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Central role of IL-17/Th17 immune responses and the gut microbiota in the pathogenesis of intestinal fibrosis

Shuvra Ray^{1,2}, Carlo De Salvo¹, and Theresa T. Pizarro¹

¹Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, USA

²Department of Gastroenterology, Guy's & St. Thomas' Hospitals/University College London, London, United Kingdom

Abstract

Purpose of review—Intestinal fibrosis is a serious, yet common, outcome in patients with inflammatory bowel disease (IBD). Despite advances in developing novel treatment modalities to control chronic gut inflammation characteristic of IBD, no effective anti-fibrotic therapies exist to date. As such, a deeper understanding of the molecular mechanisms underlying intestinal fibrosis and the availability of relevant animal models are critical to move this area of investigation forward.

Recent findings—Emerging concepts in the pathogenesis of intestinal fibrosis include the central role of interleukin (IL)-17 and Th17 immune responses, although their precise contribution to chronic inflammation and IBD remains controversial. Other novel mediators of intestinal fibrosis, such as tumor necrosis factor (TNF)-like ligand 1A (TL1A) and components of the renin-angiotensin system, support the importance of IL-17. Additionally, recent studies utilizing novel mouse models highlight the significance of the gut microbiota and link components of bacterial sensing, including nucleotide-binding oligomerization domain-containing protein 2 (NOD2), to IL-17/Th17 immune responses in the development of inflammation-associated intestinal fibrosis.

Summary—Recent progress in identifying key mediators, novel animal models, and important mechanistic pathways in the pathogenesis of intestinal fibrosis hold promise for the development of effective anti-fibrotics in an area of significant, unmet clinical need.

Keywords

Intestinal fibrosis; IL-17; TL1A; renin-angiotensin system; SAMP1/YitFc model

Corresponding Author: Theresa T. Pizarro, Ph.D., Case Western Reserve University School of Medicine, Department of Pathology, Wolstein Research Building, Rm. 5534, 2103 Cornell Rd., Cleveland, OH 44106, USA; Phone: (216) 368-3306; Fax: (216) 368-0494; theresa.pizarro@case.edu.

Conflict of interest

None declared.

Introduction

Intestinal fibrosis is a common outcome in patients with IBD that results in progressive tissue architectural distortion, loss of function, luminal narrowing, and is a major cause of morbidity. Over a third of patients with Crohn's disease (CD) will undergo at least one surgical procedure, and many will require recurrent resections; currently, there are no effective anti-fibrotic therapies in IBD (1). The fundamental principle underlying fibrosis is the imbalance between extracellular matrix (ECM) deposition and degradation, wherein excessive deposition coincides with decreased degradation, commonly by matrix metalloproteinase (MMP) and its regulator, tissue inhibitors of MMPs (TIMPs). Chronic inflammation is tightly linked to intestinal fibrosis, wherein the current dogma focuses on upstream profibrogenic Th2 immune responses. Th2 cells are important against extracellular parasites and produce IL-4, IL-5 and IL-13 (2); of these, IL-13 is most strongly implicated in fibrogenesis (3). IL-13-producing lymphoid cells are abundant in intestinal strictures of CD patients and inhibit fibroblast MMP synthesis, leading to excess ECM deposition and fibrosis (4). IL-13 also induces transforming growth factor β 1 (TGF β 1) (5), which is a potent profibrogenic molecule and key mediator in many fibrotic diseases, including IBD. TGF β 1 induces activation and proliferation of profibrogenic cells, such as intestinal myofibroblasts, and stimulates ECM production (6).

One of the major difficulties investigating the underlying etiology of intestinal fibrosis is the relatively late presentation of clinical signs and symptoms. By the time patients become symptomatic, fibrosis is generally well-advanced, limiting the ability to study critical, early changes that drive fibrogenesis. Therefore, animal models are important tools to perform mechanistic studies to investigate the initiation and progression of disease. Numerous animal models of gut fibrosis have been proposed (Table I), but many are limited in the extent and distribution of fibrosis and/or have little relevance to the human condition.

In this review, we will discuss recent studies that expand current paradigms in the pathogenesis of intestinal fibrogenesis, incorporating alternative immune pathways, such as Th17 responses, interactions with the gut microbiome, and highlight novel mouse models that integrate these concepts.

IL-17 and associated mediators

Th17 cells define a subset of T helper cells that mainly produce IL-17A, but also IL-17F, IL-21, and IL-22, and are increasingly recognized as paramount in several chronic inflammatory disorders, including IBD (7). IL-17A has been implicated in fibrosis in multiple organs, including lung (8), liver (9), and heart (10); recent studies also support its role in the intestine, linking IL-17/Th17 immune responses and other associated mediators in the pathogenesis of gut fibrosis.

IL-17

In recent years, the precise role of IL-17/Th17 in IBD has been controversial, with dichotomous reports promoting both IL-17-dependent inflammation and protection. Early evidence for a proinflammatory role include findings that IL-23 via IL-17A is an important

pathway in T cell-mediated colitis (11), and that IL-17A is upregulated in the inflamed mucosa of CD patients (12). In addition, patients with active CD have high fecal IL-17A content and increased numbers of IL-17A-producing cells within the lamina propria (13). Conversely, IL-17A appears to be protective in the CD45RBhi model of colitis, wherein transfer of T cells from IL17A^{-/-} vs. wild-type mice greatly worsens disease (14). Dige *et al.* reported that while circulating IL-17A-producing CD45R0⁺CD4⁺ T cells are significantly increased in CD patients vs. normal controls, patients who respond to treatment with the anti-TNF agent, adalimumab, have a 2–3-fold increase in IL-17A⁺ cells, suggesting a possible protective function (15). Moreover, a recent clinical trial with the anti-IL-17A antibody, secukinumab, in active CD not only failed to show efficacy, but also resulted in deterioration of symptoms (16), in contrast to successful phase II trials in other inflammatory conditions (17–19). The authors speculate that this outcome may be due to inhibiting IL-17A's protective effects in the intestine as observed in earlier animal studies (16). Colombel *et al.* alternatively suggest that this effect may be due to IL-17A countering yeast infections, a suspected provocation in CD-associated inflammation, and exacerbation may be due to overgrowth of *Candida* species, thus provoking a flare (20).

While the complex role of IL-17 during chronic gut inflammation remains unresolved, the contribution of IL-17A in the development of fibrosis has evolved. IL-17A promotes activation of hepatic stellate cells (HSCs) (21) and proliferation of cardiac fibroblasts (22). Additionally, IL-17A^{-/-} mice show reduced fibrosis in inflammatory skin models (23) and IL-17A is essential to fibrogenesis in pulmonary fibrosis models (8). The role of IL-17 in CD fibrosis has recently been reported by Biancheri and colleagues (24), wherein IL-17 is overexpressed in strictures, wherein myofibroblasts possess IL-17A receptors, have reduced migratory ability, and produce more collagen and TIMP1 in response to IL-17A. As such, IL-17A appears to be important in promoting intestinal fibrosis, but given the multifaceted functions of IL-17 and the deleterious outcomes observed with secukinumab, patient selection in future clinical studies inhibiting IL-17A should be carefully considered. Targeted treatment to cohorts of patients with fibrosis and limited inflammatory activity may be a safer strategy. Interestingly, recent evidence suggests that only a specific subpopulation of IL-17A-producing Th17 cells with transient *c-kit* expression and stable MDR1 activity are proinflammatory in CD patients (25). It remains to be seen if this novel subpopulation plays an important role in fibrosis. Selective inhibition of these specific cells may prove to be more therapeutically efficacious than nonspecific inhibition of IL-17A.

TL1A

TL1A/TNF superfamily 15 (TNFSF15) is the only recognized ligand for its functional receptor, death receptor 3 (DR3). Previous studies report its importance in the pathogenesis of IBD where its overexpression is localized to macrophages, dendritic cells (DCs), and lymphocytes, in both active CD and ulcerative colitis (26, 27). Genome wide association studies (GWAS) in IBD patients have identified and confirmed the existence of protective and at-risk genetic polymorphisms in *tnfrsf15* (TL1A) and *tnfrsf6b* (DcR3) (28–30). Interestingly, subgroup analysis from the secukinumab anti-IL-17A study established an association between lack of response and absence of the rs4263839 *tnfrsf15* polymorphism, believed to increase TL1A activity, and suggests a link between IL-17A and TL1A-driven

inflammation (16). Wallace *et al.* investigated this further using the T cell transfer colitis model and showed that donor T cells from IL-17^{-/-} mice transferred into RAG^{-/-} recipients reiterated the results of the secukinumab study by exacerbating colitis, while donor T cells from TL1A-transgenic (Tg) mice produced even more severe disease (31). Colitis was improved following transfer of T cells from IL-17^{-/-}TL1A-Tg vs. IL-17^{+/+}TL1A-Tg mice, supporting earlier work in experimental encephalitis where TL1A was critical for Th17 differentiation and proliferation, and worsened disease severity (32). Together, these data corroborate the concept that IL-17/Th17 immune responses are involved in TL1A-driven inflammation.

Increased expression of both TL1A and DR3 has been reported in other animal models of colitis, and treatment with neutralizing antibodies against TL1A are effective in treating DSS-induced and G-protein $\alpha 2$ deficient colitic mice (33). Shih *et al.* first reported that global overexpression of TL1A results in increased collagen deposition in both the small intestines and colon of TL1A-Tg mice (34). In a follow-up study, specific TL1A overexpression in either myeloid, lymphoid, or both lineages also induced collagen deposition and overt, albeit mild, colonic fibrosis (35). Performing either DSS-induced or T cell transfer colitis on lymphoid- or myeloid-specific TL1A-Tg mice exacerbates inflammation as well as small and large intestinal fibrostenosis that is not associated with elevated levels of IL-13, but with IL-17 (36). Fibrosis can be reversed upon treatment with a neutralizing TL1A antibody and is associated with decreased connective tissue growth factor (CTGF), TGF β 1, and insulin-like growth factor-1 (IGF-1), while also reducing fibroblast and myofibroblast numbers (37). Thus, TL1A-Tg mice represent a novel model and TL1A may serve as a future therapeutic target to modulate intestinal fibrosis.

The renin-angiotensin system

The renin-angiotensin system (RAS) is classically considered a regulator of blood pressure and body fluid homeostasis. However, with the discovery of local RAS in various tissues and an evolving nexus of active peptides and receptors, the pleiotropic role of this system is increasingly recognized in other biologic functions. The key effector peptide in RAS is angiotensin II (Ang-II), which is produced by cleavage of angiotensin I by the angiotensin converting enzyme (ACE), and exerts its effects through the AT1 receptor. Angiotensin receptor blockers (ARBs) block AT1 and were initially developed for the management of hypertension. Nonetheless, Ang-II has been shown to have multiple proinflammatory and profibrogenic roles in various organs. Ang-II increases reactive oxygen species in the heart (38) and kidney (39), and promotes chemotaxis of inflammatory cells, an effect abrogated by the ARB, losartan (40). The proinflammatory actions of Ang-II are also partially enacted through DCs, which have enhanced migration, maturation, and antigen presenting ability when stimulated by Ang-II, a phenomenon also reversed by ARBs (41, 42).

Recently, association between IL-17 and Ang-II is becoming evident. Ang-II modulates T cell responses in experimental autoimmune encephalitis, wherein CD4⁺ T cells produce elevated Ang-II levels and increase secretion of IL-17 and IFN γ that is reversed by ARBs or ACE-inhibitors (43). Ang-II also promotes IL-17 production from splenic T cells following malaria infection in mice, which is largely attenuated by losartan (44). Together, these data

suggest that the proinflammatory actions of RAS are likely mediated through T cell responses and enhanced IL-17 production.

In regard to fibrosis, Ang-II promotes renal and cardiac fibrosis through induction of TGF β , leading to the activation of profibrotic pathways (6, 45). Pharmacotherapies that suppress RAS decrease disease progression, morbidity and mortality, and are now fundamental to the clinical management of chronic kidney and cardiac disease (46, 47). In the liver, Ang-II promotes HSC proliferation and TGF β production, and is in turn, produced locally by HSCs (48, 49). Ang-II is upregulated in human cirrhotic livers and ARBs have been shown to attenuate fibrosis in animal models (50) and pilot human liver disease studies (51).

Within the gastrointestinal tract, all components of RAS are expressed (52) and mucosal levels of Ang-II are elevated in Crohn's colitis (53). The angiotensinogen-6 AA genotype is associated with CD (54) and serum levels of RAS subcomponents are altered in IBD (55). Experimentally, the ACE-inhibitor, enalaprilat, reduces inflammation in colitic IL-10-deficient mice (56). Recently, Wengrower *et al.* investigated the effects of losartan in the chronic TNBS colitis model (57). Oral administration of losartan significantly reduced fibrosis and dramatically decreased mucosal TGF β levels. While the relevance to human IBD and fibrosis in this model may have limitations, significant reduction of fibrosis and TGF β in this study suggests that targeting RAS using ARBs is an important and potential anti-fibrotic strategy.

Novel models of intestinal fibrosis

Novel, as well as modifications of existing, animal models of intestinal fibrosis are rapidly emerging (Table I) and enhance investigation of mechanistic pathways, particularly for the early stages of fibrogenesis. The role of microbes in IBD and intestinal fibrosis has rapidly become a key area of interest as results from GWAS repeatedly highlight genes responsible for innate immunity, mucosal barrier integrity, and bacterial sensing (58). Moreover, multiple studies demonstrate gut bacterial dysbiosis in IBD patients compared to healthy controls (59). In fact, gut inflammation in almost all animal models of colitis, as well as in human CD, is either ameliorated or absent under germ-free (GF) conditions and/or decreased after antibiotic treatment, respectively, emphasizing the central role of host-microbial interactions in the pathogenesis of intestinal inflammation and fibrosis (60).

Adherent invasive *E. coli* (AIEC) infection

A number of IBD animal models utilize specific microbes or microbial components to generate inflammation and subsequent fibrosis, including the AIEC infection model. Members of the *E. coli* family represent normal constituents of a healthy intestinal microbiota. *E. coli* can acquire virulence factors and become pathogenic; AIEC are an example of such a pathogenic subtype (61). AIEC are able to adhere to, and invade, the gut epithelium and provoke a chronic inflammatory response. Their presence is frequently observed in CD patients and is suspected to play a role in the initiation and/or maintenance of inflammation. In the chronic AIEC infection model, mice are pretreated with streptomycin prior to receiving NRG857 (a human CD isolate of AIEC) by oral gavage. Small *et al* (61) showed that this infection results in ileal and colonic inflammation

involving Th1 and Th17 immune responses and protection by CD8⁺ cells. The resulting inflammation leads to cecal and colonic fibrosis in multiple mouse strains, in varying degrees, and progresses to transmural fibrosis after 63 days post-infection. This model shares significant similarities with CD and utilizes bacteria of likely relevance to the human condition. Conversely, while ileitis is observed in some mouse strains, it is significantly milder than in the colon and does not result in fibrosis. Strictures are also absent and the effects of abrogating inflammation or treatment with conventional pharmacotherapeutics has not been reported. Further investigation of these factors and genetic susceptibility loci may yield a relevant and easily replicable model of intestinal fibrosis, with particular utility in studying host responses to infection.

SAMP1/YitFc (SAMP) mouse strain

SAMP mice represents a model of Th1/Th2-driven chronic enteritis, with Th1 events predominating early and increasing as disease severity progresses, while Th2 immune responses are observed later when chronic inflammation is established (62, 63). Disease in SAMP is localized to the ileum, occurs spontaneously without chemical, genetic or immunological manipulation, and shares common histological features and response to therapy with human CD (reviewed in 64). Similar to CD patients, both TL1A and DR3 are upregulated in the inflamed ilea of SAMP mice, with lamina propria DCs serving as the major source of TL1A, which likely contributes to Th1-mediated inflammation through induction of IFN γ (65). Concurrent with the prevalence of Th2 immune responses observed during the later, chronic stages of ileitis, hypertrophy of the ileal muscularis propria, extensive collagen deposition, and frank stricture formation occur in SAMP with advanced disease (66). However, the progression and potential mechanisms of intestinal fibrosis in these mice have never been fully characterized. Preliminary findings from our laboratory suggest that fibrosis in SAMP is first observed at 20 weeks of age, and reaches peak levels by 50 weeks, with a dramatic increase in the expression of collagen, IGF-1, and CTGF vs. AKR (parental control strain) mice (67).

Interestingly, ileitis in SAMP mice raised under GF conditions persists, but is markedly attenuated, both in incidence and severity, which is dependent on Th2 immune responses (68). These findings, similar to that observed in CD patients, highlight the importance of gut microbial constituents in chronic intestinal inflammation; foremost of which is the intracellular bacterial sensing receptor, NOD2. *Nod2* polymorphisms confer an increased risk of CD and are associated with a more aggressive disease course, in addition to a fibrostenotic phenotype and consequent increased surgery rate (69). Although SAMP mice do not appear to have mutations within the *nod2* gene, Corridoni *et al.* recently demonstrated that SAMP mice possess a functional defect in NOD2 signalling and fail to respond to muramyl dipeptide, a bacterial product recognized by NOD2 (70). Furthermore, this defect is restricted to the hematopoietic compartment and leads to impaired clearance of *Salmonella* infection. NOD2 has also been linked to IL-17 in experimental colitis. Erman *et al.* investigated the role of IL-17A-secreting innate lymphoid cells in a mouse model of microbiota-driven immune-mediated colitis (71), reporting that pathogenic IL-17A-dependent immune responses were induced by microbial stimulation of DCs through NOD2 and that deletion of NOD2 prevents the development of colitis. Although the role of IL-17

has not been extensively studied in SAMP mice, a clear advantage for using this model is the ability to investigate the natural course of disease over time. Future studies using SAMP and other novel, relevant animal models (reviewed in 72) will serve as useful tools to investigate potential mechanisms involved in the development and progression of intestinal fibrosis and stricture formation, such as that observed in IBD.

Conclusion

Current advances in the field of inflammation-associated intestinal fibrosis propose a central role of IL-17/Th17-dependent immunity and associated mediators, including TL1A and components of RAS, as an upstream alternative pathway from the established paradigm of IL-13/Th2-dependent immune responses (summarized in Figure 1). In addition, the emergence of novel mouse models, including AIEC infection, and TL1A-Tg and SAMP mice, highlight the importance of the gut microbiome and will facilitate future mechanistic studies to investigate intestinal fibrosis.

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*of special interest

**of outstanding interest

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Key points

- Despite the failure of the anti-IL-17A antibody, secukinumab, in treating active CD, the importance of IL-17 and Th17 immune responses in fibrosis is becoming clear and it may be possible to design future clinical trials targeting a more specific patient population.
- The discovery of proinflammatory Th17 subpopulations provides the opportunity to interfere with proinflammatory Th17 responses without triggering the deleterious effects of inhibiting regulatory anti-inflammatory functions.
- The central role played by TL1A in inflammation-associated fibrosis and the improvement observed with anti-TL1A antibody treatment in animal studies, despite residual inflammation, heralds a novel putative target for anti-fibrotic pharmacotherapy in CD.
- Evidence supports a significant role for angiotensin II in gut fibrosis as well as inflammation; the availability of widely used, safe and well-tolerated oral antagonists suggest that these medications could rapidly enter human translational studies.
- Recent advances in the development of novel and relevant animal models of intestinal fibrosis, such as the AIEC infection model and the SAMP mouse strain, will facilitate future investigation to study important contributions of the gut microbiota, dissect critical mechanisms during the natural course of disease over time, as well as aid in evaluating potential anti-fibrotic strategies.

Fig 1. Emerging role of IL-17/Th17-dependent immune responses and the contribution of the gut microflora in the pathogenesis of intestinal fibrosis

Fibrosis results from an imbalance between ECM deposition and degradation, which is under the control of the MMP:TIMP expression ratio. Th2 immune responses and Th2 cytokines, particularly IL-13, have been implicated in upstream events leading to inflammation-associated gut fibrosis. Recent evidence, however, suggests that IL-17/Th17 immunity are also central to the process of fibrogenesis and links established profibrogenic molecules and pathways, including TGFβ and various growth factors, as well as novel profibrogenic mediators, such as TL1A/DR3 and Ang-II, to myofibroblast proliferation and collagen deposition, resulting in intestinal fibrosis. In addition, the impact of the gut microflora and bacterial sensing receptors, such as NOD2, are emerging as important contributors not only to the pathogenesis of IBD, but also to gut fibrosis. Platelet-derived growth factor (PDGF).

Table 1

Animal models of gut fibrosis.

CATEGORY	MODEL	MODE OF ADMINISTRATION/OUTCOME
Chemically-Induced	DSS	Administered in drinking water, causing epithelial damage and permeabilization of the colonic mucosa with subsequent acute inflammation; cycling of DSS causes chronic inflammation and ensuing fibrosis
	TNBS	Colonic enema administration of TNBS/ethanol damages epithelial barrier and causes a T cell-dependent transmural inflammation; long-term administration results in colonic fibrosis
Microbial	PG-PS	Injection directly into the cecal or small bowel wall induces granulomatous enterocolitis with significant fibrosis
	Fecal injection	Injection of fecal suspension directly into the bowel wall of colon causes aggressive colitis and transmural fibrosis
	Chronic <i>Salmonella</i> infection	Oral administration after pre-treatment with streptomycin causes colonic mucosal and transmural inflammation with significant fibrosis
	AIEC infection	Gavage of the human CD isolate of AIEC, NRG857, after pre-treatment with streptomycin causes ileo-colonic inflammation and Th1- and Th17-mediated and fibrosis
Genetically-Manipulated Mice	TGFβ1-Tg TβRII k-fib-Tg	Enema administration of these adenoviral vectors overexpressing TGFβ into the colon leads to acute and chronic inflammation, ECM deposition and thickening of muscularis layers
	MCP-1-Tg	Intramural injection of adenoviral vector carrying MCP-1 in the rectum leads to transmural inflammation, collagen deposition, and fibrosis
	IL-10 deficient	Genetic deletion of IL-10 results in transmural inflammatory lesions of the colon, crypt abscesses, and thickening of the bowel wall; evidence of increased susceptibility to developing post-surgical fibrosis in small intestines after ileo-cecal resection
Immune-Mediated	T cell transfer	Transfer of donor CD4 ⁺ /CD45RB ^{high} T cells into immunodeficient recipient mice results in wasting disease, colitis and mild fibrosis
Spontaneous	SAMP1/YitFc mouse strain	Develops spontaneous terminal ileitis and subsequent fibrosis

dextran sodium sulphate (DSS); trinitrobenzene sulfonic acid (TNBS); adherent invasive *E. Coli* (AIEC); macrophage chemoattractant protein-1 (MCP-1)