region. The LTGF-β is usually linked to latent TGF-β-binding proteins (LTBP), requiring activation into a mature form for receptor binding and subsequent...
activation of signal transduction pathways. The LTBP is removed extracellularly either by proteolytic cleavage by various proteases such as plasmin, thrombin, plasma transglutaminase, or endoglycosidases, or by physical interactions of the LAP with other proteins, such as thrombospondin-1 [80].

The actions of the TGF-β family are mediated through at least three types of TGF-β receptors, TGF-β R I, R II and R III. TGF-β R I and R II are two different transmembrane serine/threonine kinase receptors with single transmembrane domains, which form homo- and heterodimer complexes that bind TGF-β, inducing phosphorylation of a family of proteins designated as Smads that transduce the ligand signal from the cell surface to the nucleus [80] (Figure 2).

Smads are a family of 8 related proteins, which function as signaling intermediates for the TGF-β superfamily of ligands. Type I receptors specifically recognize and phosphorylate the ligand-specific receptor-activated Smad (R-Smad) and include Smad1, Smad5 and Smad8 (downstream of the BMP), and Smad2 and Smad3 (downstream of TGF-β and activin), that are recruited to activated TβRI by a membrane-bound cytoplasmic protein called Smad anchor for receptor activation (SARA). Upon ligation of TGF-β to its receptors, the phosphorylated Smad2 and 3 form a complex with the common mediator Smad (Co-Smad), such as Smad4. Co-Smad acts as a convergent node in the Smad pathways downstream of the TGF-β superfamily receptors, complexing R-Smad.

R-Smad/Smad4 complexes are then translocated into the nucleus by a mechanism involving the cytoplasmic protein importin; they may then function as transcription factors, binding DNA either directly or in association with other DNA-binding proteins [80] (Figure 2).

A third group of Smad proteins, the inhibitory Smads such as Smad6 or Smad7, antagonize TGF-β signaling by interfering with the ligation of Smad2/3 to the activated receptor complex [80].

Using a combined cDNA microarray/promoter transactivation approach, several new Smad gene targets have been identified, among which are COL1A1, COL3A1, COL5A2, COL6A1, COL6A3, and TIMP-1, indicating that the Smad signaling pathway is crucial for the simultaneous activation of several fibrillar collagen genes by TGF-β [80]. About 60 other ECM-related genes were also identified as immediate-early gene targets downstream of TGF-β [81].

Several studies suggest that disruption of the TGF-β/Smad signaling pathway, either by the loss of Smad3 or the increase of Smad7 expression, prevents the development of tissue fibrosis in various organs, including the skin, kidney, lungs, liver and intestine [82-84]. Differences in small bowel and colonic morphology, as well as in the expression of collagens I-VI, α-SMA, TGF-β1, Smad7, and other factors, have been reported in Smad3 knockout mice compared to the littermate wild-type controls [85]. Using this tool made it possible to define the key role of the TGF-β/Smad signaling pathway in chronic intestinal inflammation and fibrosis [85]. In fact, a significant reduction of trinitrobenzene sulfonic acid (TNBS)-induced intestinal fibrosis has been reported in knockout mice as compared to wild-type mice [84].
Smad3 may take part in recruitment of fibroblasts to the site of injury, as well as in differentiation of fibroblasts to myofibroblasts and regulation of collagen synthesis. Furthermore, the loss of Smad3 interferes with the effects of TGF-β on chemotaxis and auto-induction of inflammatory cells\cite{94,95}. These findings indicate that the TGF-β/Smad signaling pathway is the key element in determining the progressive nature of intestinal fibrosis.

The effects of TGF-β on ECM gene expression and subsequent development of tissue fibrosis may be related to additional mechanisms, such as CTGF, the expression of which is controlled by TGF-β1 in a Smad-dependent manner\cite{96}.

TGF-β preferentially transduces intracellular signaling through Smad proteins, but signal mechanisms that stimulate ECM accumulation can also be activated by other molecules such as members of the mitogen-activated protein kinase (MAPK) family including extracellular signal-regulated kinase (ERK), c-Jun N terminal kinase and p38 kinases, as well as members of the JAK and STAT protein family\cite{95}.

Studies on liver fibrosis demonstrate that both MAPK and Smad signaling, independently and in synergy, stimulate HSC activation, thus increasing collagen and α-SMA gene expression\cite{95,97}.

The JAK-STAT signaling pathway, activated by TGF-β, is involved in fibrogenesis and can be negatively regulated on multiple levels\cite{95}. Suppressors of cytokine signaling may inhibit STAT phosphorylation by binding and inhibiting JAKs or competing with STATs for phosphorytosome binding sites\cite{97}. Protein inhibitors of activated STATs (PIAS) may act directly in the nucleus on STAT proteins. PIAS1 and PIAS3 inhibit transcriptional activation by STAT1 and STAT3, respectively, by binding and blocking their access to the DNA sequences\cite{97}.

TGF-β1 may also influence the imbalance between enhanced production and deposition and impaired degradation of ECM components. In IBD, the tissue expression of MMP-1, 2, 3 and 9 is increased in relation to that of TIMP-1 and 2, compared with controls\cite{97}. How the balance of MMPs and TIMPs is regulated is still not clear, but blocking TGF-β1 in cultures of intestinal biopsies upregulated the expression of MMP-3 but not of TIMP-1, thus suggesting a new role for TGF-β1 in IBD tissue remodeling\cite{97}.

**Activins**

The activins are members of the TGF-β superfamily with broad and complex effects on cell growth and differentiation. The activins interact with heterodimeric serine/threonine kinase receptor complexes to activate Smad transcription factors and the MAP kinase signaling pathways. Important functions of activins, in particular of activin A, in tissue inflammation, repair and fibrosis of several organs, including the intestine, have been reported\cite{98,99}.

Activin is a dimeric protein consisting of βA and βB subunits connected by disulfide linkages resulting in three different configurations with similar functions: the homodimeric activin A (βAβA), and activin B (βBβB), and the heterodimeric activin AB (βAβB).

Their action is mediated by two heteromeric receptor complexes consisting of two transmembrane receptors (type I or II) with intrinsic serine/threonine kinase activities, similar to the TGF-β mechanism of signal transduction, while its biological effects seem to be inhibited by another activin-binding protein, namely follistatin\cite{98}.

Several studies have shown an increase in activin levels in IBD and in many other inflammatory diseases, thus giving rise to the hypothesis that it plays a significant role in the inflammatory response as well as in fibrosis\cite{95}.

Munza et al\cite{96} reported an increase in the expression of activin, particularly the activin βA-subunit, in surgical specimens from the gut of patients with IBD, showing a strong correlation with the degree of inflammation. In situ hybridization studies revealed the highest levels of activin mRNAs in the mucosa and submucosa of highly inflamed areas. These findings suggest that activins play a novel and important role during the inflammatory processes of the gut\cite{95}.

**CTGF**

TGF-β has been regarded as a pivotal growth factor in the formation and maintenance of connective tissues but it has been clearly proven that the effects on ECM gene expression and subsequent development of tissue fibrosis are related to other factors, such as CTGF, the expression of which is controlled by TGF-β1 in a Smad-dependent manner. Northern blot and in situ hybridization studies have demonstrated that CTGF is co-expressed with TGF-β1 in principally every fibrotic disorder and CTGF has been considered a possible key determinant of progressive fibrosis\cite{95}.

CTGF is a cysteine-rich mitogenic peptide that binds heparin and is secreted by fibroblasts after activation with TGF-β. In the adult mammal, CTGF acts as a downstream mediator of TGF-β action on connective tissue cells, where it stimulates cell proliferation and ECM synthesis. It does not appear to act on epithelial cells or immune cells. Since the biological actions of TGF-β are complex and affect several different cell types, CTGF may serve as a more specific target for selective intervention in processes involving connective tissue formation during wound repair or fibrotic disorders\cite{97}.

CTGF is an interesting molecule for future anti-fibrotic therapies as it is possible that inhibition of CTGF might block the pro-fibrotic effects of TGF-β, without affecting TGF-β’s immunosuppressive and anti-inflammatory effects. In addition to TGF-β, a number of other regulators of CTGF expression have been identified, including VEGF, TNF-α, shear stress, cell stretch and static pressure, hydrogen peroxide (H₂O₂), superoxide (O₂⁻), hydroxyl radical (HO ·) and nitric oxide (NO)\cite{99}.

Outlining the mechanisms that underlie CTGF gene regulation in normal and fibrotic cells might help in the design of future intervention strategies aimed at targeting specific interference with CTGF expression at sites...
of progressive fibrosis. In addition, forms of treatment targeting CTGF effects have been proposed which might lead to a favorable outcome of wound repair.

**PDGF**

PDGF consists of two related peptide chains, PDGF-A or PDGF-1 (16 kDa, 124 amino acids), and PDGF-B or PDGF-2 (14 kDa, 140 amino acids) linked by disulfide bonds. All possible isoforms, i.e., PDGF-αA, PDGF-BB and PDGF-AB, are biologically active. More recently, two additional PDGF genes and proteins have been identified, namely PDGF-C and PDGF-D\(^9\).

PDGF is synthesized mainly by megakaryocytes. It is stored in the α granules of platelets from which it is released after platelet activation. Other cell types also synthesize PDGF, including macrophages, endothelial cells, fibroblasts, glial cells, astrocytes, myoblasts, smooth muscle cells, and a number of tumor cell lines. Several factors induce PDGF synthesis, including hypoxia, thrombin and several cytokines and growth factors such as IL-1, IL-6, TNF-α, TGF-β, FGF-β and EGF\(^10\).

Two tyrosine kinase receptors, called PDGFRα and PDGFRβ, have been described. Binding of the ligand leads to receptor dimerization, which initiates signaling. Ligand configuration and pattern of receptor expression influence the formation of different receptor dimers. Generally, mesenchymal cell expression of PDGFRs is low in normal conditions, but increases dramatically during inflammation.

PDGF plays crucial roles during development. Increased PDGF activity has been linked with several diseases and pathological conditions. Its expression is significantly increased in the inflamed intestine of patients with IBD. Intestinal fibroblasts, intestinal subepithelial myofibroblasts and the interstitial cells of Cajal are activated and proliferate in response to the PDGF family (mainly PDGF-BB)\(^31\). PDGF also enhances migration of fibroblasts, and its effects seem to be fibronectin-dependent. Increased activity of PDGF is also responsible for an excessive deposition of ECM in fibrotic processes within several organs, including the intestine.

**IGFs**

IGFs (IGF- I and - II) are polypeptides composed of a single chain of 70 and 67 amino acids, respectively. They are potent mitogens since they have stimulatory effects on proliferation and inhibitory effects on apoptosis in epithelial cells. IGF- I is the predominant postnatal IGF; whereas IGF- II is a predominantly fetal IGF. IGF- I interacts with IGF- I receptor type I (IGF- I R) on the cell surface. Subsequent phosphorylation by a receptor tyrosine kinase initiates the intra-cellular signaling process. IGF- II also interacts with a receptor type II (mannose-6-phosphate IGF- II R)\(^101\).

In the intestine, IGF- I interacts principally with fibroblasts and epithelial and endothelial cells. The entire GI tract expresses IGF- I and - II and their respective receptors, the latter being localized in both the mucosal and muscularis layers. Expression of insulin-like growth factor binding protein (IGFBP)-6 in the intestine has also been described; IGFBPs are proteins that, by binding to IGF, are able to modulate its bioavailability and activity.

Expression of IGF- I is locally increased in several bowel diseases. During bowel inflammation, pro-inflammatory cytokines are able to induce IGF- I expression by mesenchymal cells. The IGF- I, in turn, could regulate the proliferation of these or other cell types acting in a paracrine manner on epithelial cells or in an autocrine manner on intestinal mesenchymal cells\(^102\). IGF- I plays a relevant role in the deposition of collagen and fibrosis. It has been shown to be up-regulated in the bowel of animals with experimental intestinal fibrosis and of patients with CD\(^103\). In an experimental model of rat colitis, an up-regulated IGFBP and collagen expression and down-regulated collagenase expression were shown, confirming the important role of IGF- I in collagen synthesis in colitis, mediated by IGFBP\(^3\)\(^103\). It has been hypothesized, moreover, that IGF- I, through IGFBP-5, is able to modulate proliferation of fibroblasts/myofibroblasts and collagen synthesis\(^104\).

In addition, in contrast with the local expression, the circulating levels of IGF- I and IGFBP-3 seem to be reduced in patients with IBD\(^108\).

**EGF**

EGF is the prototype member of a family comprising different peptides with a similar primary structure that bind to a family of EGF receptors and have similar biological effects: they are potent mitogens and are also able to modify several properties of non-proliferating cells\(^109\).

Several tyrosine kinase receptors are now known for EGF family members, such as human EGF receptors 1-4 (HER1, HER2, HER3 and HER4). Since these receptors can heterodimerize, they can form at least 10 different dimers that likely bind to distinct EGF-like molecules and deliver different signaling when activated\(^106\).

Binding of EGF induces the phosphorylation of several intracellular proteins regulating transcription, translation, cell architecture, cell proliferation, and the production of inflammatory mediators.

EGF is a 53-residue peptide and is also known as urogastrone, since it was initially isolated from urine and was found to inhibit gastric acid secretion\(^107\); it is expressed in exocrine pancreas, duodenum, breast milk, colostrum and gastric juice\(^108\).

EGF can be isolated from the lumen of the intestine; its receptors have been detected in the bowel, especially on monocytes and myofibroblasts.

EGF has numerous functions within the GI tract: it stimulates cell proliferation, inhibits gastric acid secretion, up-regulates intestinal electrolyte and nutrient transport, induces expression of several enzymes, enhances epithelial restitution and stimulates angiogenesis.

EGF stimulates fibroblast proliferation and ECM production in idiopathic pulmonary fibrosis; moreover, it stimulates a more pronounced ECM production in inter-
stital pneumonia fibroblasts than in normal fibroblasts, thus suggesting an important role of this growth factor in fibrotic processes\[^{[10]}\].

The role of EGF in IBD is not clear. EGFR expression is generally upregulated in TNBS-induced colitis\[^{[119]}\]. EGF administered intraperitoneally and subcutaneously reduces the severity of TNBS-induced colitis in rats\[^{[119]}\]. EGFR appears to be involved in the regulation of migration of human colonic fibroblasts and myofibroblasts\[^{[11,112]}\]. Further studies are needed to fully elucidate the role of EGF and EGFR in IBD and intestinal fibrosis.

**Endothelins**

Endothelins are a group of polyfunctional cytokines. So far, four endothelins (ET-1, ET-2, ET-3 and ET-4) and two endothelin receptors (ET\(_A\) and ET\(_B\)) have been recognized.

Endothelins strongly induce vasoconstriction and may provoke local ischemia. They can also induce the release of pro-inflammatory cytokines, alter the intestinal permeability and stimulate leukocyte adhesion in submucosal venules of the intestine, probably through up-regulation of cell adhesion molecules on the endothelium and leukocytes\[^{[113-115]}\].

Adhesion of circulating leukocytes to the endothelium could be one of the initial steps in the pathogenesis of IBD, since adhesion is a crucial event in the recruitment of leukocytes to the inflamed tissue. In addition, several of the pro-inflammatory cytokines playing a key role in IBD, such as IL-1\(\beta\) and TNF-\(\alpha\), can remarkably increase endothelin production\[^{[116,117]}\].

It has been shown that bosentan, a non-selective endothelin receptor antagonist, reduces intestinal inflammation in a murine model of IBD\[^{[10]}\]. Probably the reduction of inflammation induced by this drug was due to a reduction in adherent leukocytes leading to a reduction of the mucosa infiltrate of inflammatory cells.

*In vivo* studies performed on cardiac, renal and pulmonary tissue have shown the role of endothelins in fibrosis\[^{[119]}\]. ET1 stimulates colonic myofibroblast activation (differentiation, migration and contraction), effects prevented using an ET-1 antagonist\[^{[123]}\]. These findings suggest a possible role for ET1 also in the development of intestinal fibrosis.

**RAS**

The relevance of the renin-ANG system, usually viewed as an endocrine system regulating physiological and vital cardiovascular processes, has expanded in the last decade to include independently regulated local systems in several tissues, new functions of the RAS and new active products of ANG II\[^{[127,128]}\] (Figure 3).

Besides circulating RAS, local RASs also exist in various organs and tissues, such as the heart, blood vessels, kidney, liver, pancreas, intestine, nervous system, reproductive system, as well as lymphatic and adipose tissue\[^{[123,129]}\]. It has been reported that the ANG converting enzyme (ACE) produces the decapeptide ANG I and octapeptide ANG II, respectively. In addition, alternative enzymes involved in their generation (such as ACE2, aminopeptidase A or prolyl endopeptidase) and receptors (such as ANG II type 4 receptor and Mas) are released in the RAS system. RAS, therefore, is not represented by a simple linear proteolytic cascade, but by a series of extremely complex reactions (Figure 3). All components of the RAS exist in the large bowel in adults\[^{[128]}\]. In the colon, many cells, such as epithelial cells, vascular endothelial cells, mesenchymal cells and inflammatory cells, express ANG II receptors.

Local RAS has novel functions including the regulation of cell growth, differentiation, proliferation and apoptosis, generation of ROS, expression of cytokines (such as IL-6, TNF-\(\alpha\)), activation of endothelial cells, as well as tissue inflammation, ECM production and fibrosis\[^{[123]}\].

These different roles make some of the RAS components attractive therapeutic targets in various chronic diseases, including fibrosis.

ANG II, moreover, plays a role in the pathogenesis of chronic fibrogenetic diseases of various organs, including kidney, heart, lung, pancreas, liver and intestine, through the regulation of both inflammatory and fibrotic processes\[^{[127,128]}\]. In such conditions, fibrosis is mediated, at least in part, through ANG II induction of TGF-\(\beta\)\[^{[13]}\].

Several components of RAS are over-expressed in the fibrotic liver, both in human and animal models\[^{[129]}\]. In particular, it would appear that ANG II is able to induce proliferation of myofibroblasts and stellate cells,
to stimulate inflammatory cells and to induce the release of several profibrotic molecules, such as TGF-β, CTGF and IL-1β. Blocking RAS, by using ACE inhibitors or ANG II type I (AT1) receptor antagonists, reduces hepatic fibrosis.

In human lung fibroblasts, ANG II is able to modify PDGF-D, IL-4 and IL-7 expression and to induce higher levels of collagen and elastin. Using candesartan, an inhibitor of AT1 receptor, these effects have been shown to be suppressed.

In experimental models of kidney damage, RAS inhibitors (ACE inhibitors and AT1 antagonists) have shown beneficial effects on proteinuria, cell growth, inflammation and fibrosis, thus suggesting that ANG II could be involved in the fibrotic process activating monocellular cells, increasing proinflammatory mediators and regulating matrix degradation.

New insights into the role of RAS in the development and modulation of chronic intestinal inflammation and related fibrosis have been reported. The direct effect of ANG II on the pathogenesis of immune-mediated colitis was assessed using mice genetically deficient in angiotensinogen (Ang−/−), which is the precursor of ANG II. TNBS-induced acute colitis was less severe in the Ang−/− mice compared to wild-type (Ang+/+) mice.

Both ANG II and TGF-β1 are overexpressed in intestinal fibrosis and stenosis, particularly in Crohn’s disease. The production of TGF-β1, indeed, is strongly stimulated by the local activation of ANG II. An up-regulation of ANG II in the colon of rats and mice with experimentally induced colitis also supports the role of RAS in intestinal inflammation and fibrosis.

Daily administration of the ACE inhibitor captopril in rats with chronic TNBS-induced colitis significantly reduced the macroscopic and microscopic pattern of both colonic inflammation and fibrosis, decreased the colon collagen content, and reduced TGF-β1 mRNA levels by about 60%. The antifibrotic mechanism of captopril could be related to the inhibition of ANG II-mediated TGF-β1 overexpression, and/or to a direct downregulation of TGF-β1 transcripts. Likewise, the use of losartan, a specific AT1 receptor antagonist, significantly improved the macro- and microscopic scores of experimentally-induced colorectal fibrosis and reduced TGF-β1 concentration, thus suggesting that this drug has a preventive effect on colorectal fibrosis complicating TNBS-induced chronic colitis by a downregulation of TGF-β1 expression.

In view of these data, RAS could be considered as a future target for new antifibrotics in IBDs.

### Cytokines

Cytokines regulate the inflammatory process, mediating the interactions between activated immune cells and non-immune cells. They can enhance an inflammatory response or reduce inflammation and promote healing.

IBDs are mainly characterized by an enhanced CD4+ T cell proliferation and trafficking into the intestinal mucosa and by alterations in the cytokine profile of the main activated Th cells, resulting in a disturbed balance between pro- and anti-inflammatory cytokines. The abnormal lamina propria T-cell activation and the consequent resistance to apoptosis are, indeed, considered as key events in the pathogenesis of IBD.

Upon activation, naïve CD4+ T cells can differentiate into different subsets depending on the surrounding cytokine milieu. CD4+ Th cells are currently divided into four major subsets, based on their expression profile of transcription factors and secreted cytokines: Th1, Th2, Th17 and Treg.

Th1 cells are characterized by the secretion of interferon γ (INF-γ). The differentiation of Th1 cells is mainly induced by IL-12 and can be further enhanced by INF-γ. Th1 cells express the transcription factors T-bet and STAT4. INF-γ is a pro-inflammatory cytokine which together with IL-12 induces the differentiation of macrophages and the production of other pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNF-α.

Th2 cells produce primarily IL-4, IL-5 and IL-13. Th2 cells develop in the presence of IL-4 and require the transcription factors GATA3 and STAT6.

Th1 and Th2 negatively regulate each other through their specific cytokine actions: IL-12 represses the induction of Th2 cells, whereas IL-4 inhibits Th1 cell development.

Microarray experiments have established that the various gene-expression profiles associated with different pathological conditions are strongly influenced by Th1- and Th2-polarization of the chronic inflammatory responses.

The Th1 response seems to be involved mainly in CD, whereas the Th2 response occurs mainly in UC. However, during the late stages of IBD, both Th1 and Th2 cytokines play a pivotal role in the progression of intestinal fibrosis. Several findings have demonstrated that IL-1β and TNF-α, two Th1 pro-inflammatory cytokines, stimulate fibroblast proliferation and collagen deposition, whereas the development of a Th2 cell response, and particularly IL-4 and IL-13, upregulates the expression of several genes including those that encode procollagen-I, procollagen-III, MMP-2 and MMP-9 as well as TIMP-1.

In addition, the other two subsets of CD4+ T cells, represented by the Th17 and Treg cells, are involved in the development of intestinal fibrosis in alternate ways. Th17 cells, which are strongly associated with autoimmune pathology, are characterized by the production of IL-17A, IL-17F, IL-21, IL-22 and TNF-α. Differentiation of Th17 cells needs the combined action of TGF-β, IL-6 and IL-21 in mice, whereas IL-6 and IL-21 can be replaced by IL-23 or IL-1β in humans. These cytokines induce the transcription factors RORγ (mice) or RORc (human) and STAT3. Development of Th17 cells is suppressed by INF-γ and IL-4, which promote Th1 and Th2
IL-1 and IL-33: IL-1 belongs to a family of proinflammatory cytokines that are rapidly expressed following tissue damage. Besides its role in acute and chronic inflammation, IL-1 is involved in tissue remodeling following chronic inflammation in various organs.

IL-1 contributes to the development of fibrosis during chronic intestinal inflammation through various mechanisms, such as regulating intestinal myofibroblast activation, the induction of chemokines (e.g., IL-8, MCP-1) and MMP secretion, and the turnover of ECM.[144,145]. Furthermore, IL-1, in combination with TNF and INF-γ, is able to increase the TGF-β-induced epithelial-mesenchymal transition (EMT), an important cellular process of fibrogenesis.[146].

Recently, it has been reported that IL-33, a novel member of the IL-1 family, induces mucosal pathology in vitro and may lead to the development of fibrosis and angiogenesis.[147]. TLR-3 is one of the strongest promoters inducing IL-33, which activates myofibroblasts and pericytes.

IL-4 and IL-13: Some Th-2 cytokines (IL-4 and IL-13) are present in elevated levels in fibrotic processes and induce activation and differentiation of fibroblasts to myofibroblasts and also induce production of collagens[147,148]. IL-4 appears to be involved in the development of pulmonary fibrosis; in particular, activation of the IL-4Rα pathway in macrophages seems to be fundamental in silica-induced pulmonary fibrosis[147]. Furthermore, it was demonstrated that IL-4 increases the mRNA expression of collagen I, III and IV in cultured human hepatic fibroblasts; these effects require STAT-6 activation[148]. IL-4 level, finally, correlated with cardiac fibrosis in patients with heart failure[149].

Inhibition of IL-13 reduces hepatic and skin fibrosis[150,151]. In experimental studies a marked reduction of hepatic fibrosis by IL-13 blockade has been reported[150,151]. IL-13 signaling through the corresponding receptor IL-13Rα2 induces production of latent TGF-β (TGF-β bound to LAP) in macrophages and, indirectly, contributes to its activation by stimulating the synthesis of enzymes, such as MMPs and cathepsins, able to remove LAP[152]. In TNBS-induced colitis, fibrosis development is dependent upon IL-13 binding to the IL-13 receptor to induce TGF-β[153]. In the same way, if IL-13 signaling is inhibited TGF-β is produced in reduced amounts and fibrosis does not occur[153]. Soluble IL-13Rα2-Fc is a highly effective decoy receptor of IL-13 which can reduce the progression of established fibrotic disease. IL-10 has also been shown to inhibit fibrosis in numerous experimental models[1]. The IL-13 decoy receptor and IL-10, by suppressing collagen deposition, act as endogenous factors that slow the progression of fibrosis.

Both IL-4 and IL-13 appear to be responsible for promoting fibrocyte differentiation from CD14+ peripheral blood monocytes, without induction of their proliferation. In dermal fibroblasts, IL-4 and IL-13 stimulate ERK1/2 pathways, which are involved in the modulation of cells, respectively. Once Th17 cells have developed, IL-23 is needed for stabilization and further expansion of these cells. Also, IL-1β and IL-6 can act to enhance the development and expansion of these cells. The intestine of patients with IBD presents higher IL-17 levels than in healthy subjects. There is emerging evidence that IL-17 strongly interacts with IL-23, the expression of which is considerably increased in the inflamed intestine; its selective depletion with monoclonal antibodies has been demonstrated to greatly attenuate T-cell mediated colitis in mice[143].

Table 3: Predominant pro-fibrotic cytokines

| Cytokines | Role in fibrosis | Ref. |
|-----------|-----------------|-----|
| IL-1/IL-33 | Activate myofibroblasts | [144,146] |
|           | Stimulate TGF expression | [145] |
|           | Regulate chemokines, cytokines and MMPs secretion | [144] |
| IL-4/IL-13 | Activate myofibroblasts | [147] |
|           | Increase deposition of collagen I, II and IV | [148] |
|           | Induce production of latent TGF-β and activate TGF-β | [152,153] |
|           | Promote fibrocyte differentiation | [154] |
| IL-6      | Stimulates proliferation of fibroblasts | [157] |
|           | Modulates TGF-β and TGF-βR II | [156] |
|           | Its neutralization improves fibrosis | [155] |
| IL-21/IL-22 | Stimulate the expression of IL-4, IL-13 and IL-17 | [159] |
|           | Increase secretion of ECM degrading enzymes | [160] |
|           | Maintain the integrity of the epithelial barrier | [161] |
|           | Inhibit deposition of collagen | [163] |
| IL-17/IL-23 | Maintain chronic inflammation in the gut | [143] |
|           | Induce activation and proliferation of myofibroblasts | [165] |
| TNFα      | Stimulate deposition of collagen | [164] |
|           | Stimulates intestinal myofibroblast proliferation | [170] |
|           | Stimulates deposition of collagen | [171] |
|           | Inhibits collagen degradation through TIMP-1 induction | [172,173] |

TNF: Tumour necrosis factor; ECM: Extracellular matrix; IL: Interleukin; TGF-β: Transforming growth factor-β; TIMPs: Tissue inhibitors of metalloproteinases; MMPs: Matrix metalloproteinases.

Treg cells, which control effector T-cell responses, produce the anti-inflammatory cytokines IL-10 and TGF-β. The differentiation of Treg is induced by TGF-β but inhibited in the presence of pro-inflammatory cytokines. Treg are characterized by the expression of the transcription factor Foxp3 and STAT5 and the expression of CD25 on their surface.

There is a growing body of evidence proving that the inflammatory cascade which influences the progression of intestinal fibrosis can involve many Th1, Th2, Th17 and Treg cytokines, such as IL-1β, IL-4, IL-6, IL-13, IL-17, IL-21, IL-22, IL-23, IL-33 and TNF-α (Table 3). Consequently, the use of biological therapies antagonizing these cytokines could be of value in the treatment of intestinal fibrosis and, therefore, may represent the targets of new antifibrotic drugs.
of collagen gene expression\sup{[154]}.

**IL-6:** In addition to a potent proinflammatory action, IL-6 may also have a relevant role in the development of fibrosis. In the cardiac allograft model, IL-6 neutralization improved graft fibrosis\sup{[155]}. The role of IL-6 in fibrosis has been further confirmed by the finding of its ability to modulate TGF-\(\beta\) and TGF-\(\beta R\) II mRNA and protein levels in mouse skin\sup{[156]} and to strongly stimulate proliferation in normal and keloid fibroblasts, as well as increase levels of STAT-3, which contributes to several processes including collagen production\sup{[157]}. IL-6 is markedly increased in CD where it appears to stimulate fibrogenetic mesenchymal cells\sup{[158]}.

**IL-21 and IL-22:** IL-21 appears to be related to IBD-associated intestinal fibrosis. IL-21 promotes fibrosis by enhancing the development, survival and migration of Th2 cells. Moreover, it stimulates the expression of IL-4 and IL-13 receptors in macrophages, inducing their activation\sup{[159]}, stimulates the secretion of ECM-degrading enzymes by fibroblasts and the secretion of the T cell chemoattractants by epithelial cells\sup{[160]}. IL-21 is produced in excess in CD compared to controls. IL-21 together with IL-6 is critical in the development of Th17 cells.

IL-22, primarily produced by Th17 cells, presents bifunctional characteristics, pro- and anti-inflammatory, depending on the local milieu. In the intestine, it is involved both in mucosal defense and in wound healing processes. IL-22 plays a protective role in IBD by enhancing barrier integrity and epithelial immunity of the intestinal tract\sup{[161]}. A mouse model of UC has, in addition, demonstrated that IL-22 is able to activate the innate immune pathway by enhancing STAT3 activation, particularly within colonic epithelial cells, and by inducing both the restitution of mucus-producing goblet cells and the STAT3-dependent expression of mucus-associated molecules. These studies might, therefore, suggest a protective role for this cytokine in intestinal inflammation\sup{[162]}. The involvement of IL-22 in the regulation of fibrotic processes has been shown in a mouse model of hypersensitivity pneumonitis (that generally progresses to lung fibrosis), in which direct blockade of IL-22 enhanced the deposition of collagen in the lung. These findings reveal a protective pathway of this cytokine in the development of lung fibrosis and could suggest an analogous role also in the intestine\sup{[163]}.

**IL-17 and IL-23:** The IL-23/IL-17 axis plays a role in normal intestinal homeostasis, although the precise actions of these cytokines remain to be fully elucidated. In the normal intestine, constitutive production of small amounts of both IL-23 and IL-17 may act to protect the epithelial layer fortifying tight junction formation between epithelial cells and inhibiting bacterial colonization. In inflammation, activated dendritic cells produce large amounts of IL-23, which could activate innate immune cells to produce pro-inflammatory cytokines such as IL-1\(\beta\), TNF-\(\alpha\) and IL-6. IL-23 also induces an increased production of IL-17 and INF\(\gamma\), both by T cells and non-T cells. Recently, it has been shown that targeting IL-23 by employing a p40 peptide-based vaccine improves TNBS-induced acute and chronic murine colitis with a significant decrease in collagen deposition\sup{[164]}. The main action of IL-17 may be to promote the production of chemokines that recruit and activate granulocytes, further increasing inflammation\sup{[165]}. IL-17 was also found to be a potent activator of mesenchymal cells, especially of myofibroblasts\sup{[166]}.

In vivo, fibrotic models show enhanced IL-17A expression and activation of IL-17A-associated signaling pathways. Studies performed on pulmonary fibrosis models showed evidence that IL-17A increased synthesis and secretion of collagen and induced epithelial-mesenchymal transition in alveolar epithelial cells in a TGF-\(\beta\)-dependent manner\sup{[167]}. Moreover, it has been demonstrated that IL-1\(\beta\)-mediated pulmonary fibrosis is IL-17A-dependent\sup{[168]} and that IL-17A blocking is able to attenuate myocarditis-induced cardiac fibrosis and ameliorate ventricular function; further confirming its role in the fibrogenetic process\sup{[169]}.

A study on Th17 cells in human peripheral blood demonstrated that the expansion of IL-23R+ CD4+ cells was promoted by the combination of IL-7 and IL-12, which are also able to increase the secretion of IFN-\(\gamma\), while IL-12 alone stimulated these cells to secrete predominately IL-17\sup{[169]}. On the basis of these data, IL-7 and IL-12 have similarly been identified as anti-fibrotic cytokines, even if their effective role in the fibrotic process remains to be established.

**TNF-\(\alpha\)**

Another central mediator of the fibrotic process in IBD is TNF-\(\alpha\), a proinflammatory cytokine with important immunomodulatory properties, abundantly expressed in the intestine of patients with CD and UC. This cytokine is produced from various cells such as monocytes/macrophages, adipocytes and T cells, and has different effects including further macrophage and T cell activation, stimulation of granuloma formation and expression of adhesion molecules in endothelial cells with consequent recruitment of neutrophils and other immune cells at the site of inflammation.

TNF-\(\alpha\) is able to induce intestinal fibrosis by up-regulating collagen accumulation; moreover, it has mitogenic effects in intestinal myofibroblasts and is able to extend the inflammatory state thus increasing the expression of other inflammation mediators, such as IL-6 and IL-1\(\beta\), and activating NF\(\kappa\)B-dependent pathways, a transcription factor that increases the expression of cytokines, enzymes and adhesion molecules\sup{[165]}.

TNF-\(\alpha\) acts by binding to two immunologically distinct TNF-\(\alpha\) receptors of approximately 55 kDa (TNF-R1) and 75 kDa (TNF-R2). Studies performed on intestinal myofibroblasts have shown that TNFR2 is essential for TNF-\(\alpha\)-induced cell proliferation and collagen synthesis\sup{[171]}.
Furthermore, TNF-α induces TIMP-1 expression and reduces MMP-2 activity and collagen degradation. Inhibition of collagen degradation, through TIMP-1, and induction of collagen gene transcription are responsible for the effects of TNF-α on total collagen accumulation; moreover, TNF-α also appears to have additional effects on collagen synthesis when combined with IGF-I, a growth factor implicated as a key pro-fibrogenic mediator during intestinal inflammation in vivo. IGF-I and TNF-α synergistically stimulate intestinal myofibroblast proliferation and collagen production [172,173].

The role of TNF-α in IBD was confirmed by the therapeutic benefits of anti-TNF-α monoclonal antibodies. In several clinical trials, anti-TNF-α drugs, such as infliximab, adalimumab and certolizumab pegol, induced clinical remission and healing of mucosal lesions in about one third of patients with CD [174-176].

There is not strong evidence regarding the effectiveness of anti-TNF-α drugs to reduce intestinal fibrosis in IBD.

ROS
Infiltrating leucocytes in the inflamed mucosa produce a large amount of ROS. ROS include various species (O₂⁻, H₂O₂, HO·) and are involved in several processes, such as regulation of signal transduction, activity of phagocytes, oxidative damage of proteins, lipids and nucleic acids [178].

Superoxide is mainly generated from the uncoupling of the mitochondrial electron-transport system and from several enzymes including NADPH oxidases, cyclooxygenases, lipoxigenases and nitric oxide synthases. Hydrogen peroxide is directly obtained from oxygen, spontaneously or enzymatically (through the action of the enzyme superoxide dismutase); it can also be the result of lipid metabolism in peroxisomes. In the presence of metals, hydrogen peroxide can be involved in HO· formation [178].

ROS, moreover, can negatively affect cell signaling by altering the transcription process and phosphorylation of proteins, including transcription factors.

ROS are involved in acute and chronic inflammatory processes that include a neutrophil or macrophage infiltrate, such as acute and chronic infections, autoimmune conditions such as IBD, arthritis and other inflammatory conditions [179]. In several pathological states the increase of ROS depends on an over-expression or increased activity of enzymes involved in their production. Increased activity of the NADPH oxidase enzyme is associated with pulmonary, cardiac, hepatic and intestinal fibrosis [179].

Patients with idiopathic pulmonary fibrosis have a lower antioxidant capacity compared to healthy subjects [180,182]. It has been reported that antioxidants protect rats against experimental pulmonary fibrosis [183,184]. It is thus likely that attenuating oxidative stress in tissues could prevent fibrosis caused by ROS.

A recent study showed that mice with NADPH oxidase deficiency had phagocytes which did not produce ROS and they did not develop lung fibrosis induced by bleomycin [185]. Inhibition of ROS production in this experimental model was associated with alterations in IL-6 levels and in the MMP-9/TIMP-1 ratio, molecules involved in pulmonary fibrosis and remodeling [185].

The role of ROS has been widely studied in liver fibrosis, and substantial data suggest its importance in the transformation of HSCs into activated collagen-producing cells [186]. ROS appear to be a key mediator in collagen gene regulation [187].

ROS could also be involved in intestinal fibrosis. It has been shown that inhibition of oxygen radical secre-
PPAR-γ

Novel molecules involved in IBD and in tissue fibrogenesis appears to be PPARs[189]. Three different isoforms of PPARs have been identified, termed PPAR-α, PPAR-γ and PPAR-δ, each one encoded by specific genes and with distinct patterns of tissue distribution but with similar structure and function. PPARs are nuclear receptors, which regulate gene transcription by binding to retinoid X receptors (RXR) as functional heterodimers in response to a variety of endogenous and exogenous ligands. The PPAR/RXR complex modulates gene target expression by binding to the PPAR response elements (PPREs) in the gene promoter[190]. These nuclear receptors present three main domains: the N-terminal domain containing a ligand-independent transactivation function; the DNA-binding domain containing two zinc-finger motifs responsible for binding on PPREs to regulate transcription of PPAR-responsive genes; the ligand-binding domain (LBD) containing a ligand-dependent activation function. The LBD shows the presence of a wide hydrophobic region which could explain the increased ability of PPARs to bind a wide variety of natural and synthetic ligands[190,192] (Figure 4).

In particular, the PPAR-γ isoform, identified mainly in the colorectal mucosa but also in adipocytes, the liver, vascular tissue and several inflammatory cells (monocytes and macrophages, dendritic cells, B and T cells), seems to be involved in several physiological processes, such as differentiation of adipocytes, glucose homeostasis, lipid metabolism, inflammatory and immune processes, as well as fibrosis[189,190]. Several ligands, either natural, such as arachidonic acid metabolites (15-d-PGJ2) and some eicosanoids, or synthetic, such as thiazolidinediones and some non-steroidal anti-inflammatory drugs, appear to be responsible for the activation of PPAR-γ (Figure 4).

PPAR-γ activation seems to be strongly related to the TGF-β/Smad pathway. The stimulation of PPAR-γ with specific ligands, indeed, interferes with the Smad3 pathway by directly antagonizing Smad3 or downregulating the CTGF expression that promotes TGF-induced synthesis of collagen[188,193]. PPAR-γ agonists inhibit fibroblast migration and proliferation[184] as well as the transdifferentiation of epithelial and mesenchymal cells in activated myofibroblasts[194], one of the key points in fibrosis development. PPAR-γ ligands repress TGF-β-induced myofibroblast differentiation and activation by targeting the PI3K/Akt and Smad3 pathways, respectively[195,196]. Overexpression of PPAR-γ prevents the development of tissue fibrosis, whereas its loss increases susceptibility to fibrosis[190,199]. All these findings could explain the ability of PPAR-γ to interfere in multiple phases of the tissue fibrotic processes. Therefore, PPAR-γ should be regarded as providing innate protection from excessive fibrogenesis and as a potential new target for the development of novel compounds with anti-fibrotic properties[200]. Several PPAR-γ ligands with selective activity are under development. Experimental studies have shown that PPAR-γ agonists attenuate fibrosis in various organs including lung, kidney, pancreas, liver and intestine, antifibrotic effects that are abolished by the use of PPAR-γ selective antagonists[201-206]. The PPAR-γ coding gene is considered a potential susceptibility gene for IBD[190].

Mammalian target of rapamycin

In the 1970s, during a discovery programme for novel anti-microbial agents from natural sources, a soil sample from Easter Island (known as Rapa Nui) was found to contain Streptomyces hygroscopicus, a bacterium that produces a potent antifungal metabolite; the antibiotic was isolated and called rapamycin (Rapa + mycin). TOR was originally identified in Saccharomyces cerevisiae and, in subsequent biochemical studies, also in mammals and therefore was named the mTOR.

mTOR is a 289 kDa phosphatidylinositol 3-kinase-related kinase[207], mTOR signaling is activated by hormones, growth factors, amino acid levels, stress and alterations in cellular energy status[208] (Figure 5). Among the signal inputs, growth factor- and hormone-induced mTOR activation is the best characterized and is believed to be mediated through the PI3K pathway and PKB/Akt.

mTOR forms at least two distinct complexes. mTOR complex 1 (mTORC1) is composed of mTOR, GβL and Raptor and is responsible for sensing the nutrient; mTORC2 consists of mTOR, GβL and Rictor and is involved in the organization of actin. mTORC1 is sensitive to rapamycin whereas mTORC2 is insensitive to rapamycin.

The best characterized downstream targets of mTORC1 are S6K1 and 4E-BPI which control protein synthesis and cell growth and proliferation, respectively, as
well as autophagy, angiogenesis and fibrosis[208] (Figure 5). Rapamycin induces a translational arrest by preventing phosphorylation of S6K-1 and 4E-BPI by mTOR[209].

It has been demonstrated that mTOR strongly enhances expression of hypoxia-inducible factor (HIF)1-α, a subunit of HIF which is a transcription factor that mediates expression of several genes, the products of which play a considerable role in inducing angiogenesis[210]. Two of the key gene products induced by HIF are VEGF and angiotensin-2, which represent the main driving factors of neo-angiogenesis.

It has been shown that mTOR signaling is required for angiogenesis and plays a key role in endothelial cell proliferation in response to hypoxia. Hypoxia, indeed, rapidly promotes and sustains mTOR phosphorylation, whereas mTOR inhibition by rapamycin specifically abrogates hypoxia-mediated amplification of endothelial proliferation and angiogenesis[211-213]. Moreover, it has been recognized that mTOR-dependent HIF1-α expression is sensitive to rapamycin. Inhibition of mTOR with rapamycin can reduce the process of angiogenesis by blocking VEGF, TNF-α and PDGFα production through inhibition of HIF1-α expression and its transcriptional activation[213,214]. mTOR might, therefore, play an important role in the abnormal angiogenesis associated with IBD. It has been reported that mTOR is an effector of EGFR- and ANG type 1 receptor-induced fibrosis[216,217].

mTOR signaling is considered an attractive target for antifibrotic intervention. mTOR inhibitors constitute a relatively new category of immunosuppressive and antineoplastic drugs[218]. These share a unique mechanism of action that is focused on the inhibition of the mTOR. Their clinical applications have recently expanded significantly to cover a wide spectrum of immune and non-immune-mediated disorders, including IBD as well as solid organ transplantation, various solid organ and hematological malignancies, metabolic problems such as diabetes mellitus and obesity, and even fibrotic conditions, including skin, pulmonary, renal, hepatic and intestinal fibrosis[215,217,219,223].

It has been reported that rapamycin exerts direct antifibrotic activities both by reducing the number of fibroblasts and myofibroblasts and by down-regulating the production of fibrogenic cytokines, such as IL-4, IL-6, IL-17 and TGF-β1, and the synthesis of type I and III collagen[224,225]. Application of rapamycin to cultures of fibroblasts dose- and time-dependently downregulated the expression of cytoplasmic PCNA, cyclin D1, α-SMA, fibronectin and collagen[227]. Rapamycin may improve the defective autophagy associated with fibrostenotic CD[208,215,216,217].

mTOR might, therefore, also play important roles in IBD-associated fibrosis. Combined immunosuppressive and anti-fibrotic actions of rapamycin and its analogues may result in a promising treatment approach to fibrotic chronic enteropathies such as CD. This has been confirmed by two case reports in which two patients with severe refractory CD were successfully treated with two different analogues of rapamycin: sirolimus and everolimus[228,229]. In addition, a recent clinical trial demonstrated the effectiveness of everolimus to maintain steroid-induced remission in patients with moderate-to-severe active CD[230].

**MMPs**

An increased turnover of ECM components leads to an intensive remodeling of connective tissue. A delicate balance exists between synthesis and degradation of ECM components. Disturbance of this balance may result either in progressive organ destruction, as seen in formation of ulcers and fistulae, or excessive deposition of collagen, resulting in fibrosis[231-233]. Degradation of all ECM components is regulated by the enzymatic activity of the predominant and large family of MMPs. In addition to playing a central role in ECM turnover, MMPs proteolytically activate or degrade a variety of non-matrix substrates including chemokines, cytokines, growth factors and junctional proteins. Thus, they are increasingly recognized as critical players in inflammatory response and fibrogenesis[231-233].

MMPs are a group of calcium-activated and zinc-dependent endopeptidases that are secreted as proforms (inactive zymogens)[231-233]. They are produced by various cell types, including mesenchymal cells, T-cells, monocytes, macrophages and neutrophils, in response to inflammatory stimuli, such as IL-1, TNF-α, or TGF-β[234]. The MMP family consists of at least 25 distinct members[234], which can be subclassified according to their substrate specificities: collagens (e.g., MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -11), elastase (MMP-12), membrane types (MMP-14, -15, -16, -17, -24, -25) and others (MMP-19, -20, -23, -26, -27, -28). All MMPs become activated via proteolytic cleavage and are regarded as the major enzyme group capable of degrading ECM components such as collagens, laminins and fibronectins, including basement membranes. Once one type of MMP is activated it can catalyze activation of other MMP proforms.

MMP activity is controlled by specific and non-specific inhibitors such as TIMPs and α2-macroglobulin, respectively[236,238,239]. TIMPs are produced by the same cell types as MMPs and form a 1:1 complex with activated MMPs. The fine balance between MMPs and TIMPs regulates the turnover of ECM both under physiological conditions and in tissue remodeling during inflammation and wound healing. Thereby, the imbalance, due to reduced MMP activity and/or increased expression of TIMPs, may lead to excessive deposition of ECM proteins driving fibrogenesis[231].

In IBD, overexpression of several MMPs has been reported, which might lead to inflammation and tissue injury, facilitating the migration and invasion of fibroblasts into the bowel wall, ultimately leading to fibrosis[235,236]. Addition of recombinant MMP-3 to an ex vivo gut model caused extensive tissue injury, and inhibition of MMPs almost completely abolished tissue destruction[239]. The expression of MMP-1, -3, -7 and -13, as well as TIMP-3,
was increased in the wound edges of ulcerated tissue in UC and CD\cite{18}. Levels of MMP-1, -2, -3 and -9 relative to TIMP-1 and -2 are increased in inflamed, compared to non-inflamed, IBD tissue homogenates, regardless of the presence of fibrosis\cite{9,23}.

TGF-β1 may lead to fibrosis by creating an imbalance of TIMP-1/MMP expression in favor of TIMP-1\cite{241}. Stricture-derived myofibroblasts in CD overexpress TIMP-1\cite{242} and the expression of TGF-β1 and TIMP-1 is increased in the mucosa overlying strictured compared to non-structured intestine, while MMP-3 and -12 are downregulated\cite{243}. In support of this notion, blocking TGF-β1 in cultures of intestinal biopsies upregulates the expression of MMP-3, but not of TIMP-1\cite{10}. In a murine model of chronic inflammation, fibrosis is associated with an increase in TIMP-1\cite{244}.

A possible genetic basis has been explored: SNPs in genes encoding MMPs and TIMPs have been reported. An SNP at the TIMP-1 site is associated with increased susceptibility for CD, and an SNP at the MMP-3 site may increase the chance of stenotic complications\cite{245}.

An imbalance in MMP:TIMP expression and enhanced levels of the messages for fibrogenic cytokines and ECM proteins were also reported in late radiation enteritis and Schistosoma mansoni-induced chronic colitis\cite{246,247}. Despite the fact that the expression of collagens, MMPs and TIMPs simultaneously increased, quantification of net collagen deposition showed an overall accumulation of collagen. These findings indicate that the intestine affected by chronic inflammation is subjected to an active process of fibrogenesis as well as fibrolysis, with a balance toward fibrogenesis. This demonstrates that established fibrotic tissue is not scarred fixed tissue but is subjected to a dynamic remodeling process.

Taken together, all these data strongly support the hypothesis that an imbalance of tissue-degrading enzymes and their inhibitors may cause intestinal fibrosis\cite{242,25,243}, MMPs and TIMPs may, therefore, be considered as targets for new anti-fibrotic treatment approaches.

CONCLUSION

Intestinal fibrosis is a highly complex process involving the dynamic actions of numerous molecules which are able to regulate activation of ECM-producing cells during tissue damage and repair. The specific molecules determining the balance between physiologic repair following acute inflammation versus the excessive accumulation of ECM leading to fibrosis remain to be identified.

Strong evidence indicates that inflammation triggers fibrosis, which, once established, may progress independently. Available anti-inflammatory drugs have been shown to be ineffective in the prevention and treatment of the fibrosis. It is critical to elucidate the cellular signals promoting fibrogenesis that act independently of inflammatory pathways and immuno-inflammatory response.

Definition of the cellular and molecular mechanisms involved in intestinal fibrosis will represent the key to the development of new therapeutic approaches for the treatment of fibrostenosing enteropathies, particularly CD.

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REFERENCES

1. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008; 214: 199-210
2. Van Assche G, Geboes K, Rutgeerts P. Medical therapy for Crohn’s disease strictures. Inflamm Bowel Dis 2004; 10: 55-60
3. Burke JP, Mulso J, O’Keane C, Docherty NG, Watson RW, O’Connell PR. Fibrogenesis in Crohn’s disease. Am J Gastroenterol 2007; 102: 439-448
4. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity 2009; 30: 646-655
5. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity 2009; 30: 80-91
6. von Lampe B, Barthel B, Coupland SE, Riecken EO, Rosewitz S. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. Gut 2000; 47: 63-73
7. Faubion WA, Loftus EV, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. Gastroenterology 2001; 121: 255-260
8. Cosnes J, Nion-Larmurier I, Beaugerie L, Afnan P, Tirez E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn’s disease on the need for intestinal surgery. Gut 2009; 58: 237-241
9. Toy LS, Scherf EF, Kornbluth A, Marion JF, Greenstein AJ, Agus S, Gerson C, Fox N, Present DH. Complete bowel obstruction following initial response to infliximab therapy for Crohn’s disease: A series of a newly described complication. Gastroenterology 2000; 118 (Suppl 2): A569
10. Gardiner KR, Dasari BV. Operative management of small bowel Crohn’s disease. Surg Clin North Am 2007; 87: 587-610
11. Hwang JM, Varma MG. Surgery for inflammatory bowel disease. World J Gastroenterol 2008; 14: 2678-2690
12. Ajlouni Y, Iser JH, Gibson PR. Endoscopic balloon dilation of intestinal strictures in Crohn’s disease: safe alternative to surgery. J Gastroenterol Hepatol 2007; 22: 486-490
13. Wengrower D, Zaminnelli G, Pappo O, Latella G, Sestieri M, Villanova A, Faitelson Y, Pines M, Goldin E. Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. Inflamm Bowel Dis 2004; 10: 536-545
14. Latella G, Sferra R, Vetuschi A, Zaminnelli G, D’Angelo A, Catitti V, Caprilli R, Gaudio E. Prevention of colonic fibrosis by Boswellia and Scutellaria extracts in rats with colitis induced by 2,4,5-trinitrobenzene sulphonic acid. Eur J Clin Invest 2008; 38: 410-420
15. Rieder F, Brenmoehl J, Loeb S, Schölmerich J, Rogler G. Wound healing and fibrosis in intestinal disease. Gut 2007; 56: 130-139
16. Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491-21494
17. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007; 117: 524-529
18. McCartney-Francis NL, Chan J, Wahl SM. Inflammatory joint disease: clinical, histological, and molecular parameters
of acute and chronic inflammation and tissue destruction. Methods Mol Biol 2003; 225: 147-159

19 Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 2009; 17: 153-162

20 Liew FY, Xu D, Brint KE, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. Nat Rev Immunol 2005; 5: 446-458

21 Szébeni B, Veres G, Dezsőfi A, Rusai K, Vannay A, Mraz M, Majorova E, Arató A. Increased expression of Toll-like receptor (TLR) 2 and TLR4 in the colonic mucosa of children with inflammatory bowel disease. Clin Exp Immunol 2008; 151: 14-21

22 Carlo E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68: 7010-7017

23 Franchimont D, Vermeire S, El Housni A, Pierik M, Van Steen K, Gustot T, Querini MM, Abramowicz M, Van Gossum A, Deviere J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR4)-Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut 2004; 53: 987-992

24 Török HP, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of the Toll-like receptor 4 gene with ulcerative colitis. Clin Immunol 2004; 112: 85-91

25 Dubinsky MC, Kugathasan S, Mei L, Picornell Y, Nebel J, Wrobel I, Quiros A, Silber G, Wahbeh G, Ketzir L, Vasiliauskas E, Bahar R, Otuay A, Mack D, Evans J, Rosh J, Hemker MO, Leleiko N, Crandall W, Langton C, Landers C, Taylor KD, Targan SR, Ott J, Markowitz J, Hyams J. Increased immunity predicts aggressive complicating Crohn's disease in children. Clin Gastroenterol Hepatol 2008; 6: 1105-1111

26 Rieder F, Lawrance IC, Leite A, Sans M. Predictors of fibrostenotic Crohn's disease. Inflamm Bowel Dis 2011; 17: 2000-2007

27 Rieder F, Fiocchi C. Intestinal fibrosis in IBD--a dynamic, multifactorial process. Nat Rev Gastroenterol Hepatol 2009; 6: 226-235

28 Meneghin A, Dassopoulos T, Targan SR, Annese V, Wijmenga C, Pettersson S, Rotter JI, Sharma Y, Silverberg MS, Cho JH, Wu J, Roeder K, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Tatt D, Green T, Griffiths AM, Kistner EO, Muriitha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhardt AH, Targan SR, Xavier RJ, Liouliou C, Sardor C, Lathrop M, Belaiche J, Hewitt O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Omnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Trelleming M, Deloukas P, Manfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008; 40: 955-962

29 McGovern DP, Gardet A, Törkvist L, Goyette P, Essers J, Taylor KD, Neale BM, Ong RT, Lagace C, Li C, Green T, Stevens CR, Beauchamp C, Fleschner PR, Carlson M, D'Amato M, Halfvarson J, Hibberd ML, Lördal M, Padyukov L, Andriulli A, Colomba E, Latiano A, Palmieri O, Bernard EJ, Deslanges C, Hommes DW, de Jong DJ, Stokkers PC, Weersma RK, Sharma Y, Silverberg MS, Cho JH, Wu J, Roeder K, Brant SR, Schumm LP, Duerr RH, Dubinsky MC, Glazer NL, Harinckens T, Ippoliti A, Melmed GY, Sicosvick DS, Vasiliauskas EA, Targan SR, Amse S, Wijmenga C, Petterson S, Rotter JI, Xavier RJ, Daly MJ, Riosx JD, Seielstad M. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 2010; 42: 332-337

30 Meijer MJ, Mieremet-Ooms MA, van Hogezaand RA, Lamers CB, Hommes DW, Verspaget HW. Role of matrix metalloproteinase, tissue inhibitor of metalloproteinase and tumor necrosis factor-alpha single nucleotide gene polymorphisms in inflammatory bowel disease. World J Gastroenterol 2007; 13: 2960-2966

31 Weersma RK, Stokkers PC, Cleynen I, Wolkamp SC, Henk aerts L, Schreiber S, Dikstra G, Franke A, Nolte IM, Rutz geerts P, Wijmenga C, Vermeire S. Confirmation of multiple Crohn's disease susceptibility loci in a large Dutch-Belgian cohort. Am J Gastroenterol 2009; 104: 630-638

32 Henckaerts L, Van Steen K, Verstreken I, Cleynen I, Franke A, Schreiber S, Rutgeerts P, Vermeire S. Genetic risk profiling and prediction of disease course in Crohn's disease patients.
Mechanisms of intestinal fibrosis

Clin Gastroenterol Hepatol 2009; 7: 972-980.e2

Danese S. Nonimmune cells in inflammatory bowel disease: from victim to villain. Trends Immunol 2008; 29: 555-564

Chidlow JH, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. Am J Physiol Gastrointest Liver Physiol 2007; 293: C5-C18

Danese S. Inflammation and the mucosal microcirculation in inflammatory bowel disease: the ebb and flow. Curr Opin Gastroenterol 2007; 23: 384-389

Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease - Current knowledge and future perspectives. J Crohns Colitis 2008; 2: 279-290

Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease: progress in basic and clinical science. Curr Opin Gastroenterol 2008; 24: 462-468

Pucilowska JB, Williams KL, Lund PK. Fibrogenesis. IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models. Am J Physiol Gastrointest Liver Physiol 2007; 297: G655-G659

Lawrence IC, Maxwell L, Doe W. Altered response of intestinal mucosal fibroblasts to profibrogenic cytokines in inflammatory bowel disease. Inflammm Bowel Dis 2001; 7: 226-236

Simmons JG, Pucilowska JB, Keku TO, Lund PK. IGF-I and TGF-beta1 have distinct effects on phenotype and proliferation of intestinal fibroblasts. Am J Physiol Gastrointest Liver Physiol 2002; 283: G809-G818

Leeb SN, Vogl D, Falk W, Schölmerich J, Rogler G, Gelbmann CM. Regulation of migration of human colonic myofibroblasts. Growth Factors 2002; 20: 81-91

Leeb SN, Vogl D, Grossmann J, Falk W, Schölmerich J, Rogler G, Gelbmann CM. Autocrine fibroblastin-induced migration of human colonic fibroblasts. Am J Gastroenterol 2004; 99: 335-340

Leeb SN, Vogl D, Gunckel M, Kiessling S, Falk W, Göke M, Schölmerich J, Gelbmann CM, Rogler G. Reduced migration of fibroblasts in inflammatory bowel disease: role of inflammatory mediators and focal adhesion kinase. Gastroenterology 2003; 125: 1341-1354

Rieder F, Georgieva M, Schirbel A, Artinger M, Zügner A, Blank M, Brenmoehl J, Schölmerich J, Rogler G. Prostaglandin E2 inhibits migration of colonic lamina propria fibroblasts. Inflammm Bowel Dis 2010; 16: 1505-1513

Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 2003; 200: 500-503

Desmoulière A, Champion M, Gabbiani G. Tissue repair, contraction, and the myofibroblast. Wound Repair Regen 2005; 13: 7-12

Powell DW, Milfin RC, Valentich JD, Crowe SE, Saada JL, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol 1999; 277: C1-C9

Powell DW, Milfin RC, Valentich JD, Crowe SE, Saada JL, West AB. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Physiol 1999; 277: C185-C201

Sanders KM, Ordög T, Ward SM. Physiology and pathophysiology of the intestinal cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of intestinal cells of Cajal. Am J Physiol Gastrointest Liver Physiol 2002; 282: G747-G756

Tilió O, Panetieri RA. Regulation of inflammation by airway smooth muscle. Curr Allergy Asthma Rep 2008; 8: 262-268

Ng EK, Panesar N, Longo WE, Shapiro MJ, Kaminski DL, Tolman KC, Mazuski JE. Human intestinal epithelial and smooth muscle cells are potent producers of IL-6. Mediators Inflamm 2003; 12: 3-8

Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, Ramadori G. Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 1999; 117: 1205-1221

Apte MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut 1999; 44: 534-541

Gerhardt H, Behrends C. Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res 2003; 314: 15-23

Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol 2006; 172: 973-981

Flier SN, Tanjore H, Kokotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem 2010; 285: 20202-20212

Rieder F, Kessler SP, West GA, Bielhoça S, de la Mote C, Sadler TM, Gopalan B, Stylianou E, Fiocchi C. Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of intestinal fibrosis. Am J Pathol 2011; 179: 2660-2673

Umar S. Intestinal stem cells. Curr Gastroenterol Rep 2010; 12: 340-348

Ishii G, Sangai T, Sugiyama K, Ito T, Hasebe T, Endoh Y, Magae J, Ochiai A. In vivo characterization of bone marrow-derived fibroblasts recruited into fibrotic lesions. Stem Cells 2005; 23: 699-706

Mifflin RC, Pinchuk IV, Saada JL, Powell DW. Intestinal myofibroblasts: targets for stem cell therapy. Am J Physiol Gastrointest Liver Physiol 2011; 300: G684-G696

Quan T, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. Curr Rheumatol Rep 2006; 8: 145-150

Fiocchi C, Lund PK. Themes in fibrosis and gastrointestinal inflammation. Am J Physiol Gastrointest Liver Physiol 2011; 300: G677-G683

Bucala R. Fibrocytes: new insights into tissue repair and systemic fibrosis. London: World Scientific Publishing Company, 2007

Gersemann M, Stange EF, Wehkamp J. From intestinal stem cells to inflammatory bowel diseases. World J Gastroenterol 2011; 17: 3198-3203

Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiah P, Kim SJ, Sporn MB. Transforming growth factor-beta: multifunctional regulator of differentiation and development. Philos Trans R Soc Lond B Biol Sci 1990; 327: 145-154

Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. J Invert Dermatol 2002; 118: 211-215

Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-beta /Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. J Biol Chem 2001; 276: 17058-17062

Inazaki K, Kanamaru Y, Kojima Y, Suedoishi N, Okumura K, Kaneko K, Yamashiro Y, Ogawa H, Nakao A. Smad3 deficiency attenuates renal fibrosis, inflammation, and apoptosis after unilateral ureteral obstruction. Kidney Int 2004; 66: 597-604

Inagaki Y, Okazaki I. Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. Gut 2007; 56: 284-292

Latella G, Vetuschi A, Serra R, Zanninelli G, D’Angelo A, Cattivi V, Caprilli R, Flanders KC, Gaudio E. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colocolitis fibrosis. Gut J Clin Invest 2009; 39: 145-156

Zanninelli G, Vetuschi A, Serra R, D’Angelo A, Fratticci A, Continenza MA, Chiaramonte M, Gaudio E, Caprilli R, Sporn MB. Smad3 knock-out mice as a useful model to study intestinal fibrogenesis. Inflamm Bowel Dis 2002; 8: 1505-1513

Verrecchia F, Mauviel A. Identification of novel TGF-beta /Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. J Biol Chem 2001; 276: 17058-17062

Inagaki Y, Okazaki I. Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. Gut 2007; 56: 284-292

Latella G, Vetuschi A, Serra R, Zanninelli G, D’Angelo A, Cattivi V, Caprilli R, Flanders KC, Gaudio E. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colocolitis fibrosis. Gut J Clin Invest 2009; 39: 145-156

Zanninelli G, Vetuschi A, Serra R, D’Angelo A, Fratticci A, Continenza MA, Chiaramonte M, Gaudio E, Caprilli R, Sporn MB. Smad3 knock-out mice as a useful model to study intestinal fibrogenesis. Gut 2007; 56: 284-292
Bonniaud P, Margotts PJ, Ask K, Flanders K, Gaudile J, Kolb M. TGF-beta and Smad3 signaling link inflammation to chronic fibrogenesis. J Immunol 2005; 175: 5390-5395

Tsukada S, Westwick JK, Ikejima K, Sato N, Rippe RA, SMAD and MAPK signaling pathways independently regulate alpha(I) collagen gene expression in unstimulated and transforming growth factor-beta-stimulated hepatic stellate cells. J Biol Chem 2005; 280: 10055-10064

Hayashida T, Schnaper HW. High ambient glucose enhances sensitivity to TGF-beta1 via extracellular signal--regulated kinase and protein kinase Cdelta activities in human mesangial cells. J Am Soc Nephrol 2004; 15: 2002-2011

Pfeifer AC, Timmer J, Klingmüller U. Systems biology of JAK/STAT signalling. Essays Biochem 2008; 45: 109-120

Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol 2002; 2: 410-416

Meijer MJ, Mieremet-Ooms MA, van der Zon AM, van Duijn W, van Hogezand RA, Sier CF, Hommes DW, Lamers CB, Verspaget HW. Increased mucosal matrix metalloproteinase-1,-2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. Dig Liver Dis 2007; 39: 733-739

Di Sabatino A, Pickard KM, Rampton D, Kruidenier L, Rovedatt L, Leakey NA, Corazza GR, Monteleone G, MacDonald TT. Blockade of transforming growth factor beta upregulates T-box transcription factor T-bet, and increases T helper cell type 1 cytokine and matrix metalloproteinase-3 production in the human gut mucosa. Gut 2008; 57: 605-612

Kumagai S, Takio K, Eto Y, Shibai H, Titani K, Sugino H. Activin-binding protein from rat ovary is follistatin. Science 1990; 247: 836-838

Werner S, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev 2006; 17: 157-171

Munz B, Hübner G, Tretter Y, Alzheimer C, Werner S. A novel role of activin in inflammation and repair. J Endocrinol 1999; 161: 187-193

Grotendorst GR. Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. Cytokine Growth Factor Rev 1997; 8: 171-179

Blom IE, Goldschmeding R, Leask A. Gene regulation of connective tissue growth factor: new targets for antifibrotic therapy? Matrix Biol 2002; 21: 473-482

Andrea J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev 2008; 22: 1276-1306

Kumagai S, Ohnita H, Nagai T, Funu K, Hiwatashi NO, Shimosegawa H. Platelet-derived growth factor and its receptors are expressed in both active inflammation and active fibrosis in inflammatory bowel disease. Tohoku J Exp Med 2001; 195: 21-33

Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Path 2001; 54: 311-316

Simmons LG, Ling Y, Wilkins H, Fuller CR, D’Ercoloe AJ, Fagin J, Lund PK. Cell-specific effects of insulin receptor substrate-1 deficiency on normal and IGF-1-mediated colonic growth. Am J Physiol Gastrointest Liver Physiol 2007; 293: G995-1003

Zeeh JM, Riley NE, Hoffmann P, Reinschagen M, Goebell H, Gerken G. Expression of insulin-like growth factor binding proteins and collagen in experimental colitis in rats. Eur J Gastroenterol Hepatol 2001; 13: 851-858

Zimmermann EM, Li L, Hou YT, Mohapatra NK, Paciowksa JB. Insulin-like growth factor 1 and insulin-like growth factor binding protein 5 in Crohn’s disease. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1022-G1029

Katsanos KH, Tsatsoulis A, Christodoulou D, Challa A, Katsaraki A, Tsianos EV. Reduced serum insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 levels in adults with inflammatory bowel disease. Growth Horm IGF Res 2001; 11: 364-367

Earp HS, Calvo BF, Sartor CI. The EGF receptor family--multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER2. Trans Am Clin Climatol Assoc 2003; 114: 315-334: discussion 333-334

Gregory H, Willishe IR. The isolation of the urogastrones - inhibitors of gastric acid secretion - from human urine. Hoppe Seylers Z Physiol Chem 1975; 356: 1765-1774

Konturek JW, Bielanski W, Konturek SJ, Bogdal J, Oleksy J. Distribution and release of epithelial growth factor in man. Gut 1989; 30: 1194-1200

Hetzel M, Bachem M, Anders D, Trischler G, Faehling M. Different effects of growth factors on proliferation and matrix production of normal and fibrotic human lung fibroblasts. Lung 2005; 183: 225-237

Hoffmann P, Reinschagen M, Zeeh JM, Lakshmanan J, Wu VS, Goebell H, Gerken G, Eysselein VE. Increased expression of epithelial growth factor-receptor in an experimental model of colitis in rats. Scand J Gastroenterol 2000; 35: 1174-1180

Brenmühl J, Mäller SN, Hofmann C, Vogl D, Falk W, Schülmchier J, Rogler G. Transforming growth factor-beta1 induces intestinal myofibroblast differentiation and modulates their migration. World J Gastroenterol 2009; 15: 1431-1442

Kong Q, Majeska RJ, Vazquez M. Migration of connective tissue-derived cells is mediated by ultra-low concentration gradient fields of EGF. Exp Cell Res 2011; 317: 1491-1502

Cunningham ME, Huribal M, Bala R, McMillen MA. Endothelin-1 and endothelin-4 stimulate monocyte production of cytokines. Crit Care Med 1997; 25: 958-964

Boros M, Massberg S, Baranyi L, Okada H, Messmer K. Endothelin 1 induces leukocyte adhesion in submucosal venules of the rat small intestine. Gastroenterology 1998; 114: 103-114

Zouki C, Baron C, Fourrier A, Filep JG. Endothelin-1 enhances neutrophil adhesion to human coronary artery endothelial cells: role of ET(A) receptors and platelet-activating factor. Br J Pharmacol 1999; 127: 969-979

Klemm P, Warner TD, Hofhild T, Corder R, Vane JR. Endothelin 1 mediates ex vivo coronary vasoconstriction caused by exogenous and endogenous cytokines. Proc Natl Acad Sci USA 1995; 92: 2691-2695

Warner TD, Klemm P. What turns on the endothelins? Inflamm Res 1996; 45: 51-53

Anthoni C, Menningen RB, Rijken EJ, Laukköter MG, Spiegel HU, Senninger N, Schürmann G, Krieg T, Bosentan, an endothelin receptor antagonist, reduces leucocyte adhesion and inflammation in a murine model of inflammatory bowel disease. Int J Colorectal Dis 2006; 21: 409-418

Kriegl T, Abraham D, Lajfatis R. Fibrosis in connective tissue disease: the role of the myofibroblast and fibroblast-epithelial cell interactions. Arthritis Res Ther 2007; 9 Suppl 2: S4

Kernochan LE, Tran BN, Tangkijvichyan P, Melton AC, Tam SP, Yee HF. Endothelin-1 stimulates human colonic myofibroblast contraction and migration. Gut 2002; 50: 65-70

Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007; 292: C82-C97

Kumar R, Singh VP, Baker KM. The intracellular renin-angiotensin system: implications in cardiovascular remodeling. Curr Opin Nephrol Hypertens 2008; 17: 168-173

Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev 2006; 86: 747-803

Leung PS. The physiology of a local renin-angiotensin system in the pancreas. J Physiol 2007; 580: 31-37

Laviole JL, Sigmund CD. Minireview: overview of the renin-angiotensin system—an endocrine and paracrine system. Endocriornology 2003; 144: 2179-2183

Hirasawa K, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Im-
munohistochemical localization of angiotensin II receptor and local renin-angiotensin system in human colonic mucosa. J Histchem Cytochem 2002; 50: 275-282

127 Sironi L, Nobili E, Gianella A, Gelosa P, Tremoli E. Anti-inflammatory properties of drugs acting on the renin-angiotensin system. Drugs Today (Barc) 2005; 41: 609-622

128 Ruiz-Ortega M, Rupérez M, Esteban V, Rodríguez-Vita J, Sánchez-López E, Carvajal G, Egido J. Angiotensin II: a key factor in the inflammmatory and fibrotic response in kidney diseases. Nephrol Dial Transplant 2006; 21: 16-20

129 Yoshiji H, Kurita Y, Soshi J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, Fukui H. Angiotensin II type I receptor interaction is a major regulator for liver fibrosis development in rats. Hepatology 2001; 34: 745-750

130 Okada M, Suzuki K, Matsumoto M, Takada K, Nakanishi T, Horikoshi H, Higuchi T, Hosono Y, Nakayama M, Obusuzu F. Effects of angiotensin on the expression of fibrosis-associated cytokines, growth factors, and matrix proteins in human lung fibroblasts. J Clin Pharmacol Ther 2009; 34: 288-299

131 Kurikawa N, Suga M, Kuroda S, Yamada K, Ishikawa H. An angiotensin II type 1 receptor antagonist, olmesartan medoxomil, improves experimental liver fibrosis by suppression of proliferation and collagen synthesis in activated hepatic stellate cells. Br J Pharmacol 2003; 139: 1085-1094

132 Velez JC. The importance of the intrarenal renin-angiotensin system. Nat Clin Pract Nephrol 2009; 5: 89-100

133 Inokuchi Y, Morohashi T, Kawanaka I, Nagashima Y, Kihara M, Umemura S. Amelioration of 2,4,6-trinitrobenzene sulfonic acid induced colitis in angiotensinogen gene knockout mice. Gut 2005; 54: 349-356

134 Babystalsky MW, Rossiter G, Podolsky DK. Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease. Gastroenterology 1996; 110: 975-984

135 Jaszewski R, Tolia V, Ehrnpirex MN, Bodzin JH, Peelman RR, Konlipara R, Reinstock JW. Increased colonic mucosal angiotensin I and II concentrations in Crohn’s colitis. Gastroenterology 1990; 98: 1543-1548

136 Katada K, Yoshida N, Suzuki T, Okuda T, Mizushima K, Takagi T, Ichikawa H, Naito Y, Cepinskas G, Yoshikawa T. Dextran sulfate sodium-induced acute colonic inflammation in angiotensin II type 1a receptor deficient mice. Inflamm Res 2008; 57: 84-91

137 Wengrower D, Zanninelli G, Latella G, Necoceo S, Metanes I, Israeli E, Lysy J, Pines M, Papo O, Goldin E. Losartan reduces trinitrobenzene sulfonic acid-induced colocolitis in rats. Can J Gastroenterol 2012; 26: 33-39

138 Latella G, Fiocchi C, Caprilli R. News from the “5th International Meeting on Inflammatory Bowel Diseases” CAPRI 2010. J Crohns Colitis 2010; 4: 690-702

139 Wynn TA. Fibrotic disease and the TH1/TH2 paradigm. Nat Rev Immunol 2004; 4: 583-594

140 Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2005; 3: 521-533

141 Hoffmann KF, McCarty TC, Segal DH, Chaarmonn M, Hesse M, Davis EM, Cheever AW, Meltzer PS, Morse HC, Wynn TA. Disease fingerprinting with cDNA microarrays reveals distinct gene expression profiles in lethal type 1 and type 2 cytokine-mediated inflammatory reactions. FASEB J 2001; 15: 2452-2457

142 Harrington LE, Mangan PR, Weaver CT. Expanding the effector CD4 T-cell repertoire: the Th17 lineage. Curr Opin Immunol 2006; 18: 349-356

143 Maloy KJ. The Interleukin-23 / Interleukin-17 axis in intestinal inflammation. J Intern Med 2008; 263: 584-590

144 Gieling RG, Wallace K, Han YP. Interleukin-1 participates in the progression from liver injury to fibrosis. Am J Physiol Gastrointest Liver Physiol 2009; 296: G1324-G1331

145 Liu X. Inflammatory cytokines augments TGF-beta1-induced epithelial-mesenchymal transition in A549 cells by up-regulating TbetaR-I. Cell Motil Cytoskeleton 2008; 65: 955-944

146 Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, Kasperczyk M, Sorensen DR, Nilsen HR, Küchler AM, Vatn MH, Haraldsen G. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. Am J Pathol 2010; 177: 2804-2815

147 Migliaccio CT, Buford MC, Jessop F, Holian A. The IL-4Ralpha pathway in macrophages and its potential role in silica-induced pulmonary fibrosis. J Leukoc Biol 2008; 83: 630-639

148 Audoujehane L, Pissia A, Scatton O, Podevin P, Massault PP, Chouzenoux S, Soubrane O, Calmus Y, Conti F. Interleukin-4 induces the activation and collagen production of cultured human intrahepatic fibroblasts via the STAT-6 pathway. Lab Invest 2008; 88: 973-985

149 Roselló-Lleti E, Rivera M, Bentomeu V, Cortés R, Jordán A, González-Molina A. [Interleukin-4 and cardiac fibrosis in patients with heart failure]. Rev Esp Cardiol 2007; 60: 777-780

150 Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-15 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. J Clin Invest 1999; 104: 777-785

151 Chiaramonte MG, Cheever AW, Malley JD, Donaldson DD, Wynn TA. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. Hepatology 2001; 34: 273-282

152 Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med 2006; 12: 99-106

153 Fichtner-Feigl S, Strober W, Geissler EK, Schlitt HJ. Cytokines mediating the induction of chronic colitis and colitis-associated fibrosis. Mucosal Immunol 2008; 1 Suppl 1: S24-S27

154 Bhogal RK, Bona CA. Regulatory effect of extracellular signal-regulated kinases (ERK) on type I collagen synthesis in human dermal fibroblasts stimulated by IL-4 and IL-13. J Biol Chem 2008; 283: 472-496

155 Diaz JA, Booth AJ, Lu G, Wood SC, Pinsky DJ, Bishop DK. Critical role for IL-6 in hypertrophy and fibrosis in chronic cardiac allograft rejection. Am J Transplant 2009; 9: 1773-1783

156 Lucket-Chastain LR, Gallucci RM. Interleukin (IL)-6 modulates transforming growth factor-beta expression in skin and dermal fibroblasts from IL-6-deficient mice. Br J Dermatol 2009; 161: 237-248

157 Liao W, Yu C, Wen J, Jia W, Li G, Ke Y, Zhao S, Campell W. Adiponectin induces interleukin-6 production and activates STAT3 in adult mouse cardiac fibroblasts. Biol Cell 2009; 101: 263-272

158 Itoh H. IL-6 and Crohn’s disease. Curr Drug Targets Inflamm Allergy 2003; 2: 125-130

159 Pesce J, Kaviratne NL, Ramalingam TR, Thompson RW, Urban JF, Cheever AW, Young DA, Collins M, Grusby MJ, Wynn TA. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest 2006; 116: 2044-2055

160 Fina D, Caruso R, Pallone F, Monteleone G. Interleukin-21 (IL-21) controls inflammatory pathways in the gut. Endocr Metab Immune Disord Drug Targets 2007; 7: 288-291

161 Zenewicz LA, Yanacopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 2008; 29: 947-957

162 Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, Blumberg RS, Xavier RJ, Mizoguchi A. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest 2008; 118: 534-544

163 Simonian PL, Wehrmann F, Roark CL, Born WK, O’Brien RL, Fontenot AP, y6 T cells protect against lung fibrosis via IL-22. J Exp Med 2010; 207: 2239-2253
PPARγamma agonists prevent TGFbeta1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 2006; 350: 385-391

198 Nan YM, Han F, Kong LB, Zhao SX, Wang QG, Wu WJ, Yu J. Adenosine-released peroxisome proliferator activated receptor gamma overexpression prevents nutritional fibrotic steatohepatitis in mice. Scand J Gastroenterol 2011; 46: 358-369

199 Kapoor M, McCann M, Liu S, Huh K, Denton CP, Abraham DJ, Leask A. Loss of peroxisome proliferator-activated receptor gamma in mouse fibroblasts results in increased susceptibility to bleomycin-induced skin fibrosis. Arthritis Rheum 2009; 60: 2822-2829

200 Wei J, Ghosh AK, Sargent JL, Komura K, Wu M, Huang QQ, Jain M, Whitfield ML, Feghali-Bostwick C, Varga J. PPARγ downregulation by TGFβ in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. PLoS One 2010; 5: e13778

201 Yang L, Stimpson SA, Chen L, Wallace Harrington W, Rockey DC. Effectiveness of the PPARγ agonist, GW570, in liver fibrosis. Inflamm Res 2010; 59: 1061-1071

202 Wu M, Melichan DS, Chang, E, Warner-Blankenship M, Ghosh AK, Varga J. Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. Am J Pathol 2009; 174: 519-533

203 Kawai T, Masaki T, Doi S, Arakawa T, Yokoyama Y, Doi T, Kohno N, Yorioka N. PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. Lab Invest 2009; 89: 47-58

204 Aoki Y, Maeno T, Aoyagi K, Ueno M, Aoki F, Aoki N, Nakagawa J, Sando Y, Shimizu Y, Suga T, Arai M, Kurabayashi M. Pioglitazone, a peroxisome proliferator-activated receptor gamma ligand, suppresses bleomycin-induced acute lung injury and fibrosis. Respiration 2009; 77: 311-319

205 Talukdar R, Tandon RK. Pancreatic stellate cells: new target in the treatment of chronic pancreatitis. J Gastroenterol Hepatol 2008; 23: 34-41

206 Chen H, He YW, Liu WQ, Zhang JH. Rosiglitazone prevents murine hepatic fibrosis induced by Schistosoma japonicum. World J Gastroenterol 2008; 14: 2905-2911

207 Tsang CK, Qi H, Liu LF, Zheng XF. Targeting mammalian target of rapamycin (mTOR) for health and diseases. Drug Disc Today 2007; 12: 112-124

208 Wang X, Proud CG. The mTOR pathway in the control of protein synthesis. Physiology (Bethesda) 2006; 21: 362-369

209 Chung I, Kuo CI, Crabtree GR, Blesij J. Rapamycin-FKB specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 1992; 69: 1227-1236

210 Land SC, Tee AR. Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. J Biol Chem 2007; 282: 20534-20543

211 Li W, Petripol M, Molle KD, Hall MN, Battegay EJ. Human R. Hypoxia-induced endothelial proliferation requires both mTORC1 and mTORC2. Circ Res 2007; 100: 79-87

212 Humar R, Kiefer FN, Berms H, Resnik TJ, Battegay EJ. Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling. FASEB J 2002; 16: 771-780

213 Toschi A, Lee E, Gadir N, Ohh M, Foster DA. Differential dependence of hypoxia-inducible factors alpha 1 and alpha 2 on mTORC1 and mTORC2. J Biol Chem 2008; 283: 34495-34499

214 Del Bufalo D, Ciuffreda L, Trisciuoglio D, Desideri M, Cognetti F, Zupi G, Milella M. Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. Cancer Res 2006; 66: 5549-5554

215 Lieberthal W, Levine JS. The role of the mammalian target of rapamycin (mTOR) in renal disease. J Am Soc Nephrol 2009; 20: 2493-2502

216 Korfhragen TR, Le Cras TD, Davidson CR, Schmidt SM, Ikegami M, Whitsett JA, Hardie WD. Rapamycin prevents transforming growth factor-alpha-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2009; 41: 562-572

217 Whalley-Connell A, Habibi J, Fanllli Z, Hayden MR, Bagree S, Nistala R, Ryder S, Krueger B, Demarco V, Pulakat L, Ferraro CM, Parrish A, Sowers JR. Angiotensin II activation of mTOR results in tubulointerstitial fibrosis through loss of N-cadherin. Am J Nephrol 2011; 34: 115-125

218 Sofroniadiou S, Goldsmith D. Mammalian target of rapamycin (mTOR) inhibitors: potential uses and a review of haematological adverse effects. Drug Saf 2011; 34: 97-115

219 Zhu J, Wu J, Friell E, Liu SL, Bashey R, Rubin R, Norton P, Zern MA. Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. Gastroenterology 1999; 117: 1198-1204

220 Neef M, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. J Hepatol 2006; 45: 786-796

221 Fatsenker E, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. J Hepatol 2011; 55: 388-398

222 Geissler EK, Schlitt HJ. The potential benefits of rapamycin on renal function, tolerance, fibrosis, and malignancy following transplantation. Kidney Int 2010; 78: 1075-1079

223 Yoshizaki A, Yanaba K, Yoshiizaki A, Inoue Y, Komura K, Ogawa F, Takenaka M, Shimizu K, Asano Y, Hasegawa M, Fujimoto M, Sato S. Treatment with rapamycin prevents fibrosis in tight-skin and bleomycin-induced mouse models of systemic sclerosis. Arthritis Rheum 2010; 62: 2476-2487

224 Wang S, Wilkes MC, Leof EB, Hirschberg R. Noncanonical TGF-beta pathways, mTORC1 and Abl, in renal interstitial fibrogenesis. Am J Physiol Renal Physiol 2010; 298: F142-F149

225 Pouallhon N, Farge D, Roos N, Tacheau C, Neuzillet C, Michel L, Mauvel A, Verrecchia F. Modulation of collagen and MMP-1 gene expression in fibroblasts by the immunosuppressive drug rapamycin. A direct role as an antifibrotic agent? J Biol Chem 2006; 281: 33045-33052

226 Osman B, Akool e-S, Doller A, Muller R, Pfeifschelter J, Erhardt W. Differential modulation of the cytokine-induced MMP-9/TIMP-1 protease-antiprotease system by the mTOR inhibitor rapamycin. Biochem Pharmacol 2011; 81: 134-143

227 Ong CT, Khoa YT, Mukhopadhyay A, Do DV, Lim JJ, Aalami O, Phan TT. mTOR as a potential therapeutic target for treatment of keloids and excessive scars. Exp Dermatol 2007; 16: 394-404

228 Massey DC, Bredin F, Parkes M. Use of sirolimus (rapamycin) to treat refractory Crohn’s disease. Gut 2008; 57: 1294-1296

229 Dumortier J, Lapalus MG, Guillaume O, Poncet G, Gagnier MC, Bartens C, Sozaey J. Everolimus for refractory Crohn’s disease: a case report. Inflamm Bowel Dis 2008; 14: 874-877

230 Reinsch W, Panés J, Léman M, Schreiber S, Feagan B, Schmidt S, Sturniolo GC, Mikhailova O, Alexeeva O, Sanna L, Haas T, Korom S, Mayer H. A multicenter, randomized, double-blind trial of everolimus versus azathioprine and placebo to maintain steroid-induced remission in patients with moderate-to-severe active Crohn’s disease. Am J Gastroenterol 2010; 105: 2284-2291

231 Warnaar N, Hofker HS, Matthuis MH, Niesing J, Bruggink AH, Dijkstra G, Ploeg RJ, Schuurs TA. Matrix metalloproteinases as profibrotic factors in terminal ileum in Crohn’s disease: a case report. Inflamm Bowel Dis 2008; 14: 774-777

232 Ravi A, Garg P, Sitarasan VM. Matrix metalloproteinases in inflammatory bowel disease: boon or a bane? Inflamm Bowel Dis 2007; 13: 97-107

233 Pender SL. Do metalloproteinases contribute to tissue destruction or remodeling in the inflamed gut? Inflamm Bowel Dis 2006; 12: 863-869
Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol Rev* 2007; 87: 69-98

Louis E, Ribbens C, Godon A, Franchimont D, De Groote D, Hardy N, Boniver J, Belaiche J, Malaise M. Increased production of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 by inflamed mucosa in inflammatory bowel disease. *Clin Exp Immunol* 2000; 120: 241-246

Gao Q, Meijer MJ, Kuepper F, Sier CF, Kruidenier L, van Duijn W, van den Berg M, van Hogezand RA, Lamers CB, Verspaget HW. Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases. *Dig Liver Dis* 2005; 37: 584-592

Heuschkel RB, MacDonald TT, Monteleone G, Bajaj-Elliott M, Smith JA, Pender SL. Imbalance of stromelysin-1 and TIMP-1 in the mucosal lesions of children with inflammatory bowel disease. *Gut* 2000; 47: 57-62

Stawowy P, Margeta C, Kallisch H, Seidah NG, Chrétien M, Fleck E, Graf K. Regulation of matrix metalloproteinase MT1-MMP/MMP-2 in cardiac fibroblasts by TGF-beta1 involves furin-convertase. *Cardiovasc Res* 2004; 63: 87-97

Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT. A major role for matrix metalloproteinases in T cell injury in the gut. *J Immunol* 1997; 158: 1582-1590

Vaalamo M, Karjalainen-Lindsberg ML, Puolakainen P, Kere J, Saarialho-Kere U. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. *Am J Pathol* 1998; 152: 1005-1014

Ma C, Chegini N. Regulation of matrix metalloproteinases (MMPs) and their tissue inhibitors in human myometrial smooth muscle cells by TGF-beta1. *Mol Hum Reprod* 1999; 5: 950-954

McKaig BC, McWilliams D, Watson SA, Mahida YR. Expression and regulation of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinases by intestinal myofibroblasts in inflammatory bowel disease. *Am J Pathol* 2003; 162: 1355-1360

Di Sabatino A, Jackson CL, Pickard KM, Buckley M, Rovedatti L, Leakey NA, Picariello L, Cazzola P, Monteleone G, Tonelli F, Corazza GR, MacDonald TT, Pender SL. Transforming growth factor beta signalling and matrix metalloproteinases in the mucosa overlying Crohn’s disease strictures. *Gut* 2009; 58: 777-789

Lawrance IC, Wu F, Leite AZ, Willis J, West GA, Fiocchi C, Chakravarti S. A murine model of chronic inflammation-induced intestinal fibrosis down-regulated by antisense NF-kappa B. *Gastroenterology* 2003; 125: 1750-1761

Meijer MJ, Merebet-Ooms MA, van Duijn W, van der Zon AM, Hanemaaijer R, Verheijen JH, van Hogezand RA, Lamers CB, Verspaget HW. Effect of the anti-tumor necrosis factor-alpha antibody infliximab on the ex vivo mucosal matrix metalloproteinase-1 proteolytic phenotype in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; 13: 200-210

Strup-Perrot C, Mathé D, Linard C, Violot D, Milliat F, François A, Bourhis J, Vozenin-Brots MC. Global gene expression profiles reveal an increase in mRNA levels of collagens, MMPs, and TIMPs in late radiation enteritis. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G875-G885

Singh KP, Gerard HC, Hudson AP, Boros DL. Differential expression of collagen, MMP, TIMP and fibrogenic-cytokine genes in the granulomatous colon of Schistosoma mansoni-infected mice. *Ann Trop Med Parasitol* 2006; 100: 611-620

Clutterbuck AL, Asplin KE, Harris P, Allaway D, Mobasher A. Targeting matrix metalloproteinases in inflammatory conditions. *Curr Drug Targets* 2009; 10: 1245-1254